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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

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გამოიცემა თბილისის სახელმწიფო სამედიცინო უნივერსიტეტთან
თანამშრომლობითა და მისი პატრონაჟით

ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

ТБИЛИСИ - НЬЮ-ЙОРК

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Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ, ინგლისურ და გერმანულ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები, რეცენზიები.
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2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 6 pages and not exceed the limit of 15 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

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Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

6. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

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In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

8. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

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Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

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4. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

5. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

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7. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით.

8. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

9. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

10. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: შესავალი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

Содержание:

НАУКА

ОПРЕДЕЛЕНИЕ ФАКТОРОВ РИСКА РАЗВИТИЯ ПЛАЦЕНТАРНОЙ ДИСФУНКЦИИ У ЖЕНЩИН С НЕВЫНАШИВАНИЕМ БЕРЕМЕННОСТИ В АНАМНЕЗЕ С ПРИМЕНЕНИЕМ РЕТРОСПЕКТИВНОГО, СРАВНИТЕЛЬНОГО И СТАТИСТИЧЕСКОГО АНАЛИЗОВ

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Невынашивание беременности является одной из актуальных проблем современной перинатальной медицины, обусловленное не только медицинскими (анатомические, эндокринные, инфекционные, хромосомные и иммунные аномалии, тромболитические нарушения, экстрагенитальные заболевания идиопатической этиологии), но и социальными факторами (снижение прироста населения, повышение уровня перинатальной и детской смертности, отрицательное влияние на детородную функцию женщин) [1].

Множеством исследователей доказано, что первичное невынашивание беременности, осложненное плацентарной дисфункцией, в 47,6-77,3% наблюдений ведет к дистрессу и задержке роста плода [1,2].

Плацентарная дисфункция (ПД) имеет мультифакторную природу, сопровождает все осложнения беременности, реализуясь на молекулярном, клеточном, тканевом и органном уровнях и нарушает способность плаценты поддерживать адекватный обмен между организмом матери и плода [4,7].

По данным ряда авторов [1,4], дистресс плода индуцирует преждевременные роды, несмотря на мероприятия, принятые по оздоровлению женщин. Преждевременные роды являются одной из основных проблем перинатологии не только в Украине, но и во всем мире. Согласно данным исследования Bord Too Soon и при участии более 50 организаций, включая Глобальный альянс по предотвращению преждевременных родов - GAPPS, доказано, что каждый десятый новорожденный рождается раньше положенного срока [4].

Преджевременные роды являются основной причиной перинатальной заболеваемости и смертности новорожденных, составляя 75-80%, причем около 40% этих наблюдений приходится на роды сроком меньше 32 недель. Следует также отметить, что выжившие после преждевременных родов дети сталкиваются с целым рядом неблагоприятных неонатальных исходов, включая тяжёлую травму головного мозга, хронические заболевания лёгких, ретинопатию недоношенных, некротический колит, неонатальный сепсис [1,3,4].

По мнению исследователей, риск преждевременных родов увеличивается при каждой последующей беременности [1,7].

Современная акушерская стратегия по снижению перинатальной заболеваемости и смертности основана на изучении и предупреждении факторов риска, оказывающих неблагоприятное воздействие на внутриутробное развитие плода [2].

Количественный и качественный анализ факторов риска позволил установить симптомы и признаки задержки развития плода при скрининге беременных [1,2,7].

Разработан и применяется ряд диагностических и лечебных методов, оказывающих положительное влияние на состояние беременной и плода [2-4]. Из них в настоящее время широко применяется изучение сердечной деятельности плода с помощью антенатальной кардиотокографии (КТГ) плода, что позволяет получить информацию о состоянии плода, как во время беременности, так и при родах, а, следовательно, дать оценку деятельности фетоплацентарной системы.

По данным ряда авторов [2,5,6], перспективным в оценке функционального состояния плода после 29-30 недель беременности считается исследование его биофизического профиля, так как именно с помощью этого метода можно с высокой достоверностью определить внутриутробное состояние плода и прогнозировать исход родов.

Целью исследования явилось на основании данных ретроспективного и сравнительного анализов выявить факторы риска, влияющие на развитие плацентарной дисфункции и исходы беременности у женщин с невынашиванием беременности в анамнезе.

Материал и методы. Для решения поставленной цели проводили ретроспективный и клинико-статистический анализы течения беременности и ее исходов у 1184 женщин с невынашиванием беременности в анамнезе, при двух и более самопроизвольных абортах или

преждевременных родах на основании медицинской документации (индивидуальные карты беременной).

С целью сравнительной оценки данных, женщины с угрозой прерывания беременности (ПБ) были разделены на 3 группы: I группу составили 592 женщины с угрозой прерывания беременности, осложнённой ПД в анамнезе, II группу - 592 женщины с угрозой прерывания беременности без ПД в анамнезе, III (контрольную) группу составили 100 соматически здоровых фертильных женщин с одними и больше своевременными родами в анамнезе.

Все женщины были репродуктивного возраста, комплексно клинико-лабораторно и функционально обследованы.

КТГ плода проводилась в динамике в течение III триместра беременности, начиная с 25 недели, поскольку к этому сроку имеет место сочетание всех типов двигательной активности плода. Исследование проводилось с помощью аппарата фирмы «Sonicad» (Япония) по двум каналам мониторинга: частота сердечных сокращений плода и сокращение матки. Исследование осуществлялось путем записи КТГ в положении беременной на боку в течение 30-60 минут в дневное время в интервале между 10 и 15 часами со скоростью движения бумаги 20 мм в минуту. Каждой пациентке проводили 15 кардиотокографических исследований.

Расшифровка кардиотокограмм осуществлялась по балльной системе шкалы W. Fischer (1976), с помощью которой определяли: базальную частоту сердечных сокращений (ЧСС), уд/мин; вариабельность сердечных сокращений, уд/мин, частоту осцилляций/мин; амплитуду осцилляций/мин. ЧСС оценивалась на основании количества акцелераций и децелераций в течение 30 мин, а также их длительностью, сроком и характером появления с использованием функциональных проб, к которым относится нестрессовый тест (НСТ). Суть теста заключается в появлении акцелераций сердцебиения плода при повышенной двигательной активности, что характеризует его нормальное состояние (реактивный тест), либо децелерации сердцебиения плода.

Биофизический профиль плода (БПП) оценивали с 30 недель беременности путем суммирования баллов отдельных биофизических параметров (реактивность сердечной деятельности плода на НСТ, дыхательные движения, тонус, двигательная активность плода, объем околоплодных вод). Каждый параметр БПП оценивался в баллах - максимально 2 балла, минимально 0 баллов, оценка в 7-10 баллов свидетельствует о нормальном состоянии плода. НСТ плода изучался с помощью кардиотокографии, остальные параметры состояния плода оценивались с помощью УЗИ. УЗИ проводилось на аппаратах SAL-35 и U-2000 (фирмы «Toshiba» и «Fukuda», Япония с применением линейных и секторных датчиков частотой 3,5 и 5 Мгц.

Проведен статистический анализ рассматриваемой проблемы. Известно, что распределение медицинских наблюдений значительно отличается от нормального распределения. В этих случаях необходимо проверить исследуемую выборку на нормальность распределения. С этой целью производили вычисления показателей ассиметрии и эксцесса. В противном случае вычисления могут быть необоснованными и ведут к ошибочным выводам.

Индекс достоверной разницы p определялся по таблицам Стьюдента: сравнивали t вычисленное с t табличным для соответствующего числа n обследованных.

Статистическая обработка данных исследования проводилась с помощью стандартного программного обеспечения Statistic 5, MS Excel 2000.

Результаты и их обсуждение. Физиологическим нормативом базальной ЧСС, согласно нормам, установленным Приказом №900 МОЗ Украины от 27.12.2006 г. об утверждении клинического протокола оказания акушерской помощи «при дистрессе плода при беременности и во время родов», определено 110-170 уд/мин. Полученные в результате проведенного анализа данные, представленные в таблице 1, показывают, что базальная ЧСС 120-160 уд/мин по шкале W. Fischer установлена в контрольной группе у всех женщин, в I – у 427 (72,1%), во II группе - у 509 (86,0%) женщин ($p<0,05$). Сравнение вариабельности ЧСС в пределах от 160 до 180 уд/мин между контрольной, I и II группами выявило достоверное повышение данного показателя в контрольной и II группах ($p<0,001$); акцелерации за 30 мин были спорадическими у 1036 беременных, из них: в контрольной группе - у всех женщин, в I группе – у 427 (72,1%), во II группе - у 509 (86,0%) женщин. Совокупность таких параметров как: базальная ЧСС <160 уд/мин или >120 уд/мин, вариабельность - >180 уд/мин, акцелерации за 30 мин - периодические, свидетельствует в пользу дистресса плода в I группе - у 165 (27,1%) женщин, во II группе – у 83(14%) женщин, т.е. в I группе выявлено почти в два раза больше случаев дистресса плода, чем во II.

Акцелерация, частота и амплитуда осцилляций достоверно больше были в контрольной и во II группах ($p<0,001$, $p<0,05$). Необходимо отметить, что параметры сердечной деятельности плода в изучаемых группах ни в одном случае не оценены в 0 баллов. Остальные параметры сердечной деятельности плода находились в пределах нормы и оценены в 2 балла во всех группах.

Таблица 1. Сравнительная оценка состояния плода у женщин с невынашиванием беременности по шкале W.Fischer (1976)

Параметры		Баллы	Группы женщин					
			контрольная группа		I группа		II группа	
			абс.	%	абс.	%	абс.	%
базальная ЧСС, уд/мин	<100	0	-	-	-	-	-	-
	100-120	1	-	-	165	27,9	83	14
	120-160	2	100	100	427	72,1	509	86
вариабельность ЧСС, уд/мин	>180	0	-	-	165	27,9	83	14
	160-180	1	100	100	427	72,1	509	86
частота осцилляций/мин	<3	0	-	-	-	-	-	-
	3-6	1	-	-	165	27,9	83	14
	>6	2	100	100	427	72,1	509	86
амплитуда осцилляций/мин	<3 или синусоидальная	0	-	-	-	-	-	-
	5-9 или >25	1	100	100	592	100	592	100
акцелерации за 30 мин	отсутствуют	0	-	-	-	-	-	-
	периодические	1	-	-	165	27,9	83	14
	спорадические	2	100	100	427	72,1	509	86
децелерации за 30 мин	поздние	0	-	-	-	-	-	-
	поздние	1	-	-	-	-	-	-
	отсутствуют	2	100	100	592	100	592	100
длительность	длительные	0	-	-	-	-	-	-
	кратковременные	1	-	-	-	-	-	-
срок появления	ранние	2	-	-	-	-	-	-
характер	вариабельные	0	-	-	-	-	-	-
	невариабельные	1	-	-	-	-	-	-

При оценке нестрессового теста у всех беременных диагностировали реактивный тест.

Выявлено, что у всех беременных контрольной и II групп и 416 (70,3%) беременных I группы БПП составил от 7 до 10 баллов, что указывает на удовлетворительное состояние плода. У 176 (29,7%) беременных I группы БПП был неудовлетворительным и составил 5-6 баллов. Этим беременным проведена активная терапия лечения сопутствующих заболеваний, направленная на оптимизацию функции плаценты, улучшение маточно-плацентарного кровотока. 2-3 дня спустя были получены удовлетворительные результаты (8-10 баллов).

При детальном рассмотрении каждого параметра состояния плода следует отметить, что НСТ у 176 (29,7%) женщин I группы и у 83 (14%) женщин II группы оценивался в 1 балл. Этой оценке соответствовали базальная ЧСС 100-120 уд/мин, вариабельность ЧСС - 160-180 уд/мин, частота осцилляций - 3-6 мин, амплитуда осцилляций - 5-9 или >25 мин, акцелерации за 30 минут - периодические, что указывает на дистресс плода, несмотря на отсутствие децелерации.

Учитывая, что частота дыхательных движений плода (ДДП) по данным различных авторов [1,4,8] неодинакова, оценку проводили согласно нормам, установленным МОЗ Украины. У всех женщин контрольной и II групп, у 296 (50%) женщин I группы оценка ДДП составила 2 балла. По данным УЗИ, за 30 минут наблюдений отмечалось не меньше одного ДДП длительностью 60 сек.

Однако у остальных 296 (50%) женщин I группы длительность ДДП была снижена до 30 сек. и оценена в 1 балл, что, по всей вероятности, свидетельствует о патологическом состоянии плода. Следует отметить, что в норме ДДП непостоянны, о чем указывают и другие авторы [1,8,9].

Двигательная активность плода при 30-минутном УЗИ составила не меньше 3 движений (2 балла) в контрольной группе у всех женщин, в I группе - у 427 (72,1%) женщин, во II группе - у 509 (88%) женщин, а у 165 (27,8%) женщин I группы и 83 (14%) - II группы двигательная активность была снижена до 1 или 2 движений, что оценивалось в 1 балл. Из вышеизложенного следует, что в I группе, в которой в анамнезе

выявлена дисфункция плаценты, у каждой 4 женщины была понижена двигательная активность плода, а во II группе – у каждой 7 женщины.

Тонус плода при 30-минутном УЗИ с оценкой в 2 балла при одном и более разгибаний плода с возвращением его в сгибательное положение позвоночника и конечностей отмечено в контрольной и II группах у всех женщин, в I группе - у 427 (72,1%); одно разгибание с возвращением в сгибательное положение плода было оценено в 1 балл у 165 (27,9%) женщин I группы, т.е. в I группе у каждой третей женщины с невынашиванием беременности был понижен тонус плода.

В контрольной группе у всех, в I группе - у 427 (27,1%) женщин, во II группе - у 592 (100%) женщин объем околоплодных вод измерялся в матке - вертикальный диаметр свободного участка вод в 2 см и больше оценивался в 2 балла. Лишь у 165 (27,9%) женщин I группы вертикальный диаметр свободного участка вод варьировал в пределах 1-2 см (1 балл). Это допустимая норма, как свидетельствуют и другие исследователи [9,10], однако, при понижении вертикального диаметра свободного участка вод ниже 1 см и тесном размещении частей тела плода повышается опасность сдавливания пуповины.

Дистресс плода во время родов был диагностирован у 165 (27,9%) беременных I группы; у остальных он не выявлен. У всех женщин контрольной группы роды были своевременные, через естетвенные родовые пути, осложнений при родах не выявлено, новорожденные на 1 и 5 минуте жизни по шкале Апгар оценены в 9-10 баллов, масса новорожденных составила 3000±100 г.

Анализ осложнений беременности в I и II группах выявил, что у 176 (29,7%) женщин I группы беременность завершилась преждвременными родами, у 416 (70,3%) женщин I группы и всех женщин II группы роды были своевременными. Характеристика течения родового акта представлена в таблице 2.

Данные таблицы 2 свидетельствуют, что у всех женщин II группы роды были своевременными; в I группе процент своевременных родов составил 70,2%. Роды в I группе через естественные родовые пути протекали у 213 (35,9%) беременых, путем операции кесарево сечение - у 203 (34,3%) женщин; во II группе: через естественные родовые пути - у 486 (82,1%) женщин, путем операции кесарево сечение - 106 (17,9%) жещин, т.е. во II группе было в 2 раза больше родов через естественные родовые пути и в 2 раза меньше операций кесаревого сечения, чем в I группе рожениц. Необходимо отметить, что во II группе преждевременные роды не отмечены.

Таблица 2. Особенности родов у женщин с угрозою переривания беременности

Особенности родов	**Женщины с угрозою невынашиванния беременности**				
	I группа n=592		**II группа n=592**		**Всего n=1184**
	абс.	**%**	**абс.**	**%**	**абс.**
роды своевременные из них: -через естественные родовые пути; -путем операции кесарево сечение.	416 213 203	70,2 35,9 34,3	592 486 106	100 82,1 17,9	1008 699 309
роды преждевременные из них: через естественные родовые пути; -путём операции кесарево сечение	176 115 61	29,7 19,4 10,3	- - -	- - -	176 115 61
предлежение плода: -головное; -тазовое	 581 11	 98,1 1,9	 592 -	 100 -	 1173 11
несвоевременное отхождение околоплодных вод	106	17,9	-	-	106
отслойка нормально расположенной плаценты	187	31,6	58	9,8	245
слабость родовой деяльности	214	36,1	95	16,0	309
ручное отделение плаценты и выдиления последа	256	43,2	130	21,9	386
гипотоническое кровотечение	156	26,3	-	-	156
дистрес плода во время родов	165	27,8	-	-	165
травмы мяких родовых путей	191	32,3	194	32,8	385

Преждевременные роды произошли на 36-37 неделе беременности у 176 (29,7%) женщин I группы, то есть у каждой третей женщины, из них путем операции кесарево сечение - у 61 (10,3%) женщины.

Предлежание плода в большиcтве случаев было головное, как в I, так и во II группах беременных, лишь у 1,9% женщин I группы выявлено тазовое предлежение. Наиболее часто встречались следующие осложнения: несвоевременное отхождение околоплодных вод - у 17,9% женщин I группы (каждая пятая в группе); отслойка нормально расположенной плаценты - у 31,6% женщин I группы, у 9,8% женщин II группы (в 3 раза чаще у беременных I группы); слабость родовой деятельности - у 36,1% женщин I группы и у 16,0% женщин II группы (в I группе в 2 раза чаще, чем во II). Ручное выделение плаценты и отделение последа было в 2 раза чаще в I группе (43,2%), чем во II (21,9%). Гипотоническое кровотечение – в 26,3% случаев, дистресс плода во время родов – в 27,8% только в I группе. Различий в показателях травм мягких родовых путей в обеих группах исследования не выявлено (таблица 2).

Проведение операции кесарево сечение в большенстве случаев было необходимо как для матери, так и плода. Наиболее частой акушерской патологией - показанием к оперативному родоразрешению была отслойка нормально расположенной плаценты или предлежение; а со стороны плода - дистресс при беременности.

Послеродовой и послеоперационный периоды у рожениц протекали без осложнений. Случаев родового травматизма новорожденного не выявлено. В таблице 3 приводяться данные о состоянии новорожденных.

Из таблицы 3 следует, что в I группе у 70,3% женщин новородженные были здоровые, у 29,7% женщин выявлен дистресс плода во время родов, реанимационные мероприятия были эффективными. Во II группе женщин все новорожденные были здоровыми. Антенатальной смерти ни во одном случае не зафиксировано. Общее число живых детей составило 1184.

Изучались также некоторые показатели физического развития новорожденных, матери которых перенесли угрозу прерывания беременности на разных сроках (таблица 3).

Таблица 3. Состояние новорожденных, матери которых перенесли прерывание беременности

Оценка по шкале Апгар (баллы)		Группы женщин с невынашиванием беременности								
		I группа женщин n=592				II группа женщин n=592				Всего
		Роды своевременные		Роды преждевременные		Роды своевременные		Роды преждевременные		
		абс.	%	абс.	%	абс.	%	абс.	%	абс.
8-10	1 мин.	416	70,3	-	-	592	100	-	-	1008
	5 мин.	416	70,3	-	-	592	100	-	-	1008
6-7	1 мин.	-	-	176	29,7	-	-	-	-	176
	5 мин.	-	-	176	29,7	-	-	-	-	176

Таблица 4. Масса новорожденных, матери которых перенесли прерывание беременности

Масса тела, гр	Группы женщин с невынашивантем береметности								
	I группа				II группа				
	Роды своевременные		Роды преждевременные		Роды своевременные		Роды преждевременные		Всего
	абс.	%	абс.	%	абс.	%	абс.	%	
До 1500	-	-	-	-	-	-	-	-	-
1501-2000	-	-	6	1,0	-	-	-	-	6
2001-2500	31	5,2	23	3,9	-	-	-	-	31
2501-3000	366	61,8	98	16,5	36	6,1	-	-	402
3001-3500	19	3,2	49	8,3	292	49,3	-	-	311
3501-4000	-	-	-	-	205	34,6	-	-	205
Больше, чем 4000	-	-	-	-	59	10,0	-	-	59

Анализ данных таблицы 4 выявил, что масса новорожденных была самой низкой у женщин с преждевременными родами - 6 (1,0%) новорожденных, пониженная масса тела у 31 (5,2%) новорожденного, родившегося в срок, и у 23 (3,9%) новорожденных, которые родились преждевременно у женщин I группы.

Прерывание беременности в III триместре допускает высокую вероятность рождения жизнеспособного ребенка, однако хорошо известно, что недоношенность является основной причиной перинатальной смертности.

Таким образом, установление и анализ факторов риска позволяют предотвратить осложнения при беременности и, тем самым, снизить репродуктивные потери и улучшить демографические показатели.

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SUMMARY

ESTIMATION OF RISK FACTORS OF PROGRESSION OF PLACENTAL DYSFUNCTION IN WOMEN WITH RECURRENT PREGNANCY LOSS IN PAST MEDICAL HISTORY, USING RETROSPECTIVE, COMPARATIVE AND STATISTICAL ANALYSIS

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The retrospective comparative analysis of the following three groups is presented: control group composed of 100 somatically healthy pregnant women; 592 pregnant women with recurrent pregnancy loss complicated with placental dysfunction in the past medical history – the 1st group; and 592 pregnant women with recurrent pregnancy loss without placental dysfunction in the past medical history – the 2nd group. The fetal cardiotocography was analyzed with determination of non-stress test, fetus ultrasound investigation; the fetus biophysical profile was determined; the fates of the current pregnancy and childbearing and the state of the newborn infant were studied; using the statistical analysis, the risk factors affecting the progression of placental dysfunction in women with recurrent pregnancy loss in the past medical history were discovered.

Keywords: risk factors, placental dysfunction, pregnancy, fetus.

РЕЗЮМЕ

ОПРЕДЕЛЕНИЕ ФАКТОРОВ РИСКА РАЗВИТИЯ ПЛАЦЕНТАРНОЙ ДИСФУНКЦИИ У ЖЕНЩИН С НЕВЫНАШИВАНИЕМ БЕРЕМЕННОСТИ В АНАМНЕЗЕ С ПРИМЕНЕНИЕМ РЕТРОСПЕКТИВНОГО, СРАВНИТЕЛЬНОГО И СТАТИСТИЧЕСКОГО АНАЛИЗОВ

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Изложен ретроспективный сравнительный анализ трех групп беременных: контрольная группа - 100 соматически здоровые беременные, I группа - 592 беременных с невынашиванием беременности, осложненной плацентарной дисфункцией в анамнезе и II группа -592 беременных с невынашиванием без плацентарной дисфункции в анамнезе. Проанализированы результаты кардиотокографии плода с определением нестрессового теста, ультразвуковые исследования плода, определены биофизический профиль плода, оценены исходы настоящей беременности и родов, состояние новорожденного и с помощью статистического анализа выявлены факторы риска, влияющие на развитие плацентарной дисфункции у женщин с невынашиванием беременности в анамнезе.Таким образом, установление и анализ факторов риска позволяют предотвратить осложнения при беременности и, тем самым, снизить репродуктивные потери и улучшить демографические показатели.

რეზიუმე

პლაცენტური დისფუნქციის განვითარების რისკ-ფაქტორები ქალებში შეუნარჩუნებელი ორსულობით ანამნეზში (რეტროსპექტური, შედარებითი, სტატისტიკური ანალიზი)

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მოყვანილია ორსულთა სამი ჯგუფის შედარებითი რეტროსპექტური ანალიზი: საკონტროლო

ჯგუფის 100 სომატურად ჯანმრთელი ორსული; 592 ორსული შეუნარჩუნებელი ორსულობით და გართულებული პლაცენტური დისფუნქციით ანამნეზში (I ჯგუფი) და 592 ორსული შეუნარჩუნებელი ორსულობით ანამნეზში პლაცენტური დისფუნქციის გარეშე (II ჯგუფი).

ჩატარებულ იქნა ნაყოფის კარდიოტოკოგრაფია არასტრესული ტესტის განსაზღვრით, ნაყოფის ულტრაბგერითი გამოკვლევა,განისაზღვრა ნაყოფის ბიოფიზიკური პროფილი. შესწავლილ იქნა აღნიშნული ორსულობის და მშობიარობის შედეგები, ახალშობილის მდგომარეობა. ავტორებმა სტატისტიკური ანალიზის საშუალებით გამოამჟღავნეს რისკ-ფაქტორები, რომლებიც გავლენას ახდენენ პლაცენტური დისფუნქციის განვითარებაზე. კვლევის შედეგები უზრუნველჰყოფს რეპროდუქციული დანაკარგის შემცირებას და დემოგრაფიული მაჩვენებლების გაუმჯობესებას.

FUNCTIONAL STATE OF RED BLOOD SYSTEM AS A POSSIBLE PREDICTOR OF INDIVIDUAL RADIOSENSITIVITY AND CARCINOGENESIS

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Appropriate prediction of medical consequences of radiation impact is the major condition of optimal management of emergency situation of radiological incidents [7,8,12]. In case of acute radiation syndrome it's near consequences mainly are determined by adsorbed dose and forecasting is based on analysis of reaction the lymphoid cells lines on irradiation [14], whereas the long-term effects also irradiation effects of middle and low doses mainly are caused by individual features of adaptive response the myeloid, particularly, the erythroid cells lines [4,5,11,13], whilst the research of initial, genetically determined, generated in phenotype and age-dependent features of red blood system regulation, which are causally associate with the final radiological effect, is the perspective direction of detection of predictors of individual radiosensitivity.

Methodical difficulties of researches in this field are determined by the period length between the beginning of initial derangements and the development of clinically identifiable manifestations of pathology, by selection of the irradiated individual's cohorts, retrospective assessment of exposure doses, by selection the statistical criteria of difference between forecasted and real risk of developing cancer and so on.

The present data regarding correlation between radiosensitivity and risk of developing cancer [1,3-6] clears the ways for removing obstacles.

The aim of this study was to determine the possible predictors of the individual radiosensitivity and the probability of developing cancer on the basis of the comparable characteristic of functional state of the red blood system in patients with adenoma and carcinoma of thyroid gland.

The present report is a result of complex studies of red blood system and neurovegetative statute for the purpose of detection possible criteria of assessment of individual radiosensitivity and cancer risk predictors.

Material and methods. There have been studied the functional state of red blood cells (RBC) in 9 patients (7 women and 2 men) with carcinoma of thyroid gland and in 12 patients (9 women and 3 men) with adenoma of thyroid gland. Age range 25-75 years. The inclusion criteria – follicular adenoma and carcinoma, euthyroid. Exclusion criteria – late metastases. Possible uncertainty – influence of leading and accompanied pathologies on erytropoesis and systematic error of experiment. The functional state of RBC was determined by using the specially developed method based on analysis of the dynamics of population spectrum of erythrocytes of peripheral blood (EPB) – EPB distribution according to their living resources [as described in 9].

Maintenance of a quality of the circulating erythrocytes is a target function of RBS and therefore, qualitative content of EPB and its dynamics is a reflection not of functional state of RBS only, but of the whole organism as well. Effectiveness of such approach depends completely on adequacy of choice of indices, which characterize quality of erythrocytes. Ideally they must unequivocally charac-

terize properties causally concerned with aging and death of the cells.

As minimally sufficient set of parameters, describing living resources of EPB, two parameters are being applied: Spherulation degree (Q) and volume (V). The first parameter in a good approximation could be viewed as a degree of the erythrocytes spherulation (Q), determined as the relation of cell volume (V) to the volume of sphere with the same surface area (S).

$$Q = \frac{V}{V_{\text{ш}}} = 6 * \pi^{1/2} * \frac{V}{S^{3/2}} \quad .$$

The second parameter chosen was cell volume – V.

Spherulation degree characterizes cell deformability and along with the volume determines the probability of overcoming by them of a barrier of reticuloendothelial system. These characteristics in combination with antigen features of erythrocyte membranes are the basic factors determining probability of their elimination from circulating channel.

Blood drawn from a finger in the amount 15 µl is diluted in 1ml solution of the following content: NaCl - 0,150 mM/ l, EDTA-Na_2 - 0.03 mM/L, 5% formalin - 5 ml/l, glycerin - 1,8 M/l, "HEPES" - 10 mM/l, pH=7. Fractionated hemolysis of erythrocytes is performed after 15-20 min, by means of introduction of 50µl. glycerin loaded erythrocyte suspension into 1 ml samples 1,3; 1,5; ... 1,9 M/l solution of NaCl, ethanol - 4%, "HEPES" - 10 mM//l, pH=7,3. After 3-5 min 100µl suspension from each separate sample is diluted in 5 ml basic solution.

Measurement of distribution according to the volumes of suspended particles (erythrocytes and erythrocyte ghosts) in various samples is made 10-15 min after full restoration of the volume in unhemolysed erythrocytes.

Experiments are carried out by using the special device [10] based on the conductometric method for the measurement the sizes of particles dispersed in the electrolyte.

Obtained data is presented as distributions of concentration of erythrocytes and their ghosts.

After primary processing these data by filtration the noised areas of erythrocyte ghosts, the distribution curve smoothing also transformation of coordinates it was obtained the PBE distribution by their spherulation degree and volume [9] P=P(Q,V) (Fig. 1).

Statistical analysis of obtained data (MANOVA, LINEAR DISCRIMINANT ANALYSIS**)** was carried out by mathematic program packages MATLAB and STATISTICA.

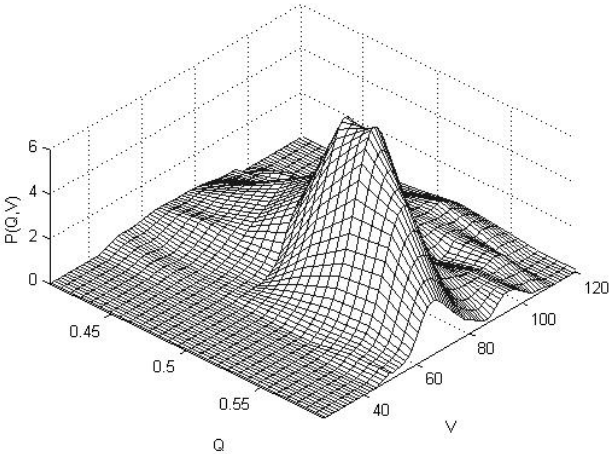

Fig. 1. Typical for a practically healthy 45-year old man PBE population spectra PBE distribution cording to the volume and degree of spherulation,
Axis of abscise: degree of spherulation in relative units.
Axis of ordinate: - volume [μ^3],
Axis of Z - standardized values of PBE concentration

Results and their discussion. On above presented way a problem of the RBC functioning regimes' classification first of all is a problem of classification the 3-D images, with consecutive statistical assessment of authenticity of the differences revealed and, finally, the medico-biological interpretation of the above differences.

The foundations of population spectra interpretation are based on modern conception of PBE aging-elimination processes in circulation bed, computational simulation of P=P(Q,V) distributions in different conditions of RBS functioning, comparative analysis of predicted and observed distributions in frames of physiological norm also different pathology.

One of the most characteristic manifestation of the PBE aging is their increased density. This event is a result of decreased surface area of a cell because loss of the membrane material (fragmentation) and concommitant dehydration of a cell in a course of the life cycle. The overall effect of these processes shows in increased cell volume against its surface area ratio – erythrocyte spherulation. From this point of view cell spherulation might be viewed as a property causally connected to chronological age of a cell (τ), its biological age Q=Q(τ).

In these coordinates the regions of minimal and maximal values of spherulation degree corresponds to distribution by volumes of least and most spherulated and hence young and old fractions of erythrocytes.

Obviously, in conditions of similar degree of spherulation, probability of cell overcoming the reticulo-endothelial barrier of the spleen must decrease during increase of their

size. This regularity clearly shows in population spectra – critical value of spherulation degree at which probability of finding the cells in circulatory bed decreases with increase of their volume.

The distributions reflect age-dependent regularity of V and S evolution in conditions of circulation; meanwhile, as seen in the graph, the cells already at entering into the bed differ by S, V and Q and they could be used for haemopoesis characterization.

Comprehensive analysis of population spectrum, requiring multiparametric statistical analysis of multidimensional arrays, is available only by means of NeuroNet algorithms, which are unsteady during analysis of small samples [9].

Therefore at the given stage in investigated cohort there were analysed only some geometric attributes of 3D images in the field of small-scale Q, characterizing haemopoesis features and PBE deterioration at the initial stage of their life cycle: mean volume, spherulation degree and surface area, difference between maximal also mean volume of young fractions of erythrocytes**,** witchshows theinitialanisocytosis, balance of velocities of volume and surface area changes. Results of dispersion analysis (MANOVA) of value which defines initial heterogenity of young fractions of PBE in patients with adenoma (a) and carcinoma(cr) of thyroid gland are shown on the Fig. 2. Statistically reliable differences in groups of discovered patients are revealed only by initial anisocytosis (Fig. 2), at the same time common anisocytosis of PBE is near to norm.

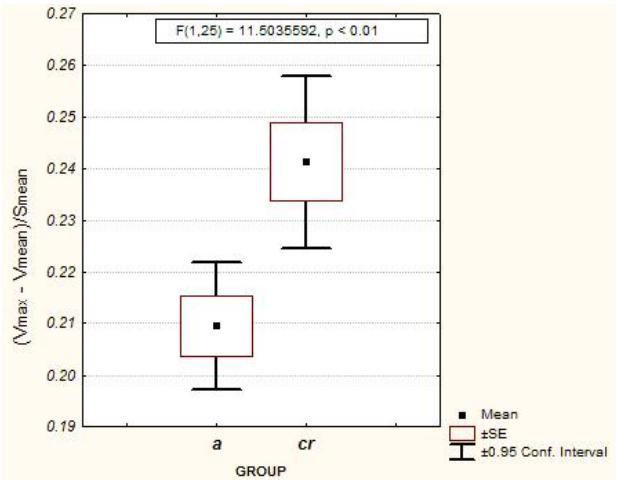

Fig. 2. Results of dispersion analysis (MANOVA) of value which defines initial heterogenity of young fractions of PBE in patients with adenoma (a) and carcinoma(cr) of thyroid gland

Revelation of differences certainly indicates high heterogeneity by the quality of young fractions of EPB in patients with cancer versus the patients with adenoma. This parameter may be determined as a marker of individual radiosensitivity and probability of developing cancer. However the issue about haemopoesis instability in patients with cancer, whether it is caused by genotype, phenotype or toxic action of products of disintegration of tumors, demands the further researches.

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SUMMARY

FUNCTIONAL STATE OF RED BLOOD SYSTEM AS A POSSIBLE PREDICTOR OF INDIVIDUAL RADIOSENSITIVITY AND CARCINOGENESIS

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Functional state of the red blood system is being studied in patients with adenoma and carcinoma of thyroid gland.

The aim of this study was to determine the possible predictors of the individual radiosensitivity and the probability of developing cancer on the basis of the comparable characteristic of **f**unctional state of the red blood system in patients with adenoma and carcinoma of thyroid gland.

Functional state of the red blood system is being studied in patients with adenoma and carcinoma of thyroid gland.

The functional state of RBC was determined by using the specially developed method based on analysis of the dynamics of peripheral blood erythrocytes distribution according to their spherulation degree (Q) and volume (V).

Spherulation degree is considered as biological age and along with the volume determines the probability of their elimination from circulating bed. In connection with these positions distribution P=P(V,Q) express mechanism of production-aging-elimination of RBC of peripheral blood in circulating conditions and according to their dynamics we can discuss about the character of these processes.

Revelation of differences certainly indicates high heterogeneity by the quality of young fractions of RBC of peripheral blood in patients with cancer versus the patients with adenoma. This parameter may be determined as a marker of individual radiosensitivity and probability of developing cancer. However the issue about haemopoesis instability in patients with cancer, whether it is caused by genotype, phenotype or toxic action of products of disintegration of tumors, demands the further researches.

Keywords: red blood system, individual radiosensitivity, cancer risk.

РЕЗЮМЕ

ФУНКЦИОНАЛЬНОЕ СОСТОЯНИЕ СИСТЕМЫ КРАСНОЙ КРОВИ, КАК ВОЗМОЖНЫЙ КРИТЕРИЙ ИНДИВИДУАДЬНОЙ РАДИОЧУВСТВИТЕЛЬНОСТИ И ОНКОГЕНЕЗА

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Изучено функциональное состояние системы красной крови (СКК) у пациентов с аденомой и карциномой щитовидной железы.

Целью исследования явилось установление возможных предикторов индивидуальной радиочувствительности и онкогенеза на основе сравнительной характеристики функционального состояния системы красной крови у пациентов с аденомой и карциномой щитовидной железы.

Функциональное состояние системы красной крови определялось с помощью специально разработанного метода, основанного на анализе динамики популяционных спектров эритроцитов периферической крови (ЭПК) - их распределения по объему (V) и степени сферуляции (Q). Степень сферуляции эритроцитов, наряду с их размерами, определяет вероятность их элиминаций из циркуляторного русла и в данном подходе рассматривается в качестве биологического возраста ЭПК. С этих позиций распределение эритроцитов P=P(V, Q) можно рассматривать как интегральный показатель процессов продукции-деструкции-эллиминации ЭПК, позволяющий судить о характере этих процессов в различных условиях и при различных патологиях.

У пациентов с карциномой щитовидной железы выявлена достоверно высокая гетерогенность по качеству фракции молодых эритроцитов переферической крови по сравнению с аденомой. Данный параметр может быть рассмотрен как предиктор высокой индивидуальной радиочувствительности и онкологического риска. Однако, выяснение вопроса - является ли данный факт следствием нестабильности гемопоэза у больных или отражением токсического воздействия продуктов распада опухолевых клеток, является предметом дальнейших исследований.

რეზიუმე

წითელი სისხლის სისტემის ფუნქციური მდგომარეობა,როგორც ინდივიდუალური რადიომგრძნობელობისა და ონკოგენეზის შესაძლო პრედიქტორი

ფ. თოდუა, გ. ორმოცაძე, დ. ნადარეიშვილი,
თ. სანიკიძე, კ. მარდალეიშვილი

ივ. ბერიტაშვილის ექსპერიმენტული ბიომედიცინის ცენტრი, ონკოლოგიის ნაციონალური ცენტრი, თბილისი, საქართველო

შესწავლილია წითელი სისხლის სისტემის (წსს) ფუნქციური მდგომარეობა ფარისებრი ჯირკვლის ადენომითა და კარცინომით დაავადებულ პაციენტებში. ნაშრომის მიზანს წარმოადგენდა ინდივიდუალური რადიომგრძნობელობისა და ონკოგენეზის შესაძლო პრედიქტორების გამოვლენა წითელი სისხლისა სისტემის ფუნქციური მდგომარეობის შედარებითი ანალიზით პაციენტთა ზემოაღნიშნულ ჯგუფებში.

წითელი სისხლის სისტემის ფუნქციური მდგომარეობა შეისწავლებოდა ორიგინალური დამუშავების მეთოდით, რომელიც ეფუძნება პერიფერიული სისხლის ერითროციტების (პსე) განაწილების ანალიზს სფერულაციის ხარისხის (Q) და მოცულობის (V) მიხედვით.

აღნიშნულ მიდგომაში სფერულაციის ხარისხი განიხილება, როგორც პსე ბიოლოგიური ასაკი, რომელიც, პსე მოცულობებთან ერთად, განსაზღვრავს ცირკულაციიდან მათი ელიმინაციის ალბათობას. ამ პოზიციებიდან ერითროციტების განაწილება შეიძლება განიხილებოდეს,როგორც პსე ინტეგრალური მაჩვენებელი P=P(V,Q), ცალსახა დამოკიდებულებაშია პსე პროდუქცია-დესტრუქცია-ელიმინაციის პროცესებთან და შესაძლებელია გამოიყენებულ იქნან მათ დასახასიათებლად.

ფარისებრი ჯირკვლის კარცინომით პაციენტებში გამოვლენილი იქნა ერითროციტთა ახალგაზრდა ფრაქციების სარწმუნოდ მაღალი ჰეტეროგენობა მათი ხარისხის მიმართ ადენომის მქონე პაციენტებთან შედარებით. აღნიშნული პარამეტრი შეიძლება განხილულ იქნას,როგორც ინდივიდუალური რადიომგრძნობელობისა და ონკოგენეზის შესაძლო პრედიქტორი. თუ რასთან არის დაკავშირებული ჰემოპოეზის არასტაბილურობა ონკოლოგიურ პაციენტებში: გენოტიპთან, ფენოტიპთან, თუ განპირობებულია სიმსიმნეთა დაშლის პროდუქტების ტოქსიური ზემოქმედებით, შემდგომი გამოკვლევის საგანს წარმოადგენს.

ПРОФИЛАКТИКА И ЛЕЧЕНИЕ ПЕРИИМПЛАНТИТА

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Современная стоматология для восстановления утраченных зубов предлагает высокотехнологичный метод протезирования - имплантацию. Зубной имплант служит опорой для искусственного зуба или зубного протеза, по функциональным и эстетическим характеристикам полностью соответствуя естественному корню зуба [4].

Срок службы имплантов у разных пациентов неодинаков и варьирует в пределах 10-25 лет в зависимости от состояния здоровья пациента и правильного ухода за имплантами. О технике и способах "домашней профилактики'' пациентов подробно консультирует врач-стоматолог. В постимплантационном периоде стоматолог осуществляет обязательные плановые осмотры - спустя 1, 3 и 6, 9, 12 месяцев с целью проверки состояния гигиены полости рта, выявления признаков воспаления или отторжения импланта, удаляет зубные отложения, неустранимые зубной щеткой. Все части импланта нуждаются в ежедневной тщательной очистке не только в раннем постимплантационном периоде, но и в течение всего времени службы, поскольку на

них скапливаются и размножаются микроорганизмы. Превенция воспалительных и деструктивных изменений тканей вокруг внутрикостного импланта весьма актуальна.

Для слизистой оболочки здоровых тканей вокруг шейки импланта и его супраструктуры характерен бледно-розовый цвет; ширина десневой ткани вокруг зубов должна составлять 2 мм, а в месте ее прикрепления к импланту - 1 мм; обязательным условием является отсутствие кровоточивости десны при зондировании десневого желобка. При пальпации имплант должен быть стабилен, а перкуссия как в горизонтальном, так и в вертикальном направлении - безболезненна.

В процессе, а также после имплантации иногда развивается воспалительный процесс костного ложа у импланта и прилегающих тканей в виде периимплантита. Периимплантит, в основном, возникает при несоблюдении определенных правил во время постановки импланта и закрытии раны, асептики и антисептики во время операции, при травмировании кости, вызванной нарушением температурного режима во время подготовки ложа под имплант, что провоцирует образование гематомы и её нагноение, отек слизистой оболочки вокруг импланта, формирование травмирующих рубцов. Периимплантит может также развиться в случае постановки импланта при наличии определенных противопоказаний - нелеченного пародонтита [9] и отсутствии удовлетворительной индивидуальной гигиены полости рта после установки импланта в период приживления и даже спустя несколько лет.

Появление неприятного запаха изо рта, неприятных ощущений и болей в десне в области импланта, кровоточивости этого участка при зондировании и чистке зубов, отечной и гиперемированной слизистой оболочки вокруг шейки или супраструктуры импланта, подвижности слизистой над внутрикостным имплантом, образование грануляций, увеличение глубины десневого кармана, выделение в небольшом количестве серозно-гнойного экссудата указывает на развитие воспалительного процесса [10]. Особенность и опасность периимплантита заключается в наличии прямого контакта области воспаления с костью. Зона соединительной ткани, характерная пародонтиту, отсутствует.

Вокруг импланта изначально содержится естественная флора ротовой полости. В случаях проникновения микроорганизмов в зону слизистой оболочки импланта развивается процесс воспаления, микробный пейзаж которого представлен разнообразием аэробов и анаэробов. В ближайшие недели после постановки имплантов в наддесневой бляшке обнаруживаются грамположительные кокки, а в поддесневой среде - бактерии, характерные для обычной флоры полости рта и десневых карманов [14].

A. Weerkamp и соавт. [16] считают, что характер поверхности имплантов определяет образование зубной бляшки. Авторы в эксперименте обнаружили, что на шероховатой поверхности образуется вдвое больше бактерий, чем на гладкой. R. Adell [10] и соавт. наблюдали более медленное образование зубной бляшки около шейки с высокой степенью полировки. Одной из концептуальных особенностей имплантов в форме корня зуба является хорошо отполированная шейка. На поверхности любого материала бляшки спустя 4 часа образуются микробы, среди которых преобладают стрептококковые виды, а спустя 48 часов число анаэробов увеличивается. G. Nakazato и соавт. [12] в эксперименте выявили отрицательное воздействие образующихся микробных ассоциаций больше на раннюю регенерацию кости, чем на полное созревание бляшек. На микробный пейзаж влияет также снижение барьерной функции пародонта у курильщиков, ослабление защитной функции организма и низкая дисциплина курильщиков в отношении рекомендаций врача [8].

C. Misch [11] выявил, что основной причиной развития воспалительного процесса вокруг импланта является стресс кости в связи с ее неадекватной нагрузкой, однако такой критерий как образование кармана, автор не считает решающим; по мнению автора, этот процесс связан с “биологической шириной” (ширина полосы соединительнотканного и эпителиального прикрепления от края кости до основания зубодесневой борозды) десны, что предотвращает атрофию кости, а решающим фактором является нагрузка на кость.

Периимплантит, также как пародонтит [9], развивается постепенно, в возрастающей степени, проявляя следующие клинические признаки: воспаление, тканевая гиперплазия, кровотечение или выделение гнойного экссудата при зондировании и пальпации, увеличение глубины десневого кармана [13], резко выраженная потеря кости, подвижность импланта, или его отторжение.

Периимплантит угрожает хорошему результату остеоинтеграции имплантов, поэтому важно своевременно выбрать необходимый и правильный метод лечения. В литературе описаны случаи успешно заверешенных лечений, однако, к сожалению, большинство из них имеют лишь временный эффект. Лечение периимплантита, также как и пародонтита, носит комплексный характер: применяется местная и общая терапия. В связи с присутствием нескольких стадий заболевания и учитывая общее состояние больного, лечение требует индивидуального подхода, однако в целом оно сводится к выполнению набора определенных лечебных мероприятий: 1) активное полоскание антисептиками, травами, антибиотикотерапия, прием эффективных терапевтических лекарственных средств; 2) проведе-

ние физиотерапевтических процедур, направленных на восстановление тканей вокруг импланта; 3) обработка десны специальными антибактериальными средствами; чистка, кюретаж и удаление десневого кармана, промывание патологических участков антисептиками, гигиена полости рта с применением соответствующих паст; 4) в случае потери кости проводится хирургическое вмешательство: удаление воспалительно-гнойного участка, пластика мягких тканей, окружающих имплант; 5) в случае некачественных конструкций необходима их переделка.

На основании литературных данных [3,7] и результатов проведенных нами многолетних исследований и клинического и практического опыта лечения пародонтитов [1], мы сочли возможным активно применить наиболее эффективный и перспективный на сегодняшний день метод, апробированный в пародонтологии - Vector-терапию для профилактики и комплексной терапии перимплантитов [13].

Система Vector наилучшим образом подходит для лечения периимплантитов. Принцип работы аппарата основан на гидродинамическом эффекте. Происходит непрямое связывание ультразвуковой энергии и одновременная пульсирующая подача жидкости с частицами гидроксиаппатита, чем обеспечивается тщательное удаление налета, качественное очищение патологического кармана от инфекции, разрушение биопленки с поверхности имплантов, в которых живут колонии микробных ассоциаций, вымывание эндотоксинов, одновременно полируется доступная часть импланта [15].

Инструменты аппарата Vector для лечения периимплантитов специально изготовленны из углеродистого волокна, которые в комбинации с полировочной суспензией Vector Fluid Polish, содержащей частицы гидроксилапатита, надежно и тщательно очищют поверхность импланта без повреждения его конструкции.

Целью исследования явилась оценка эффективности лечения периимплантитов системой Vector в сравнении со стандартным лечением.

Материал и методы. Проведено наблюдение и лечение начальной фазы заболевания, первичной причиной которой послужило поражение мягких тканей вокруг импланта в связи с недостаточной гигиеной полости рта. Наблюдение и курс лечения проводили в стоматологической клинике Royal Dent на 52 пациентах, пролеченных по поводу периимплантитов спустя 1, 3, 6, 9, 12 месяцев после имплантации. Перед лечением проведено клиническое и рентгенологическое обследование (ортопантомограмма) пациентов в области внедренных имплантов. Ортопантомограммы выполняли до начала лечения и 1, 3, 6, 9, 12 месяцев спустя после его завершения в рентгенологическом кабинете на ортопантомографе фирмы “Planmeca” (Финляндия).

Диагноз периимплантита устанавливали на основании клинических проявлений воспаления (гиперемия и отечность десны, кровоточивость, образование различной глубины патологических карманов, наличие экссудата в них, образование припухлостей, присутствие болезненных ощущений) и рентгенологических изменений кости (кратерообразная деструкция в окружности шейки и даже в верхней трети импланта). На основе контрольного рентгеновского снимка, сделанного после установки зубных протезов, оценивалась степень костной резорбции.

С точки зрения клинико-рентгенологического анализа пациенты разделены на две группы: первую группу составили 32 пациента с признаками воспаления десны с нормальной величиной десневого кармана, рецессия кости рентгенологически не выявлялась; вторую группу - 20 пациентов с признаками воспаления десны и резорбцией костной ткани.

11 пациентам первой и 8 пациентам второй группы проводилось только местное и общее противовоспалительное лечение, а 21 пациенту первой и 12 пациентам второй группы, наряду с местной и общей консервативной терапией, лечение системой Vector.

С целью профилактики и лечения воспалительных процессов были осуществлены следующие мероприятия: местные гигиенические процедуры - удаляли зубной налет, обрабатывали десневой карман 1% раствором перекиси водорода и 0,06-0,12% раствором хлоргексидина, производили аппликации мазью грамицидина С, витамином А и солкосерил-дентальной адгезивной пастой на слизистой оболочке вокруг импланта, накладывали лечебные повязки с 5% бутадиеновой мазью. Противовоспалительную терапию закрепляли комбинированным антимикробным и противопротозойным препаратом орципол 500\500 (Ciprofloxacin hydrochloride+ Ornidazole) назначая 1 таблетку 2 раза в сутки перед едой или 2 часа спустя после еды в течение 5-7 дней.

Для дополнительного домашнего ухода рекомендовали полоскания антисептическим раствором пародонтакс. Обучали пациентов правильной чистке зубов. Для тщательного очищения поверхности импланта и протеза рекомендовали пользоваться монопучковыми зубными щетками, интрадентальными ершиками [9], суперфлоссами, ирригаторами. Назначали общее лечение десен сенсибилизирующими, обезболивающими и общеукрепляющими лекарственными препаратами. Для профилактики периимплантита рекомендовали применение витамина С по 3 г в день в течение 6 недель.

Наряду с вышеуказанными гигиеническими и лечебными процедурами пациентам проводили лечение аппаратом Vector (фирма "Durr Dental", Германия). С помощью углеродо-волокнистых наконечников этого аппарата и струей высокодисперсной полировочной суспензии Vector Fluid Polish, содержащей частицы гидроксиапатита кальция, тщательно промывали десневые карманы, удаляли зубной налет и инфицированные грануляции в окружности импланта и его супраструктуры.

Отобранным 33 пациентам из I и II групп лечебные процедуры аппаратом Vector и местную и общую противовоспалительную терапию вначале проводили ежедневно, затем 2-3 раза в неделю до полной ликвидации воспалительных явлений. После каждого курса комплексного лечения клинически и рентгенологически оценивали состояние тканей и костного ложа вокруг импланта.

Профилактические мероприятия аппаратом Vector и клинико-рентгенологический контроль продолжали и после стабилизации воспалительного процесса. Пациентам назначали вышеуказанные повторные визиты для проведения местной профилактики и комплексной противовоспалительной терапии перимплантита по надобности.

В комплексном лечении заболевания предусматривалось устранение любой биомеханической перегрузки имплантов путем модификации наддесневой конструкции. Уделяли внимание также зубам и протезам - антагонистам импланта и по возможности устраняли травматическую окклюзию. При невозможности снятия протеза создавали защитные устройства в виде капп, шин, которые пациент надевал на период сна. В случае, когда на имплант опирался съемный зубной протез, то его снимали, очищали в ультразвуковой ванночке или чистили механически при помощи щетки гидрокарбонатом натрия. Хирургическую фазу проводили по показаниям; спустя 2-3 недели решали вопрос о достаточности мягких тканей для плотного разобщения супраструктуры с имплантом и его ложем. В случаях недостаточного уровня десневого прикрепления производили пластику слизистой оболочки в окружности импланта. Профилактические мероприятия проводили с помощью аппарата Vector с целью превенции повторных воспалительных явлений.

Результаты и их обсуждение. После курса консервативной и Vector-терапии из 21 пациента первой группы у 19 (90,5%) наблюдалось значительное улучшение клинического состояния: прекратилось кровотечение, выделение гнойного экссудата из очага, исчезли отечность, припухлость и болевые ощущения, уменьшилась глубина патологических карманов, увеличилось десневое прикрепление, десна стала плотной и бледно-розовой; 2 (9,5%) пациентам дополнительно потребовалось хирургическое вмешательство ввиду недостаточности мягких тканей и реконструкция протеза с целью снятия перегрузки и создания удовлетворительных условий гигиены.

Из 11 пациентов первой группы у 6 (54,5%) отмечался положительный клинико-рентгенологический результат, а у 5 (45%) - незначительное уменьшение глубины патологического кармана. С целью достижения окончательного купирования воспалительного процесса была проведена Vector-терапия. Из 12 пациентов второй группы у 7 (58%) противовоспалительное лечение в комбинации с Vector-терапией завершилось успешно: уменьшилась глубина пародонтальнго кармана, прекратилось выделение гнойного экссудата, десна стала более плотной и бледно-розовой, рентгенологически наблюдалась стабилизация процесса; у 5 (42%) - на фоне достигшей стабильности, для закрытия костного кармана в области убыли костной ткани стало возможным размещение остеосинтетического препарата. Из 8 пациентов второй группы у 3 (37%) противовоспалительное лечение оказалось эффективным; а 5 (63%) пациентам ввиду недостаточного увеличения уровня прикрепленной ткани потребовалась хирургическая коррекция десневой манжетки. С целью создания и поддержания асептической среды, что является гарантом успешного хирургического исхода, было решено перед оперативным вмешательством провести Vector-терапию (таблица 1).

Таблица 1. Результаты лечения

Количество пациентов n=52	**Положительный результат абс. %**	**Хирургическое вмешательство абс. %**
I группа (n=32) n=21 Vector + консерв. леч. n=11 консерв. леч.	 19-90,5% 6-54,5%	 2-9,5% 5-45%
II группа (n=20) n=12 Vector + консерв. леч. n=8 консерв. леч.	 7-58% 3-37%	 5-42% 5-63%

Заключение: Из 52 пациентов Vector-терапия была проведена 33 (63%), среди них у 26 (79%) наблюдался успешный исход лечения (19 пациентов - первой и 7 - второй группы), у **7** (21%) пациентов удовлетвори-

Таблица 2. Оценка эффективности лечения

Результат	Vector-терапия+консервативное лечение (n=33)			Консервативное лечение (n=19)		
	I группа	II группа	всего	I группа	II группа	всего
удовлетв.	19 (74%)	7 (26%)	26 (79%)	6 (67%)	3 (33%)	9 (47%)
неудовлетв.	2 (29%)	5 (71%)	7 (21%)	5 (50%)	5 (50%)	10 (53%)

тельный результат не получен (2 пациента - первой и 5 – второй группы). 19 (37%) пациентов обеих наблюдаемых групп (52) получили стандартное противовоспалительное (общее и местное) лечение, среди них у 9 (47%) лечение завершилось удовлетворительно (6 пациентов - первой и 3 - второй группы), а у 10 (53%) – Vector-терапия оказалась безрезультатной (5 пациентов из каждой группы) (таблица 2).

Лечение периимплантита завершилось купированием воспалительного процесса: исчезли инфекционные очаги в тканях вокруг импланта, закрылись десневые карманы, десна стала плотной, бледно-розовой, прекратилось кровотечение и выделение гнойного экссудата из десневых карманов, создались условия для предотвращения дальнейшей утраты кости; благодаря суспензии, содержащей микрочастицы гидроксиапатита кальция, эффективно очистилась коронковая часть импланта.

Таким образом, клинико-рентгенологические результаты доказали эффективность Vector-терапии в комплексном лечении периимплантитов.

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SUMMARY

PROPHYLAXIS AND TREATMENT OF PERIIMPALNTITIS

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Ultrasonic Vector System (Dürr Dental, Germany) is the best means for complex treatment of peri-implantitis. The therapeutic effect of the system proved anti-inflammatory and regenerative impact of calcium hydroxyapatite on the soft tissue around the implant. The effectiveness of the result provided full, persistent and safe removal of bacteria, dental plaque, infected granulation tissue; the possibility of regular monitoring, prevention of re-infection of pathological pockets, without damaging the hard and soft tissues around the implant, the implant itself and artificial fragile crown, securing superb cleaning and polishing of the implant surface, which is an important condition for

the further reduction and inactivation of pathogenic bacteria population. Healing peri-implantitis pockets at the expense of using conventional oral hygiene is not possible. Tools of carbon fiber in combination with a suspension Vector Fluid Polish and the Vector machine successfully accomplish the function of disinfection and polishing.

The aim of the research was to present the results of peri-implantitis treatment with a new ultrasonic device Ultrasonic Vector System.

Surgical treatment (21% of patients) was atraumatic. The risk of infection was decreased and necessary conditions were created for a significant acceleration of the tissue healing process. Relying on the successful results we recommend Vector- therapy before surgical procedures.

Complex treatment was repeated depending on the clinical manifestations of inflammation around the implant. To prevent peri-implantitis, and get timely diagnosis of unwanted changes, and to maintain implants and the soft tissues around them in a healthy condition, the patients were strongly recommended to visit the dentist 3-4 times a year. They were offered professional oral hygiene and accessible parts of the implants with the help of a Vector machine.

Relying on the results of the research we conducted during the treatment of peri-implantitis, we have seen the "magic" efficiency and the need to apply the «Vector» system into the complex treatment of this disease.

Keywords: peri-implantitis, implants, Vector system, Vector fluid polish suspension, Calcium Hydroxyapatite, orcipol, inflammation, bone destruction.

РЕЗЮМЕ

ПРОФИЛАКТИКА И ЛЕЧЕНИЕ ПЕРИИМПЛАНТИТА

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Комплексное лечение периимплантитов ультразвуковым аппаратом Vector (Dürr Dental, Германия) в 79% обеспечило отличные результаты. Лечебный эффект заключается в противовоспалительном и регенерирующем воздействии гидроокисикальция на мягкие ткани вокруг импланта, обеспечивая полное, стойкое и щадящее удаление бактерий, зубных отложений, инфицированных грануляций; создание соответствующих условий для регулярного контроля и превенции реинфекции патологических карманов. Повреждения тканей вокруг импланта, самого импланта и искусственной хрупкой коронки не наблюдалось, достигнута максимальная очистка и полировка поверхности импланта, что является необходимым условием для дальнейшего уменьшения и инактивации популяций патогенных бактерий. Инструменты аппарата Vector из углеродистого волокна в комбинации с суспензией Vector Fluid Polish успешно выполняют функцию дезинфекции и полировки.

Проведенное после Vector-терапии хирургическое вмешательство у 21% пациентов прошло в более асептических условиях, атравматично, снизился риск инфекции, создались необходимые условия для ускорения процесса заживления тканей. Результаты проведенного нами лечения периимплантитов позволяют рекомендовать применение Vector - терапии перед хирургическими манипуляциями.

Комплексное лечение повторяли в зависимости от клинических проявлений воспаления около импланта. В целях превенции периимплантита и своевременной диагностики нежелательных изменений, поддержания имплантов и мягких тканей вокруг них в здоровом состоянии пациентам настоятельно рекомендуется посещать стоматолога 3-4 раза в год; соблюдать профессиональную гигиену полости рта и доступной части импланта с помощью аппарата Vector.

Результаты проведенного исследования доказывают эффективность включения системы Vector в комплексную терапию периимплантитов.

რეზიუმე

პერიიმპლანტიტის პროფილაქტიკა და მკურნალობა

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ულტრაბგერითი აპარატი Vector (Dürr Dental, გერმანია) პერიიმპლანტიტის კომპლექსური მკურნალობის საუკეთესო საშუალებას წარმოადგენს. იგი მარტივია მოხმარებაში, ხარისხიანი, ეფექტური და მაქსიმალურად დამზოგავი. სისტემის სამკურნალო ეფექტი ეფუძნება იმპლანტის ირგვლივ რბილ ქსოვილებზე კალციუმის ჰიდროქსიაპატიტის ანთებისსაწინააღმდეგო და მარეგენერირებელ მოქმედებას. Vector-ის პრაქტიკაში გამოყენებით პერიიმპლანტიტით 33 პაციენტებში მიღებულ იქნა საუკეთესო შედეგები (79%). პათოლოგიური ჯიბეებიდან სრულად და დამზოგავად მოსცილდა ღრძილქვეშა ნადები და ინფიცირებული გრანულაციები, ბაქტერიები, ენდოტოქსინები, გასტერილდა ღრძილოვანი მიდამო და სწრაფად ალაგდა ანთება, არ დაზიანებულა იმპლანტის ირგვლივ

რბილი ქსოვილები, თვით იმპლანტი და ხელოვნური გვირგვინი; იმპლანტის სუპრასტრუქტურის ზედაპირი მაქსიმალურად გაიწმინდა და გაპრიალდა,რაც მნიშვნელოვანი პირობაა პათოგენური ბაქტერიების პოპულაციათა აქტივაციის შესამცირებლად. აპარატ Vector-ის კარბომიდის ბოჭკოსგან დამზადებული ინსტრუმენტები სუსპენზია Vector Fluid Polish-სთან კომბინაციაში,წარმატებით ადეზინფიცირებენ და აპრიალებენ იმპლანტს.

პაციენტებისთვის (21%) ჩატარებულმა აუცილებელმა ქირურგიულმა ჩარევამ Vector-თერაპიის შემდეგ ჩაიარა ასეპტიკურ პირობებში,შემცირდა ინფექციების რისკი, შეიქმნა აუცილებელი პირობები ქსოვილების შეხორცების პროცესის მაქსიმალური დაჩქარების, პათოლოგიური ჯიბეების რეგულარული კონტროლისა და რეინფიცირების პრევენციისთვის.

კომპლექსური მკურნალობა მეორდებოდა იმპლანტის გარშემო ანთებითი პროცესის გამოვლენის შემთხვევაში. პერიიმპლანტიტის პრევენციისა და არასასურველი ცვლილებების დროული დიაგნოსტიკის, ასევე, იმპლანტებისა და მათ ირგვლივ რბილი ქსოვილების სიჯანსაღის შესანარჩუნებლად მიზანშეწონილია წელიწადში სამ-ოთხჯერ პირის ღრუსა და იმპლანტატის ხელმისაწვდომი ნაწილის პროფესიონალური ჰიგიენის ჩატარება აპარატ Vector-ის საშუალებით.

ჩატარებული კვლევის შედეგები მიუთითებენ სისტემა Vector-ის მაღალეფექტურობაზე.

БИОМЕХАНИЧЕСКОЕ ОБОСНОВАНИЕ ШИНИРОВАНИЯ ПОДВИЖНЫХ ЗУБОВ С СОХРАНЕНИЕМ ИХ ПОДВИЖНОСТИ НА УРОВНЕ ФИЗИОЛОГИЧЕСКОЙ

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Распространенным способом укрепления подвижных резцов является их шинирование с использованием различных видов арматуры и ее размещения по отношению к шинированным зубам [1,2,4,7,9]. К основным факторам, на которые необходимо обращать внимание, можно отнести: степень резорбции межзубных перегородок, величина анатомической коронки зубов, степень подвижности и наклона зубов во фронтальном участке [14]. Предлагаемые способы шинирования подвижных резцов с размещением арматуры на внутренней стороне зубов имеют существенные эстетические преимущества [9,11]. Однако, при любом способе шинирования особое значение имеет определение оптимального (наиболее рационального с точки зрения биомеханики) места размещения ретенционной борозды и арматуры в зависимости от высоты коронки зуба. Максимальные окклюзионные нагрузки (внешняя сила), возникающие при жевании, зависят от индивидуальных возможностей мускулатуры и болевого порога [6]. Исходя из вышеизложенного, увеличить стабильность зуба можно за счет снижения плеча силы (*L*), например, укоротив зуб, или увеличив сопротивление (*PX*) в пародонте за счет шинирования соседних зубов. С целью направления силы реакции на нижние фронтальные зубы аксиально, режущая поверхность этих зубов должна быть наклонена примерно на 20^0 вестибулярно. Подобное моделирование окклюзионной поверхности позволит минимизировать момент силы, опрокидывающей зуб, направляя жевательную нагрузку аксиально.

Вышеизложенное указывает на актуальность биомеханического подхода к шинированию, позволяющего сохранять подвижность зубов на уровне физиологической, равномерно распределять и демпфировать нагрузку, вовлекать в регулирование периодонто-мышечный рефлекс; гарантирует сохранение целостности конструкции во время жевания и проведение лечебных мероприятий, сохраняя при этом высокие функционально-эстетические качества шины.

Целью данного исследования явилось биомеханическое обоснование шинирования подвижных зубов при пародонтите с учетом высоты и наклона коронок зубов, степени их подвижности, вида прикуса.

Материал и методы. При биомеханических расчетах рассматривали модель с интактными тканями пародонта и модельные случаи, где высота коронки - $h \geq 6$ мм. Величина данного параметра является необходимой составляющей для получения достоверных данных. В

качестве модели исследования при вертикальных нагрузках был принят стержень, испытывающий действие осевого сжатия, а при горизонтальных - консольную балку, прочно закрепленную в сагиттальной плоскости. По рентгенологическим данным определяли основные геометрические параметры опорных зубов: длину корней, разницу внеальвеолярной и внутриальве-олярной частей зуба. Внутренние усилия конструкции определяли по оценке действий суммарно приведенных вертикальных и горизонтальных внешних компонентов нагрузки. На основании определения функциональных напряжений тканей пародонта опорных зубов и их соотношения с минимальными и максимально допустимыми разработан алгоритм выбора расположения на зубах шинирующей конструкции с учетом рационального распределения жевательной нагрузки в биомеханической системе «шина - опорные зубы - пародонт».

Результаты и их обсуждение. Результаты исследования показали, что в норме расчетная схема резца представляет собой жестко зажатую консольную конструкцию, воспринимающую вертикальную и горизонтальную нагрузки, которые возникают при откусывании пищи. В качестве расчетной высоты зуба *h* принимается размер, равный сумме высоты коронковой части зуба и третьей части длины его корня.

Следует учитывать, что зубы в лунках находятся в упруго-фиксированном состоянии, имеют некоторую подвижность [8]. Независимо от направления силы, зависимость смещения зуба от нагрузки носит сложный характер. При патологической подвижности резцов I и II степени жестко ущемленная опора превращается в пружинно-шарнирную, а расчетная схема - в геометрически переменную систему, при добавлении нагрузки к которой последнюю можно рассматривать как механизм. Для восприятия внешней нагрузки такой схемы требуется дополнительная связь, которой и является шина, закрепленная на клыках (рис. 1).

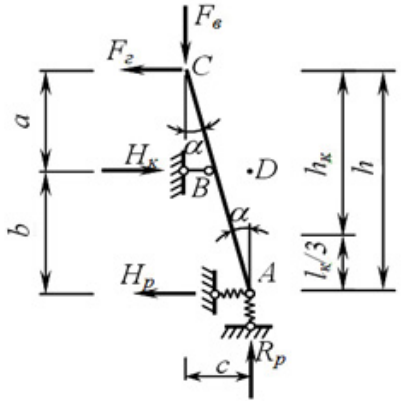

Рис. 1. Расчетная схема подвижного резца

$F_в$ - вертикальная составляющая внешней нагрузки; $F_г$ - горизонтальная составляющая внешней нагрузки; α- угол наклона резцов во фронтальном участке; *h* - расчетная высота зуба; $h_к$ - вертикальная проекция коронковой части зуба; $l_к$ - вертикальная проекция корня резца; $H_к$ - горизонтальное усилие, которое воспринимает арматура шины; H_p - горизонтальное реактивное усилие в резце; R_p - вертикальное реактивное усилие в резце.

Основным критерием оптимального размещения ретенционной борозды по высоте коронковой части зуба является усилие в корне резца и горизонтальное усилие, передаваемое непосредственно шиной на клыки при действии вертикальной и горизонтальной нагрузок на резцы. Угол наклона центральных резцов во фронтальном участке определяли с использованием параллелометрии. Для этого на моделях определяли результирующую кривой F.G. Spee для каждой из сторон. Перпендикулярно плоскости, образованной этими результирующими, действует сила *F*.

Таким образом, разделив глубину поднутрения $P_в$ вестибулярных поверхностей центральных резцов нижней челюсти к вектору силы *F* на длину коронковой части центрального резца, получаем sin α, а отношение глубины поднутрения $P_в$ к вертикальной проекции высоты коронки центрального резца h^l_k равен *tg*.

Значение угла наклона центральных резцов α во фронтальном участке может быть соответственно определено как:

$$\alpha = \arcsin \frac{p_в}{h^l_к} \quad \text{или} \quad \alpha = arctg \frac{p_в}{h_к}.$$

Для определения численных значений усилий, передаваемых шиной на клыки, и усилий, возникающих в корне резца от действия вертикальной нагрузки, использованы уравнения равновесия:

$$\sum M_A = 0 \qquad F_г \cdot h + F_в \cdot c - H_k \cdot b = 0$$

(1.1)

$$H_k = \frac{F_г \cdot h + F_в \cdot c}{b} = \frac{F_г \cdot h + F_в \cdot h \cdot tg\alpha}{b}$$

(1.2)

$$\sum Y = 0 \qquad R_p - F_в = 0 \quad \Rightarrow \quad R_p = F_в$$

$$\sum M_D = 0 \qquad F_г \cdot a + F_в \cdot c - H_p \cdot b = 0$$

$$H_p = \frac{F_г \cdot a + F_в \cdot c}{b} = \frac{F_г \cdot a + F_в \cdot h \cdot tg\alpha}{b}$$

(1.3)

Как видно из полученных зависимостей (1.1), (1.2) и (1.3), значения реактивных усилий, возникающих в резцах, и усилий, передаваемых на клыки, зависят от

анатомических размеров коронковой части резцов, их наклона и положения арматуры шины по высоте зуба. При этом, чем выше устанавливается шина (увеличивается размер *b* на рис. 1), тем меньше горизонтальное усилие H_p, возникающее в резце и усилие $H_к$, передаваемое через арматуру шины на клык (т.к. *b* в выражениях 1.1 и 1.2 находится в знаменателе).

Величины горизонтального усилия в шине и горизонтального реактивного усилия в резце зависят не только от значений горизонтальной составляющей нагрузки, но и от вертикальной. Вертикальная составляющая реактивного усилия в резце, согласно 1.2, независимо от положения шины, всегда равна значению вертикальной нагрузки.

Таким образом, согласно выражениям 1.1, 1.2 и 1.3, наиболее рациональное размещение шины соответствует максимально возможному приближению ее к режущей кромке резцов. Размещение ретенционной борозды на расстоянии 2-3 мм от верхнего среза резца обусловлено технологией изготовления шины и стремлением как можно меньше изменить форму внутренней поверхности возле режущей кромки резца. С точки зрения передачи горизонтальной нагрузки от резцов через шину на клыки положение ретенционной борозды в зоне крепления арматуры к клыку должно быть как можно ниже (ближе к нижнему краю коронковой части зуба) для уменьшения значения опрокидывающего момента в клыках, вызванного горизонтальными нагрузками, передаваемыми шиной.

Поэтому, наиболее оптимальным вариантом шинирования резцов, с точки зрения биомеханики, можно считать расположение ретенционной борозды и соответственно рабочей арматуры в верхней части первых резцов с последующим постепенным опусканием арматуры и ретенционной борозды к нижнему краю коронковой части клыков. Именно такое расположение арматуры следует считать целесообразным.

Следует также отметить, что максимально возможное верхнее положение арматуры при прогеническом прикусе определяется окклюзионным положением режущей кромки резцов верхней челюсти. Симметричность шинирования зубного ряда и прикладываемой нагрузки позволяет перейти от объемной расчетной схемы к ее плоской проекции, спроецировав все приложенные и возникающие усилия на сагиттальную плоскость (рис. 2).

В результате шинирования резцов и клыков с использованием арматуры и полимера все шинированные зубы, с точки зрения механики, можно считать жестким диском (деформациями которого можно пренебречь ввиду их незначительности), закрепленным подвижными шарнирно-пружинными опорами по продольным осям резцов и в месте соединения шины с клыками (точки *A*, *B* и *D* рис. 2) и шарнирно-неподвижной опорой в точке *C*. В качестве нагрузки *F* воспринимается сосредоточение нагрузки на один резец (в случае незначительных габаритных размеров пищевого комка, не превышающих ширины резца) или равнодействующая от распределенной по всей длине зубного ряда или некоторой его части нагрузка (при размерах пищевого комка, превышающего ширину одного резца). Менее благоприятным вариантом загрузки является случай, при котором равнодействующая нагрузка *F* расположена между центральными резцами. При этом степень наклона зубов во фронтальном участке по отношению к нагрузке задается углом α, а степень резорбции и анатомическая величина зубов - соответственно размерами h_k и l_k.

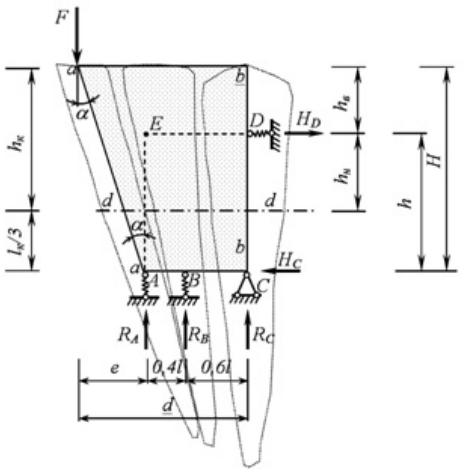

Рис. 2. Расчетная схема шинирования зубов

где $h_к$ - высота коронковой части клыка; $l_к$ - длина корня клыка; *F* - вертикальная нагрузка, возникающая при откусывании на центральном резце, *a-a* - наклонная ось центрального резца; α - угол наклона центральных резцов во фронтальном участке по отношению к нагрузке; *b-b* - ось клыка, *e* - эксцентриситет приложенной силы *F* относительно центральных резцов; *d-d* - уровень нижней кромки коронковой части клыка; *l* - расстояние между центрами сопротивления нижнего клыка и нижнего центрального резца; R_A - вертикальное реактивное усилие центрального резца; R_B - вертикальное реактивное усилие латерального резца; R_C - вертикальное реактивное усилие клыка; H_D - горизонтальное усилие, передаваемое арматурой шины на клык; H_C - горизонтальное реактивное усилие, возникающее в корне клыка, *E* - точка пересечения линий действия сил H_D и R_A; $h_в$ - расстояние от режущей кромки коронковой части зубов к оси ретенционной борозды; $h_н$ - расстояние от нижнего края коронковой части зубов к оси ретенционной борозды; *h* - расстояние от оси ретенционной борозды к точке *A*, *H* - расчетная высота зуба.

Учитывая совместную работу всех зубов, включенных в шину, величина вертикальной нагрузки на резцы мало зависит от начальной степени подвижности резцов,

и определяется, в основном, подвижностью клыков, которые и воспринимают нагрузку, передаваемую от резцов шиной. При действии нагрузки *F* жесткий диск возвращается соответственно точки *C* (пренебречь вертикальным перемещением клыков позволяет тот факт, что клык является опорой для всей конструкции шины), вызывая реактивные усилия в упругих опорах, которые прямопропорциональны их удалению от центра вращения *C*.Как видно из **рис. 2**, сила *F* вызывает реакцию корня резца R_A, вращающая шину относительно оси поворота С. При этом шина передает нагрузку H_D на клык. Предполагаем, что сила реакции самого клыка, которая представлена на рисунке силой H_C, (вследствие его большой жесткости) намного меньше реакции пародонта,

следовательно: $\frac{R_A}{l} = \frac{H_D}{h}$ отсюда: $R_A = \frac{H_D \cdot l}{h}$

Аналогично: $\frac{R_B}{0,6l} = \frac{H_D}{h}$ отсюда: $R_B = \frac{H_D \cdot 0,6l}{h}$.

Размер *l* можно выразить через *H* как *l = H•tga*. Для определения значений вертикальных и горизонтальных усилий, возникающих в зубах, используются уравнения равновесия. С учетом того, что при проецировании на сагиттальную плоскость всех усилий, проекции реактивных усилий, возникающих в зубах правой и левой сторон зубного ряда, накладываются друг на друга (т.е. количество реактивных усилий удваивается) имеем нижеуказанные показатели (1.4 - 1.7). Полученные показатели (1.4), (1.5), (1.6) и (1.7) связывают реактивные усилия, возникающие в зубах, со значениями внешней нагрузки *F*. Точное значение геометрического размера *l* может быть определено с помощью кронциркуля, как расстояние от линии, соединяющей центры тяжести поперечных сечений клыков, внешней режущей кромки первых резцов *d* за вычетом значения (или примерно как расстояние между центром продольного валика оральной поверхности нижнего клыка и язычным бугорком нижнего центрального резца), что возможно в клинических условиях.

$$\sum M_c = 0 \quad F(e+l) - 2R_A \cdot l - 2R_B \cdot 0,6l - 2H_D \cdot h = 0$$

$$H_D = \frac{0,5F(H \cdot tg\alpha + l) \cdot h}{1,36l^2 + h^2}$$

$$R_A = \frac{H_D \cdot l}{h} = \frac{0,5F(H \cdot tg\alpha + l) \cdot l}{1,36l^2 + h^2}$$

$$R_B = \frac{H_D \cdot 0,6l}{h} = \frac{0,3F(H \cdot tg\alpha + l) \cdot l}{1,36l^2 + h^2}$$

$$\sum M_A = 0 \quad -Fe - 2R_C \cdot l - 2R_B \cdot 0,4l + 2H_D \cdot h = 0$$

$$R_C = \frac{H_D \cdot h - 0,4R_B l - 0,5FH tg\alpha}{l}$$

Величина максимального значения нагрузки *F* может быть определена из выражения 1.4, как

$$F = \frac{2H_D(1,36l^2 + h^2)}{(H \cdot tg\alpha + l) \cdot h},$$

где вместо значения H_D подставляется предельно допустимая величина горизонтального усилия на клык в зависимости от его клинического состояния

$$H_D = F_z^n \frac{h_k}{h_H},$$

F_z^n (*выбрана из соответствующей таблицы*) [5] с учетом высоты приложенного усилия.

$$F = \frac{2F_z^n (1,36l^2 + h^2)h_к}{(H \cdot tg\alpha + l) \cdot h \cdot h_н}$$

Тогда: (1.8)

Независимо от длины зуба, соотношение длины коронки и корня для всех зубов в среднем равно 1:2. Более точно Д.В. Корляков представляет соотношение длины коронки клыка к длине его корня как 1: 1,5 [10]. Однако возрастные изменения и возможное наличие патологической стираемости не позволяют считать высоту коронки достоверной величиной. В свою очередь для этих целей можно воспользоваться схемой расчета, предложенной Л.М. Ломиашвили [12]. Так высота клыка нижней челюсти (H_{cor}) соотносится с мезиодистальным (MD_{cor}) и вестибуло-лингвальным (VL_{cor}) размерами его коронки как 1,40:1,00:1,11. Учитывая вариабельность форм патологической стираемости, достоверным размером для расчета можно считать только VL_{cor}. Итак, искомое длины корня клыка можно рассчитать по формуле:

$$l_k = 2 \cdot H_{cor} = \frac{2 \cdot 1,4VL_{cor}}{1,11} = 2,522VL_{cor}$$

Выразив все размеры через VL_{cor} получим:

$$h_k = \frac{1,4VL_{cor}}{1,11} = 1,261VL_{cor}$$

$$h = h_к + \frac{l_к}{3} - h_в = 1,261VLcor + 0,841VLcor - h_в = 2,102VLcor - h_в,$$

$$H = h_к + \frac{l_к}{3} = 1,261VLcor + 0,841VLcor = 2,102VLcor,$$

$$h_н = h_к - h_в = 1,261VLcor - h_в,$$

и подставив в (1.8) получим:

$$F = \frac{2,522F_z^n(1,36l^2 + (2,102VL_{cor} - h_в)^2)VL_{cor}}{(2,102VL_{cor} \cdot tg\alpha + l)(2,102VL_{cor} - h_в)(1,261VL_{cor} - h_в)}$$

(1.9)

К осноным преимуществам выражения (1.9) можно отнести то, что для вычисления величины максимально допустимого значения нагрузки *F* на шинированный резец достаточно определить вестибуло-лингвальные размеры коронки клыка VL_{cor} и значение угла наклона

центральных резцов во фронтальном участке к линии действия нагрузки. Существенными недостатками выражения (1.9) являются отсутствие возможности учета клинической высоты коронок зубов (учтены только анатомические размеры коронки), величины их возможной патологической стираемости и степени атрофии костной ткани лунок зубов.

Таким образом, применение расчетной схемы (1.9) допустимо только в случаях, проведения полной анатомической реставрации всей фронтальной группы зубов. Поэтому, как правило, на практике для расчетов более целесообразным представляется использование выражения (1.8). Окончательно величина максимально допустимого значения нагрузки F на шинированный резец определяется как меньшее из двух значений: полученного по (1.8) по Г.А. Макееву [13] и выбранного из таблицы предельно допустимых величин вертикальных усилий на опорные зубы в зависимости от их клинического состояния.

Вышеприведенная методика достоверна для клинических случаев с патологической подвижностью центральных и латеральных резцов I и II степени, с величиной атрофии менее $^{2}/_{3}$ лунки и отсутствием патологической подвижности клыков. При патологической подвижности клыков I степени с целью уменьшения значений расшатывающего момента, возникающего в клыке от нагрузки, передаваемой шиной, ретенционная борозда должна быть размещена как можно ниже по высоте коронковой части клыка. Максимальное значение нагрузки, передаваемой на клык шинированными резцами, определяется по формуле (1.4).

Выводы.

1. Создана аналитическая модель математического обоснования распределения жевательной нагрузки на передние зубы с различной степенью патологической подвижности с учетом состояния опорных зубов и выносливости тканей пародонта в зависимости от изменений в полости рта.
2. Обоснован способ шинирования фронтальных зубов с патологической подвижностью с размещением армирующего элемента шины на основе неорганического матрикса на вестибулярной поверхности зуба с учетом биомеханической целесообразности, степени подвижности зубов, резорбции межзубных перегородок и высоты клинических коронок.
3. Обоснована рациональность шинирующей конструкции, ее расположение соответственно математическим расчетам и учетом высоты клинических коронок, наклона зубов и вида прикуса после предварительного изучения гипсовых моделей в параллелометре.

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SUMMARY

BIOMECHANICAL BASIS SPLINTING OF LOOSE TEETH WHILE PRESERVING THEIR MOBILITY AT PHYSIOLOGICAL

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The aim of this study was biomechanical rationale for splinting mobile teeth with periodontitis according to the height and slope of teeth crowns, their degree of mobility, and type of occlusion. The research studied the effect of axial vertical and horizontal loads on the functioning of the proposed method of splinting. Set clear indications and contraindications for the use of the method of splinting. Biomechanically proven unreasonableness includes teeth with III degree of mobility in the splinted construction. Clinical variant of splinting is only possible at the height of the anatomical crowns of the teeth of at least 7 mm, otherwise the preferred laboratory methods for the manufacture of tires.

Keywords: tooth mobility, splinting construction adhesive splinting, biomechanical rationale.

РЕЗЮМЕ

БИОМЕХАНИЧЕСКОЕ ОБОСНОВАНИЕ ШИНИРОВАНИЯ ПОДВИЖНЫХ ЗУБОВ С СОХРАНЕНИЕМ ИХ ПОДВИЖНОСТИ НА УРОВНЕ ФИЗИОЛОГИЧЕСКОЙ

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Целью исследования явилось биомеханическое обоснование шинирования подвижных зубов при пародонтите. Разработана конструкция с учетом высоты и наклона коронок зубов, степени их подвижности, вида прикуса; изучено влияние осевых вертикальных и горизонтальных нагрузок на их функционирование. Установлены показания и противопоказания при использовании предложенного способа шинирования. Проведены биомеханические исследования.

Исследование показало, что зубы с подвижностью III степени не должны быть включены в шинирующую конструкцию. Установлено, что шинирование возможно только при высоте анатомической коронки всех зубов не менее 7 мм, в противном случае предпочтение отдается лабораторным методам изготовления шин.

რეზიუმე

მოძრავი კბილების შინირების ბიომექანიკური დასაბუთება ფიზიოლოგიურ დონეზე მათი მოძრაობის შენარჩუნებით

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გამოკვლევის მიზანს წარმოადგენდა მოძრავი (მორყეული) კბილების შინირების ბიომექანიკური დასაბუთება პაროდონტიტის დროს მათი სიმაღლის, გვირგვინის დახრილობის, მოძრაობის ხარისხის და თანკბილვის ტიპის გათვალისწინებით. შესწავლილია ღერძული ვერტიკალური და ჰორიზონტალური დატვირთვის გავლენა შემოთავაზებული მეთოდით შინირების ფუნქციონირებაზე, დადგენილია მისი გამოყენების მკაფიო ჩვენებები და უკუჩვენებები. დასაბუთებულია მორყევის III ხარისხის მქონე კბილების შინირებაში ჩართვის ბიომექანიკური მიზანშეუწონლობა. სადღეისოდ საჭრელების შინირების სხვადასხვა კონსტრუქციული მეთოდის გამოყენების შესაძლებლობა, რომელთაც თავისი უპირატესობები და ხარვეზები აქვთ, ექიმს საშუალებას აძლევს ინდივიდური თავისებურებების გათვალისწინებით შეარჩიოს შინირების ოპტიმალური მეთოდი. შინირების კლინიკური ვარიანტი დასაშვებია, თუ ყველა კბილის ანატომიური გვირგვინის სიმაღლე არაუმცირეს 7 მმ-ია; წინააღმდეგ შემთხვევაში უპირატესობა ენიჭება შინირების ლაბორატორიულ მეთოდებს.

CYTOKINES AND T REGULATORY CELLS IN THE PATHOGENESIS OF TYPE 1 DIABETES

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Type 1 diabetes is an inflammatory disease of the pancreatic islet, in which insulin producing β-cells are preferentially destroyed to varying degrees by the concerted action of autoreactive T-cells and monocytic cells. Th1-type cytokines (IL-2 and IFN-γ), and proinflammatory cytokines (TNF-α and IL-1) of monocyte cells correlate with T1D, they have cytotoxic, cytostatic (inhibits insulin synthesis and secretion), or cytocidal actions to pancreatic islets [21], whereas Th2 (IL-4 and IL-10, Th3 (TGF-β), and T regulatory cell-type cytokines (IL-10 and TGF-β) correlate with protection from T1D [11,16,22,26]. However, some investigators have shown that IFN-γ, IL-6 and TNF-α play dual role in the development of T1D [4,25,28]. IL-6 is also involved in the regulation of the balance between two T cell subsets: Tregs and Th17, which have contradictory functions in the control of inflammation [7]. Elevated concentration of proinflammatory IL-17 has pathogenic effect in pathogenesis of T1D [5]. An altered balance between the proinflammatory and regulatory T-cell responses, in which T regulatory cells lose the battle, leads to T1D [17,18].

Abnormalities of Tregs, either in the cell number or in the function, are associated with the initiation and progression of type 1 diabetes [19,20]. Although, data regarding alterations in Tregs in T1D are controversial and could be associated with age and the stage of disease and conducted treatment [2,27].

The aim of current study was to determine the role of CD4+CD25+ regulatory T cells and cytokines: IFN-γ, IL-6, TNF-α, IL-10, IL-4, IL-17 in the pathogenesis of T1D.

Material and methods. Seventy-one patients suffering from T1D (experimental group) and 60 healthy age- and sex-matched subjects (control group) of age 14-56 years were enrolled in the study. Information about their age, sex, education, income, cigarette and alcohol consumption, physical activity, diet, obesity, reproduction history, infection and chronic diseases was collected at the department of endocrinology, Tbilisi State Medical University and Diabetic Children Association. Subjects of risk group (with acute diseases, treated by steroids) were not included in this study. All participants were provided with consent letter approved by local ethics committees, and study procedures were performed in accordance with the principles of the World Medical Association Declaration of Helsinki.

Collection of blood samples: Peripheral blood samples were collected in 1 sterile vacutainers (10 ml), containing EDTA. Part of blood samples was centrifuged at 1500 x g for 15 min, and sera were aliquoted at 1 ml portions and frozen at -24^{0}C before use.

Cytokine measurement in serum: The levels of IFN-γ, IL-4, IL-6, IL-10, IL-17, and TNF-α were determined using commercially available assay such as ELISA (R&D Systems Inc., USA) according to manufacturer's protocol. A day prior to running the ELISA, we diluted the Capture Antibody and immediately coated a 96-well microplate, sealed the plate and incubated overnight at 4° C. Unbound antibodies were washed and blocked with 200 mcl 1X Assay Diluent per well. After 1 hour incubation the appropriate sample and standards were added and incubated for 2 hours at room temperature. Subsequently, biotinylated mouse anti-human antibody against cytokines (IL-10, IL-4, IL-6, INF-γ, TNF-α, IL-17, respectively) was added to each well, and the mixture incubated for an hour. Each plate was washed four times; avidin peroxidase-conjugated antibody was added, and incubated for 20 minutes. Substrate solution was added to each well following further rinsing; 20 min later, the reaction was terminated by adding stop solution. According to manufacturer's instruction, the absorbance was read at 450 nm within 30 minutes on microlpate reader (Labsystems, Multiskan, Finland). The inter- and intra-assay coefficients of variation were less than 5% and 7% respectively for all studied variables.

Evaluation of CD4+CD25+ frequency: On Ficol-Paque (density gradient 1.077-1.079) freshly isolated peripheral blood mononuclear cells (PBMCs) were immediately stained for the surface markers using anti-CD3, anti-CD4 and anti-CD25 monoclonal antibodies (mAb) (Miltenyi Biotec; Germany). Fluorochrome-labeled mAbs were added to 100 mcl of PBMCs and incubated for 20 min at 8^0 C. The tubes were centrifuged for 5 min at 400g and washed twice with 3 ml of FBS buffer. Fluorochrome-conjugated isotype-matched antibodies were used as negative controls. Data were acquired on cytofluorometer (BD FACSCalibur flow cytometer, USA).

Preliminary statistical analysis was performed using the STATISTICA 8.0 for PC (Statsoft Inc., Minneapolis, USA). Since the distributions of the studied molecules were markedly skewed, the data were log-transformed to correct for non-normality prior to further analysis. Differences in means of the plasma cytokines or Tregs between male and female groups were determined by the Mann-Whitney U-test. All correlations with potential covariates were examined separately for healthy and diabetic individuals.

Results and their discussion. Descriptive statistical analysis of basic anthropomorphic and immunological parameters (cytokines, Tregs) in T1D patients is shown in Table 1.

Cytokines (IL-6, TNF-α, IL-10, IL-17 and IL-4) levels in plasma were significantly different in health subjects and T1D patients. Particularly, concentrations of IL-6, TNF-α, IL-10 and IL-4 were decreased (1.197-, 1.188-, 1.504- and 1.840-times respectively), whereas only IL-17 plasma level was increased (2.311-times). Meanwhile, IFN-γ plasma level remained unchanged in T1D patients (refer to Table 2).

At next stage of analysis, we assessed correlations between anthropomorphic measurements, glucose concentration in blood and cytokines (IL-6, TNF-α, IL-10, IL-17 and IL-4) levels (refer to Table 3) in T1D patients.

Table 1. Baseline characteristics of the patients with diabetes type 1

	T1D	Men	Women
Age	22.80±8.37 (14.00-56.00)	22.23±7.92 (16.00-23.00)	23.06±8.62 (14.00-56.00)
Height	167.13±9.24 (140.00-193.00)	176.05±8.03 (157.00-193.00)	163.55±7.79 (140.00-177.00)
Weight	62.37±11.13 (38.00-88.00)	70.14±10.86 (54.00-88.00)	58.88±9.43 (38.00-83.00)
Systolic blood pressure	109.71±12.42 (90.00-160.00)	112.05±7.82 (90.00-140.00)	108.65±13.98 (90.00-160.00)
Diastolic blood pressure	72.57±8.11 (60.00-100.00)	72.95±6.29 (60.00-80.00)	72.40±8.87 (60.00-100.00)
Diabetes duration	10.01±6.98 (1.00-32.00)	10.26±7.53 (1.00-24.00)	10.06±6.9 (1.00-32.00)
$HbA1_C$	9.00±2.12 (6.20-14.90)	8.41±2.08 (6.20-14.00)	9.24±2.12 (6.20-14.90)
Glucose	153.60±67.67 (60.00-390.00)	145.05±60.25 (64.00-280.00)	157.40±71.03 (60.00-390.00)
IL-6	6.86±4.11 (3.69-26.78)	6.14±2.10 (4.13-13.32)	7.20±4.77 (3.69-26.78)
TNF-α	4.62±1.55 (3.11-9.86)	4.18±1.62 (3.11-9.86)	4.81±1.48 (3.20-9.79)
IL-10	5.56±9.38 (1.33-63.74)	3.54±2.54 (1.33-10.45)	6.60±11.37 (2.28-63.74)
IFN-γ	8.54±5.16 (0.69-28.05)	8.46±4.45 (0.92-17.62)	8.5±5.49 (0.69-28.05)
IL-4	8.89±26.00 (0.87-137.25)	15.46±37.23 (0.87-26.79)	5.77±18.293 (1.36-117.98)
IL-17	35.14±75.34 (0.23-540.36)	30.35±75.34 (0.23-131.41)	37.65±90.305 (0.26-540.36)

Table 2. T-test for studied cytokines between independent groups of control and T1D subjects (p<0.05 – significant difference)

	Control	T1D	p
LnIL-6	2.197±0.859	1.835±0.372	0.010
LnIL-17	0.992±0.440	2.293±1.915	0.000
LnIL-4	2.125±1.254	1.155±0.969	0.000
LnTNF-α	1.764±0.881	1.485±0.283	0.020
LnIL-10	2.023±0.904	1.345±0.683	0.000
LnINF-γ	1.672±0.899	1.925±0.742	0.106

Table 3. Correlations between age, anthropomorphic measurements, glucose blood concentration and cytokines of T1D patients (p<0.05 – significant difference)

Variables	T1D			
	Age	Height	Weight	Glucose
IL-6	-0.1026 p=0.452	-0.1724 p=0.213	-0.0310 p=0.821	-0.3540 p=0.012
TNF-α	-0.0048 p=0.969	0.0912 p=0.470	0.0709 p=0.568	0.1109 p=0.393
IL-10	0.0567 p=0.715	-0.1629 p=0.291	-0.1229 p=0.427	0.0509 p=0.752
IL-17	-0.020 p=0.871	0.143 p=0.267	0.83 p=0.510	-0.1276 p=0.335
IFN-γ	-0.0452 p=0.712	0.1653 p=0.181	0.1683 p=0.167	0.2904 p=0.021
IL-4	0.186 p=0.157	0.1025 p=0.448	0.086 p=0.516	0.2023 p=0.138

The statistical analysis of results showed that glucose blood level correlated with INF-γ and IL-6 plasma level (p=0.021 and p=0.012 respectively), whereas IL-10, TNF-α and IL-4 levels did not correlate with glucose level. We did not detect any correlation between of cytokines levels and age or anthropomorphic parameters in the studied patients.

As in T1D patients diabetes duration is a risk factor of the development of further complications, T1D patients were divided into three subgroups: I – 0-5 years, II – 5-10 years, III - more than 10 years. We compared the cytokine levels in these subgroups and revealed that the levels of anti-inflammatory cytokines: IL-4 and IL-10 decreased (respectively p=<0.01 and p=0.004) and IL-17 significantly increased in the first five years from disease on-set (p=0.004). In patients with longer duration of T1D, plasma levels of these cytokines remained the same (p<0.05). In newly diagnosed patients TNF-α plasma level decreased comparing with healthy individuals, then elevated (5-10 years) and again decreased after 10 years (p=0.05) (refer to Fig.).

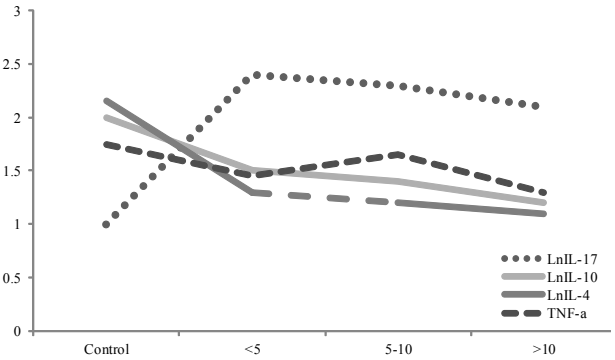

Fig. 1. Dependence of IL-4, IL-10, IL-17, TNF-α plasma levels on diabetes duration in T1D patients

At next stage of our study we evaluated CD4+CD25+ Tregs frequency in T1D patients. A Treg population having the CD4+CD25+ phenotype was identified, with the most amenable population being the so-called CD4+CD25high T-cells. Similar frequencies of CD4+CD25TOT and CD4+CD25high T-cells were observed in healthy control subjects and type 1 diabetic patients of similar age (refer to Table 4).

Table 4. T-test between studied cells in control and T1D patients (p<0.05 – significant difference)

	Control	T1D	p
CD4+ CD25high	1.168±0.769	1.098±0.896	0.787
CD4+ CD25med	4.186±3.039	3.113±2.243	0.134
CD4+ CD25high +med	5.353±3.729	3.987±2.877	0.131
CD4+ CD25Low	11.168±4.124	10.584±4.749	0.662
CD4+ CD25TOT	16.521±7.376	14.571±6.742	0.330

There was no difference in T1D subjects of recent-onset versus those with established disease in terms of their CD4+CD25TOT or CD4+CD25high T-cell frequency (refer to Table 5).

Interestingly, positive correlation was observed between increasing age and CD4+CD25+ Tregs frequency in T1D subjects group (refer to Table 6). Positive correlation was observed between plasma level of IL-4 and CD4+CD25high, CD4+CD25medium, CD4+CD25low, T cells in T1D patients, as well as between plasma level of TNF-α and CD4+CD25medium, CD4+CD25high+medium T cells. Plasma level of Il-17 negatively correlated with CD4+CD25medium cells in T1D patients (refer to Table 6).

Thus, in T1D subjects group CD4+CD25+ Tregs frequency was similar to control subjects group. In T1D patients CD4+CD25+ Tregs frequency did not correlate with diabetes duration and positively correlated with age and IL-4.

Our study revealed the dysregulated balance of inflammatory IL-6, IL-17, TNF-α and anti-inflammatory IL-4 and IL-10 cytokines on the background of almost unchanged frequency of Tregs in patients with type 1 diabetes. Plasma concentrations of IL-6, TNF-α, IL-10 and IL-4 were decreased, while only IL-17 plasma level was increased. Meanwhile, IFN-γ plasma level remained unchanged in T1D patients. Our data do not match with the results obtained by the other investigators [3,5,12], who showed significant enhancement of IL-6 and TNF-α plasma concentrations in T1D patients. They also demonstrated that

the elevated levels of these cytokines remained high in long standing T1D. However, there are papers that link IL-6 with protection of pancreatic islet β-cells from pro-inflammatory cytokines-induced cell death and functional impairment in vitro and in vivo [6]. Most of the studies investigating the pathogenic role of IL-17 in T1D [5,9,14], correspond to our results, that IL-17 is upregulated in the peripheral blood of patients.

Table 5. Dependence of Tregs frequency on diabetes duration

	Diabetes duration		
	<10 years	**10 - 20 years**	**>20 years**
CD4+CD25high	0.869±0.663 (0.130-2.100)	0.894±0.706 (0.150-2.660)	1.323±1.098 (0.090-3.920)
CD4+CD25high+med	3.654±1.797 (1.470-6.520)	4.449±3.719 (1.300-11.340)	4.680±2.590 (0.250-9.530)
CD4+CD25TOT	14.397±5.901 (4.140-24.920)	14.390±9.080 (4.150-33.680)	15.109±5.676 (5.460-24.550)

Table 6. Correlations between age, anthropomorphic measurements, glucose blood concentration, cytokines and Tregs of T1D patients ($p<0.05$ – significant difference)

	CD4+CD25high	**CD4+CD25med**	**CD4+CD25low**	**CD4+CD25high+med**
Age	0.5183 p=0.000	0.5625 p=0.000	0.4798 p=0.000	0.5949 p=0.000
Height	0.1239 p=0.417	-0.1191 p=0.391	-0.0919 p=0.509	0.0134 p=0.930
Weight	0.0278 p=0.855	-0.1251 p=0.358	-0.0107 p=0.938	-0.1005 p=0.507
Systolic blood pressure	-0.0615 p=0.688	-0.1275 p=0.354	-0.3447 p=0.010	-0.0651 p=0.671
Diastolic blood pressure	-0.0178 p=0.908	-0.0200 p=0.885	-0.1973 p=0.149	0.0411 p=0.789
$HbA1_C$	0.2754 p=0.094	0.0404 p=0.792	0.1283 p=0.401	0.0654 p=0.696
Glucose	0.0548 p=0.730	-0.0172 p=0.904	0.0573 p=0.690	0.0853 p=0.591
lnIL-6	-0.0313 p=0.856	-0.1138 p=0.457	-0.0994 p=0.516	-0.1376 p=0.424
lnIL-17	0.0040 p=0.980	-0.2803 p=0.049	-0.1410 p=0.329	-0.2296 p=0.154
lnIL-4	0.3571 p=0.028	0.3255 p=0.029	0.3799 p=0.010	0.3300 p=0.043
lnTNF-α	0.1273 p=0.410	0.4170 p=0.002	0.2328 p=0.093	0.3572 p=0.017
lnIL-10	-0.1382 p=0.466	0.1501 p=0.404	0.2160 p=0.227	0.0816 p=0.668
lnIFN-γ	-0.1440 p=0.345	-0.2111 p=0.122	-0.0623 p=0.651	-0.2894 p=0.054

Tregs play indispensable role in the maintenance of immune homeostasis by regulating inflammatory responses against invading pathogens and preventing destructive autoimmunity [18]. As it has already been mentioned according to our research frequency of CD4+CD25+ cells remain unchanged in T1D patients.

Majority of papers link T1D with lower percentage and the absolute number of regulatory T cells in diabetic type 1 patients in comparison to healthy individuals from the control group [10,12] Though our results match data of McClymont et al., 2011: CD4+CD25+ T cells do not change in T1D patients [15]. For example, diabetic children

<5 years has lower CD4+CD25highCD127lowFoxP3+, CD4+CD25highIL-10, and CD4+CD25highTGF-β Tregs compared to age-matched controls [24]. At this stage of study we did not investigate functional activity of Tregs. However, we suppose that decreased level of IL-4 and IL-10 reflects inhibited functional activity of these cells. Our data suggest that age strongly influences the frequency of CD4+CD25+ Tregs and that function, rather than frequency, may represent the means by which these cells are associated with type 1 diabetes in humans.

The balance between Tregs and Th17cells subsets is crucial for immune homeostasis. However, it is shown being impaired in various clinical disorders [1,6,12]. Inflammatory and autoimmune diseases are associated with Th17 immunity [3,5]. We suggest that shifted balance of Th17/Tregs towards inflammatory IL-17 producing cells and decreased levels of suppressive cytokines IL-4 and IL-10 played crucial role in T1D.

We suppose that duration and managment of disease can be the factors, that determine involvement of different subsets of regulatory cells in T1D. For instance, there are papers in which therapeutical potencial of regulatory cells - CD4-CD8-Tregs - in peripheral tolerence and autoimmune desease on-going is intensively discussed [8].

Future studies are needed to clarify changes in which subsets of heterogenous population of regulatory cells are associated with diabetes duration and how the therapy affects their frequency and function.

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SUMMARY

CYTOKINES AND T REGULATORY CELLS IN THE PATHOGENESIS OF TYPE 1 DIABETES

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Type 1 diabetes (T1D) is an inflammatory disease of the pancreatic islet, in which insulin-producing β-cells are preferentially destroyed to varying degrees by the concerted action of autoreactive T-cells and monocytic cells. Th1-type cytokines (IL-2 and IFN-γ) correlate with T1D, whereas Th2 (IL-4 and IL-10), Th3 (TGF-β), and T regulatory cell-type cytokines (IL-10 and TGF-β) correlate with protection from T1D. An altered balance between the proinflammatory and regulatory T-cell responses, in which T regulatory cells lose the battle, leads to T1D.

The aim of current study was to determine the role of CD4+CD25+ regulatory T cells and cytokines: IFN-γ, IL-6, TNF-α, IL-10, IL-4, IL-17 in pathogenesis of T1D.The study was carried out on 71 patients suffering from T1D at the department of endocrinology, Tbilisi State Medical University and Diabetic Children Association. The circulating levels of (IFN-γ, IL-6, TNF-α, IL-10, IL-4, IL-17) were determined by ELISA according manufactures' protocol (R&D Systems Inc., USA). Tregs - CD4+CD25+ frequency was determined on cytofluorometer (BD FACSCalibur flow cytometer, USA). Statistical analysis was performed by using STATISTICA 8.0 for PC (Statsoft Inc., Minneapolis, USA) and Mann-Whitney U-test.

Our study revealed significant decrease of IL-6, TNF-α, IL-10 and IL-4 plasma levels (1.197-, 1.188-, 1.504- and 1.840-times respectively) and increase of IL-17 plasma level (2.311-times) on the background of almost unchanged frequency of Tregs in patients with type 1 diabetes. In T1D patients CD4+CD25+ Tregs frequency did not correlate with diabetes duration and positivly correlated with age and IL-4. We supposed that decreased level of IL-4 and IL-10 reflects inhibited functional activity of these cells. We suggested that shifted balance of Th17/Tregs towards inflammatory IL-17 producing cells and decreased levels of suppressive cytokines IL-4 and IL-10 play crucial role in T1D. Future studies are needed to clarify changes in which subsets of heterogenous population of regulatory cells are associated with diabetes duration and how the therapy affects their frequency and function.

Keywords: Diabetes type 1, cytokines: IL-6, IL-4, IL-10, IL-17, INF-γ, TNF-α, CD4+CD25+ T regulatory cells.

РЕЗЮМЕ

ЦИТОКИНЫ И Т РЕГУЛЯТОРНЫЕ КЛЕТКИ В ПАТОГЕНЕЗЕ ДИАБЕТА ТИПА 1

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Диабет типа 1 является воспалительным заболеванием островков панкреаса, в процессе которого инсулиносекретирующие клетки избирательно разрушаются сочетанным действием аутореактивных Т и моноцитарных клеток. Th1- типа цитокины (IL-2 и IFN-γ) положительно коррелируют с диабетом типа 1, тогда как цитокины типа Th2 (IL-4 и IL10), Th3 (TGF-β) и Т регуляторных клеток (IL-10 и TGF-β) служат защитой от диабета типа 1. Отклонение баланса между воспалительным и Т регуляторным иммунным ответом в сторону воспаления ведет к развитию диабета типа 1.

Целью исследования является определение роли CD4+CD25+ Т регуляторных клеток (Tregs) и цитокинов (IFN-γ, IL-6, TNF-α, IL-10, IL-4, IL-17) в патогенезе диабета типа1. Исследования проведены на базе депар-

тамента эндокринологии ТГМУ и Ассоциации детей больных диабетом. Уровень циркулирующих в периферической крови цитокинов определялся методом иммуноферментного анализа по протоколу производителя (R&D Systems Inc., США). Количество CD4+CD25+ Т регуляторных клеток определялось методом цитофлюорометрии (BD FACSCalibur flow cytometer, США). Для статистического анализа использована компьютерная программа STATISTICA 8.0 (Statsoft Inc., Minneapolis, США) и Mann-Whitney U-тест.

Проведенные исследования выявили в плазме пациентов с диабетом типа 1 статистически достоверное понижение уровня цитокинов: IL-6, TNF-a, IL-10 и IL-4 (1.197-, 1.188-, 1.504- и 1.840-кратное, соответственно) и увеличение IL-17 (2.311-кратное) на фоне незначительного изменения количества Tregs. Вполне вероятно, что понижение IL-10 и IL-4 указывает на ингибированную функциональную активность регуляторных клеток. Предполагаем, что отклонение Th17/Tregs баланса в сторону IL-17-секретирующих клеток и понижение уровня супрессирующих цитокинов (IL-10 и IL-4) играют решающую роль в развитии диабета типа 1. Полученные результаты диктуют необходимость проведения дальнейших исследований для выявления субпопуляции регуляторных клеток, изменения которых ассоциируются с продолжительностью диабета, а также для оценки влияния терапии на количество и функцию этих клеток.

რეზიუმე

ციტოკინები და T მარეგულირებელი უჯრედები I ტიპის დიაბეტის პათოგენეზში

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I ტიპის დიაბეტი პანკრეასის კუნძულების ანთებითი დაავადებაა,რომლისთვისაც აუტორეაქტიული T- და მონოციტური უჯრედებით ინსულინის წარმომქმნელი β-უჯრედების დესტრუქციაა დამახასიათებელი. Th1 უჯრედების ციტოკინებს (IL-2 და IFN-γ) I ტიპის დიაბეტთან დადებითი კორელაცია ახასიათებს,მაშინ,როცა Th2 (IL-2 და IFN-γ), Th3 (TGF-β) და T-მარეგულირებელი უჯრედების ციტოკინები (IL-10 და TGF-β) დაავადებისგან დაცვას განაპირობებს. ანთებით და მარეგულირებელ T-უჯრედულ პასუხს შორის ბალანსის გადახრა ანთებისკენ I ტიპის დიაბეტის განვითარებას იწვევს.

წარმოდგენილი კვლევის მიზანი იყო CD4+CD25+ მარეგულირებელი T უჯრედებისა და ციტოკინების (IFN-γ, IL-6, TNF-α, IL-10, IL-4, IL-17) როლის შესწავლა I ტიპის დიაბეტის პათოგენეზში. კვლევა ჩატარდა თსსუ ენდოკრინოლოგიის დეპარტამენტისა და დიაბეტის მქონე ბავშვთა ასოციაციის ბაზაზე. პლაზმაში ციტოკინების დონე განისაზღვრა იმუნოფერმენტული მეთოდით მწარმოებლის პროტოკოლის მიხედვით (R&D Systems Inc., აშშ). CD4+CD25+ T უჯრედების სიხშირე განისაზღვრა ციტოფლუორომეტრიის მეთოდით კომპიუტერული პროგრამის (Statsoft Inc., Minneapolis, აშშ) და Mann-Whitney U-ტესტის გამოყენებით.

კვლევის შედეგად დადგინდა, რომ I ტიპის დიაბეტის მქონე პაციენტებში Tregs უჯრედების სიხშირის უმნიშვნელო ცვლილების ფონზე ადგილი აქვს პლაზმაში IL-6, TNF-α, IL-10 და IL-4-ის დონის კლებას (შესაბამისად, 1.197-, 1.188-, 1.504- და 1.840-ჯერ) და IL-17–ის დონის მნიშვნელოვან მატებას (2.311-ჯერ). CD4+CD25+ Treg უჯრედების სიხშირე I ტიპის დიაბეტის მქონე პაციენტებში არ კორელირებს დაავადების ხანგრძლივობასთან და დადებით კორელაციაშია პაციენტის ასაკთან და IL-4-ის კონცენტრაციასთან. IL-4 და IL-10 დონის შემცირება, შესაძლოა, მარეგულირებელი უჯრედების ფუნქციური აქტივობის დათრგუნვაზე მეტყველებდეს. სავარაუდოა,რომ Th17/Tregs ბალანსის გადახრა IL-17-ის წარმომქმნელი უჯრედებისკენ და მასუპრესირებელი ციტოკინების (IL-4 და IL-10) დაქვეითება გადამწყვეტ როლს ასრულებს I ტიპის დიაბეტის განვითარებაში.

მიღებული შედეგები მიუთითებს კვლევის გაგრძელების აუცილებლობაზე მარეგულირებელი უჯრედების იმ სუბპოპულაციის გამოსააშკარავებლად,რომელთა ცვლილება ასოცირდება დიაბეტის ხანგრძლივობასთან და ასევე ამ უჯრედების რაოდენობაზე და ფუნქციაზე თერაპიის გავლენის შესაფასებლად.

MANAGEMENT OF ACUTE ACHILLES TENDINOPATHY: EFFECT OF ETORICOXIB ON PAIN CONTROL AND LEG STIFFNESS

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Achilles tendon problems are a major cause of disability in sports people and those who undertake an active lifestyle [11,13,20,25,27,34]. Tendinopathy is defined as the clinical syndrome characterized by a combination of pain, diffuse or localized swelling, and impaired performance arising from overuse [21]. Tendinopathy is a difficult problem requiring lengthy management. Most patients with Achilles tendon complaints can be treated conservatively (rest or activity modification, cold, stretching, strengthening, NSAIDs, training correction). However, 25% of patients with persisting symptoms may require surgical treatment [23,27].

The purpose of the treatment of tendinopathies in active individuals is to reduce symptoms and improve function [13,25,27]. In athletes, an additional demand is that the recovery time should be as short as possible [27]; therefore, there seem to be an expanded pharmacological role in physical therapy practice [2].

Several factors play a role in the cause of pain in patients with Achilles tendinopathy. Mechanical loading is known to increase connective tissue blood flow of human tendons and to cause local release of vasodilatory substances [18,23].

COX-2 specific mechanism are responsible for the exercise-induced increase in prostaglandin synthesis, and that increase in tissue prostaglandin plays an important role for blood flow in peritendinous connective tissue during physical loading *in vivo* [19].

Etoricoxib (*Arcoxia, Merck & Co., Inc.*) is a selective inhibitor of cyclooxygenase-2 (COX-2), an enzyme involved in pain and inflammation. It is a member of the COX-2-selective class of non-steroidal anti-inflammatory drugs (NSAIDs) [22]. Extensive clinical trials have confirmed its analgesic and anti-inflammatory efficacy to be at least as good as and, in some cases, superior to non-selective NSAIDs in a number of diseases [22]. Etoricoxib displays improved gastrointestinal safety compared with nonselective NSAIDs and has a favorable overall safety and tolerability profile. It is rapidly and completely absorbed following oral administration providing a rapid onset of action. Its long plasma half-life allows for once-daily dosing. Etoricoxib is currently approved in a number of countries for various indications, including the treatment of acute pain, acute gouty arthritis, chronic low back pain, primary dysmenorrhea, and chronic treatment for the signs and symptoms of osteoarthritis and rheumatoid arthritis.

Mechanical stiffness is thought to influence several athletic variables, including rate of force development, elastic energy storage and utilization of sprint kinematics [7]. Stiffness is the resistance of a muscle to an increase in length and is calculated as muscle force/length [22]. Stiffness can be measured at the level of a single muscle fiber to the modeling of the entire body (spring-mass) [26]. Generally, the effect of stiffness on muscular performance is thought important with many changes in strength, power and flexibility being attributed to changes in stiffness [8,9,16]. For example, the relationship between stiffness and running speed is assumed to be strong as many authors have speculated that a stiff musculo-tendinous unit will enhance the rapid transmission of force [1,16,37].

Muscle-tendon stiffness seems to be improved by eccentric exercises [28]. and reduced by stretching [17]. Calf muscles stiffness variability may play a role in Achilles tendinopathy and in the positive effects seen of eccentric exercises on that lesion [33]. Farley and coworkers have demonstrated that modulation of ankle stiffness is the primary mechanism for adjusting leg stiffness when humans hop to different heights [12]. Recent studies have shown that leg stiffness is reduced in the involved limb in patients with unilateral Achilles tendinopathy [24]. As ankle stiffness is a function not only of the passive factors associated which each structure (i.e. viscoelasticity) but also of the level of neural influence over each structure [4,30,37], Achilles tendon pain may affect stiffness considerably.

The purpose of this study was to evaluate etoricoxib efficacy in pain control, leg stiffness of patients suffering acute Achilles tendinopathy.

It was hypothesized that etoricoxib (120mg orally/day/7 days) efficacy was not inferior to diclofenac (100mg orally /day/7 days) for pain control in subjects suffering acute Achilles tendinopathy.

Type of Study. Prospective, randomized, controlled, blinded clinical trial. Level of evidence: Level I, therapeutic study (randomized controlled trial).

Material and methods. *General.* The study protocol and informed consent form were both approved by the appropriate ethical review committees and institutional review boards. The study was conducted in full accordance with ethical standards for the treatment of patients as laid down

in the Declaration of Helsinki. Each patient gave written informed consent prior to undergoing any study procedure.

Criteria. Patients were eligible if they were male between 18 and 50 years of age and they suffered acute Achilles tendinopathy (<2 weeks). Diagnosis of Achilles tendinopathy was made by means of activity-related achillodynia, morning stiffness or pain, painful one-legged jumping test, tenderness and decreased sports performance [26]. Subjects' age, race, affected tendon (right, left), activity, height and weight were recorded.

Exclusion Criteria.
Patients were excluded from the study if they had: prior lower limb surgery or major trauma; bilateral Achilles tendinopathy; history of lower limb radiculo-neuropathy or miopathy; hypersensitivity to any NSAIDs; used analgesic agents (NSAIDs, salicilates, narcotics) within 1 week; concurrent medical/arthritic disease (e.g. gout, lupus, rheumatoid arthritis); personal history of gastrointestinal hemorrhage or gastritis; other concurrent medical conditions including diabetes, hypertension, angina or congestive heart failure, ischemic cardiopathy, malabsorption, morbid obesity, bleeding disorders; personal history of renal dysfunction, hepatic dysfunction or anemia; used corticosteroids, clopidogrel bisulphate, rifampin, quinolon antibiotics, antiepileptics, muscle relaxants, warfarin, ticlopidine, glucosamine, condroitin sulphate for < 6 months prior to the study start (patients taking low dose aspirin for cardioprotective benefit were also excluded); history of psychotic illness, dementia or depression; history of drug or alcohol abuse or dependence; participated in any previous NSAIDs study and received active treatment, or in an investigational trial within 30 days prior to the first visit; inability to communicate or to cooperate with the investigator.

Efficacy Assessment.
The pre-defined primary efficacy endpoint of the study was a 100-mm pain visual analog scale (P-VAS), where 0= no pain; 100= extreme pain. The *"Analgesic Effect"* was determined by rating values of P-VAS pre-treatment/P-VAS post-treatment x 100.

The secondary study endpoints included: the *Patients' Global Assessment Response to Therapy (PGART)* using a 5-point *Likert* scale (0=excellent/ideal response; 4=no response/treatment ineffective) and leg functional stiffness.

Other indices of efficacy included the proportion of patients with excellent or good PGART scores and the *"Initiative on methods, measurement and pain assessment in clinical trials" (IMMPACT)*.

Clinical affectation was also evaluated by the *VISA-A Questionnaire* [28], the *Achilles Tendinopathy Scoring System (ATSS)* [8].

Baseline visit (visit 1) includes: complete medical history and physical examination; vital signs; informed consent; randomization; evaluation (P-VAS, VISA-A, ATSS, leg stiffness evaluation); NSAID prescription.

Follow-up visit at day 8 includes: complete medical history and physical examination; vital signs; adverse effect/tolerability monitoring; evaluation (P-VAS, leg stiffness evaluation, PGART).

Leg stiffness was indirectly determined by modeling the vertical ground reaction force on a portable mat (*Axon Jump 2.0, Axon Bioingeniería Deportiva, Bs. As., Argentina*) measuring flight and contact time during hopping [10,15]. Each subject performed a warm-up for approximately ten minutes (jogging, cycling, skipping, and stretching) and got used to hopping at preferred frequency. The test consisted hopping in place in one leg, for ten seconds with his hands on his hips while keeping their leg as straight as possible, in three series. Randomization defined which leg was examined first (injured/non affected). A 2-minute rest period was respected between each series. The stiffness was calculated for each hop and the mean across the hops was estimated. The maximal stiffness mean value was selected for each subject. The test was repeated if the subject did not adhere to the specific jumping protocol. The *"leg stiffness ratio" (LSR)*, leg stiffness of the injured leg / leg stiffness of healthy leg, was calculated to determine the relative patient affectation and was used for intra-group comparison and correlation analysis.

Method.
Fifty-six male patients (aged 37.56± years, range 18-50) were randomly assigned to two groups by a computerized random number generator program: etoricoxib group (n=28) treated with etoricoxib 120 mg/day orally for 7 days and diclofenac group (n=28) received sodium diclofenac 100 mg/day/7 days orally. Patients' height and weight averaged 173.95±8.02 cm (range 153-192) and 75.16±13.09 kg (range 49-107), respectively.

The non-selective NSAID diclofenac was chosen as the comparator. This phenylcetic acid derivative is one of the most widely used anti-inflammatory drugs for the management of musculoskeletal painful disorders [14].

Tablets administered to the patients were of similar size, color and taste to maintain authenticity with respect to the blinded nature of the study.

No additional treatment was prescribed to both groups and the patients must avoid any weight-bearing exercise or sport activity during the anti-inflammatory therapy period.

At follow-up visit (day 8), the patient was tested in the same manner by an independent blinded examiner.

Safety and Tolerability Assessments
Throughout the study, patients were carefully monitored for clinical adverse events (AEs). All AEs were recorded at each study visit and evaluated by the investigator (whilst blinded to study medication) for intensity, seriousness and relationship to study medication (categorized as "definitely", "probably", "possibly related", "probably not-related" and "definitely not-related"). The outcome and any action taken were documented. Investigators were required to report all adverse events that occurred from the time the patient signed informed consent at visit 1 until 14 days after the study drug was discontinued. Pre-specified NSAID-related gastrointestinal (GI) adverse events were defined as acid reflux, heartburn, dyspepsia, epigastric discomfort, abdominal pain, nausea, and vomiting. AEs commonly associated with NSAID or selective COX2 inhibitor use (for example, elevations in blood pressure, lower extremity edema) and the percentage of patients who discontinued from the study due to adverse experiences were also monitored.

A power analysis showed that a sample size of 56 patients, 28 in each group, would have 80% power to detect a 10 mm difference in the VAS between groups (sd: 15) with a 0.05 1-sided significance level.

The clinically relevant equivalence range for the primary endpoint was defined as ±10 mm on the VAS pain scale (0-100 mm VAS). This comparability boundary was based upon that used in previous studies with etoricoxib.

Statistical analysis was performed using Statistica™ software (StatSoft Inc, Tulsa, Oklahoma). Student-t, Mann-Whitney´s or Wilcoxon tests were used for testing differences between groups or within groups, respectively; $p< 0.05$ was considered significant.

Results and their discussion. *Efficacy*
- Primary Endpoint
Over the 7-day treatment period, both NSAIDs (etoricoxib and diclofenac; n=56) provided significantly pain relief of Achilles tendon pain measured by the 0-100 mm P-VAS compared to that experienced at the time of randomization: 56.4±18.8 vs. 26.7±22.1, respectively; $p<0.000003$ (Wilcoxon matched pairs test) (Fig. 1). P-VAS in the etoricoxib group pre- and post treatment was 54.5±21.6 (CI 95% 44.3-64.6; range 10-80) and 24.5±24.8 (CI 95% 12.8-36.1; range 0-70), respectively. VAS in the diclofenac group pre- and post-treatment was 58.8±15.3 (CI 95% 50.9-66.7; range 30-90) and 29.4± 18.8 (CI95% 19.7-39.1; range 0-60), respectively.

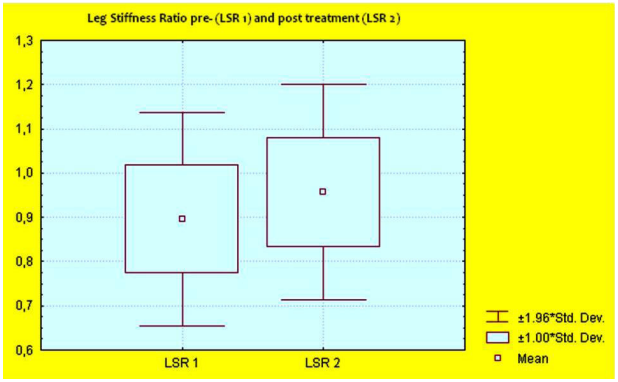

Fig. 1. Achillodynia (VAS) pre- and after treatment with NSAIDs

The *Analgesic Effect* (P-VAS post- /P-VAS pre- treatment x 100) averaged 53.77% in the complete sample (sd 38.18; range -40 to 100%; CI 95% 41.0-66.5). The *Analgesic Effect* was higher in the etoricoxib group than in the diclofenac group: 56.45±43.4% (CI 95% 36.1-76.7) and 50.61±31.9% (CI 95% 34.1-67.0), respectively. The effects were comparable based upon the predetermined comparability criteria (p=0.64; t - test for independent samples). Intra-group comparison showed that etoricoxib was not inferior to diclofenac for treatment for acute Achilles tendinopathy.

After treatment with both NSAIDs, the majority of patients referred clinical improvement in achillodynia (n=44; 80.35%); some of them showed no changes (n=9; 16.07%), while two patients referred worsen of tendon pain (3.57%) after COX inhibitor therapy.

- Additional Endpoints
IMMPACT scale showed that 7.14% of patients referred "minimally" pain relief; 14.28% referred "moderate" pain relief; 16.07% referred "substantial" pain relief; and 41.07% referred "important" pain relief.

PGART score averaged 2.05±1.35 (range 0-4). Subgroup analysis showed that patients treated with etoricoxib referred better response to therapy than those in the diclofenac group: 2.23±1.25 vs. 1.90±1.44; (p=0.46; t-test for independent samples) (Fig. 2). The percentage of patients with PGART "good to excellent" responses were 62.5% (35/56); 71.14% in the etoricoxib group (20/28) and 53.57% in the diclofenac group (15/28).

- Leg stiffness
Leg stiffness ratio (LSR) showed a significant improvement from baseline values to day-8° measurements: 0.89±0.12 (range 0.65-1.20), and 0.95±0.12 (range 0.70-1.27), respectively (p=0.038, Student test) (Fig. 3).

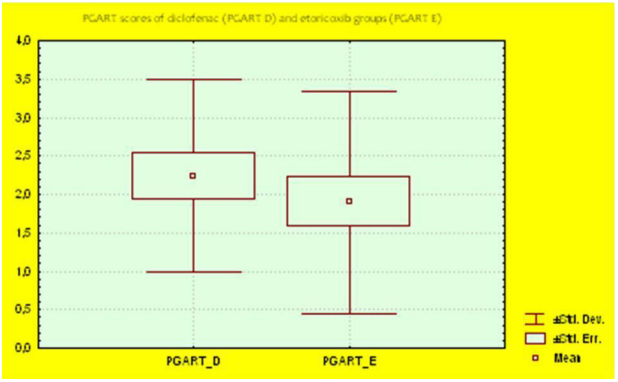

Fig. 2. PGART score of both groups: diclofenac and etoricoxib

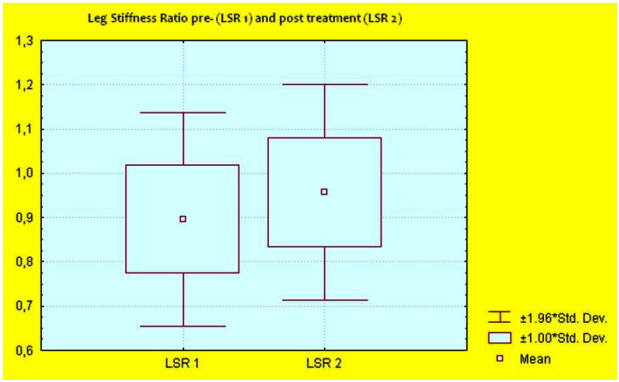

Fig. 3. Leg stiffness values pre- and after treatment with NSAIDs

- Additional results

Correlation analysis showed that pain relief after NSAIDs treatment was higher in those patients with a poor score in all clinical evaluation tests at baseline (Table 1).

Safety and tolerability.

Both treatments were generally well tolerated. The overall incidence of clinical AEs was 7.14% (4/56). Patients receiving etoricoxib reported significantly less side effects than those in the diclofenac group (0% and 14,2%, respectively, p=0.037). All four AEs effects were epigastric discomfort/gastritis; none of them required hospitalization nor further diagnostic studies. All of them were considered definitely drug-related. These patients were treated with omeprazol 40 mg/day during one week, while follow-up examination showed complete resolution of symptoms. None patient discontinued treatment due to lack of efficacy or AEs. The research protocol did not consider rescue therapy.

Table 1. Correlation of clinical evaluation and pain-relief after NSAIDs treatment

Clinical Evaluation at Baseline	**N**	***p***
P-VAS	-0.03	0.846
VISA	0.35	0.035
ATSS	0.26	0.164
LSR	0.0937	0.581

Relevant variables of both groups are summarized in Table 2.

Table 2. Summary of results of patients with acute Achilles tendinopathy treated with etoricoxib and diclofenac (P-VAS: pain visual analogue scale; PGART: Patients' Global Assessment Response to Therapy; LSR: leg stiffness ratio)

Assessment	etoricoxib	diclofenac	*p*
P-VAS Pre-treatment	54.5±21.6	58.8±15.3	0.49
P-VAS Post-treatment	24.5±24.8	29.4±18.8	0.50
Analgesic Effect (%)	56.4±43.4	50.6±31.9	0.64
PGART	2.2±1.2	1.9±1.4	0.46
LSR Pre-Treatment	0.89±0.1	0.89±0.1	0.96
LSR Post-Treatment	0.93±0.1	0.97±0.1	0.32
LSR Improvement (%)	3.4±16.5	7.8±13.9	0.37

The main finding of the present study indicated that etoricoxib is clinically effective in the treatment of acute Achilles tendinopathy providing an effect comparable to that of diclofenac. After seven days of COX inhibitor therapy, achillodynia was reduced in the majority (80%) of this sample of athletes, and they reached a significant relief (53%) of the tendon pain measured by a VAS.

Previous clinical studies have established the efficacy and tolerability of etoricoxib in several chronic musculoskeletal conditions such as osteoarthritis, rheumatoid arthritis, low back pain, and ankylosing spondylitis [6]. Etoricoxib has also been approved for the treatment of a few acute painful conditions, including primary dysmenorrhea, acute gouty arthritis, and postoperative pain [5,6,35]. To the best of our

knowledge, the present study is the first research showing the efficacy of etoricoxib in acute tendon disorders such as Achilles tendinopathy. Furthermore, it is the first report of a COX-2 inhibitor in sports-related soft tissue injuries, which are the most prevalent lesions among athletes. Results of the present trial will be specifically helpful for the athletic community given their high incidence of soft-tissue injuries and the consequent use of pain control drugs. NSAIDs were the most commonly use medications in the last editions of the Olympic Games; 33-38% of these high-performance athletes reported to use at least one NSAID. The highest prevalence was observed for diclofenac, while 10% reported consumption of more than one NSAID [36,39]. According to the present data of efficacy and safety, etoricoxib has emerged as a valid alternative for pain control in the initial phase of sport-related soft-tissue disorders like tendinopathies. However, the efficacy of these NSAIDs for the treatment of acute tendinopathies should not be directly extrapolated to other chronic tendon injuries. Most chronic tendinopathies are considered as degenerative processes rather an inflammatory condition [20,42]. Further clinical studies should be carried out to evaluate the efficacy of COX- 2 inhibitors in chronic tendinopathies.

Injuries of the Achilles tendon cause impairment in lower leg muscle tendon function, but the issue of how tendon pain affects leg mechanical abilities and performance is still unknown. Silbernagel et al [38] have found that full-symptomatic recovery in patients with Achilles tendinopathy does not ensure full recovery of muscle tendon function. Only 25% of the patients who were not longer symptomatic had full function as measured by a test battery, including jumps and hopping movements.

Leg stiffness represents the average stiffness of the overall musculoskeletal system during the ground contact phase, and it influences the mechanics and kinematics of the body´s interaction with the ground [12]. In spite of the simplicity of the spring mass model relative to the complexity of the actual neuromuscular system, it describes the mechanics of bouncing gaits remarkably well [12]. Recent studies have shown that leg stiffness is significantly decreased in patients suffering unilateral Achilles tendinopathy [24]. According to Wang et al [41], tendon pain may have the potential to influence the volitional activation of leg muscles and force production. Results of the present research support that leg stiffness is affected by acute Achilles tendon pain. After achieving significant pain relief using oral NSAIDs, leg stiffness increased significantly compared to baseline values. We considered that this objective improvement in leg function adds valuable information for using NSAIDs in the acute phase of Achilles tendinopathy in active individuals.

The source and the background of pain mechanisms associated with Achilles tendinopathy have not yet been clarified. It is hypothesized that the main cause of pain in patients with symptomatic Achilles tendinopathy does not arise from the tendon itself but is generated by its surrounding tissues [40]. The process starts with localized tendon micro-injury and degeneration, which are caused by ageing and repetitive strain below the failure threshold of the tendon. When demands of the tendon are higher than can be managed, micro-injuries develop. Due to the lack of blood vessels within the tendon instead of a chemical, a neurogenic inflammatory process is activated to repair these micro-ruptures. This neurogenic inflammation occurs in the tissue surrounding the Achilles tendon. The transition to symptomatic tendinopathy is marked by nerve proliferation accompanying the vascular ingrowth (neovascularization) to repair the defect, which arises from the paratenon. Therefore, short-term use of etoricoxib and other NSAIDs will be helpful to control pain derived from this neurogenic inflammation in the acute phase of Achilles tendinopathy.

Clinical analysis of tolerability and safety showed that both drugs were well tolerated. Patients receiving etoricoxib reported significantly less AEs than those in the diclofenac group. Several clinical trials have reported superior GI tolerability with etoricoxib compared with traditional NSAIDs even in acute musculoskeletal conditions. For instance, Rubin et al [33] have reported lower rate of GI adverse event with etoricoxib than indomethacin in an 8-day study in patients with acute gout arthritis. These results are consistent with the COX-2 hypothesis of a more favorable gastrointestinal safety profile from a COX-2 selective NSAID than a relative non-selective agent like diclofenac [29].

In summary, activity-related pain is the primary complain of athletes suffering acute Achilles tendinopathy. The main goal of initial treatment in these patients is to relieve pain. Results of the present randomized, controlled, blinded trial demonstrated that etoricoxib (120 mg/orally/1 week) is clinically effective in treatment of acute Achilles tendinopathy providing a magnitude of effect comparable to that of diclofenac with fewer side effects. Effective control of tendon pain in the acute phase of such sports-related injuries may be helpful to reduce morbidity and improve capabilities associated with high performance like leg stiffness.

Conflict of interest. The study had financial support by a grant from Merck Sharp & Dome.

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SUMMARY

MANAGEMENT OF ACUTE ACHILLES TENDINOPATHY: EFFECT OF ETORICOXIB ON PAIN CONTROL AND LEG STIFFNESS

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Tendinopathies are a major cause of disability in the athletic population; the main purpose of the treatment of these injuries is to reduce pain and improve function promptly. The objective of this randomized, active comparator controlled, blinded study was to evaluate etoricoxib efficacy in pain control and leg stiffness in athletes suffering acute unilateral Achilles tendinopathy.

Fifty-six eligible male athletes (mean age 37.5±11.0 y) suffering acute Achilles tendinopathy were randomized to receive either etoricoxib 120 mg oral once daily (n=28) or diclofenac 100 mg oral once daily (n=28). Pain (100-mm visual analogue scale-VAS), analgesic effect (percentage of 100-mm VAS reduction), satisfaction with pain management (PGART), and leg stiffness (LSR) were evaluated after one week of anti-inflammatory treatment.

Over the 7-day treatment period, both etoricoxib and diclofenac provided significantly relief of Achilles tendon pain compared to that experienced at baseline (mean VAS 26.7±2.2 and 56.4±1.8, respectively; $p<.001$). Analgesic effect averaged 53.7±38.1% (etoricoxib= 56.4% and diclofenac 50.6%, $p=0.64$). Patients referred high level of satisfaction with anti-inflammatory treatment (PGART=2.0±1.3), while leg stiffness showed a significant improvement after one-week therapy (LSR 0.89±0.1 vs. 0.95±0.1; $p=0.038$). PGART and LSR values within etoricoxib and diclofenac groups were not significant ($p=0.46$, and $p=0.37$, respectively). Both drugs were generally well tolerated; patients receiving etoricoxib reported significantly less side effects than those in the diclofenac group (0% and 14,2%, respectively, $p=0.037$).

Etoricoxib is clinically effective in treatment of acute Achilles tendinopathy providing a magnitude of effect comparable to that of diclofenac with fewer side effects. Effective control of tendon pain in the acute phase of such sports-related injuries may be helpful to reduce morbidity and improve capabilities associated with high performance like leg stiffness.

Keywords: Achilles tendinopathy, etoricoxib, diclofenac.

РЕЗЮМЕ

ЛЕЧЕНИЕ ОСТРОЙ ТЕНДИНОПАТИИ АХИЛЛЕСОВА СУХОЖИЛИЯ: ВЛИЯНИЕ ЭТОРИКОКСИБА (Etoricoxibum) НА КОНТРОЛЬ БОЛИ И ОНЕМЕЛОСТЬ НОГИ

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Тендинопатия – основная причина нетрудоспособности среди атлетов; лечение ушибов и повреждений направлено на снижение боли и улучшение функциональной способности суставов. Целью данного рандомизированного исследования явилась оценка боли и эффективности ее контроля, а также онемения ног у атлетов, страдающих от острой односторонней тендинопатии ахиллесова сухожилия.

Пятьдесят шесть атлетов (средний возраст 37.5±11.0 лет), страдающих острой тендинопатией ахиллесова сухожилия были рандомизированным методом ото-

браны для лечения эторикоксибом (etoricoxib) - 120 мг орально один раз в день (n=28) или диклофенаком (diclofenac) - 100 мг орально один раз в день (n=28). Боль и анальгетический эффект оценивались по 100-мм визуальной аналоговой шкале (VAS), удовлетворенность атлетов от лечения боли (PGART) и онемения ноги (LSR) оценивались спустя неделю после проведенного противовоспалительного лечения. Установлено, что при тендинопатии ахиллесова сухожилия как эторикоксиб, так и диклофенак обеспечивали существенное облегчение боли у атлетов после семидневного лечения (средний VAS 26,7±2,2 и 56,4±1,8, соответственно; p<,001). Анальгетический эффект достигал, в среднем, до 53,7±38,1% (эторикоксиб – 56,4% и диклофенак – 50,6%, p=0,64). Пациенты отмечали удовлетворенность от противовоспалительного лечения (PGART=2,0±1,3); при онемении ног улучшение наблюдалось после недельного курса лечения (LSR 0,89±0,1 vs. 0,95±0,1; p=0,038). Коэффициент PGART и LSR как в группе, леченной эторикоксибом, так и в группе, леченной диклофенаком, не был статистически значимым (p=0,46, и p=0,37, соотвественно). Оба препарата хорошо переносились больными, однако, у пациентов, леченных эторикоксибом отмечалось существенно меньше побочных эффектов, чем у пациентов, леченных диклофенаком (0% и 14,2%, соответственно, p=0.037).

Делается вывод о клинической эффективности эторикоксиба при лечении острой тендинопатии ахиллесова сухожилия; при его применении наблюдается меньше побочных эффектов, чем при лечении диклофенаком.

რეზიუმე

აქილევსის მყესის მწვავე ტენდინოპათიის მკურნალობა: ეტორიკოკსიბის (Etoricoxibum) ზეგავლენა ტკივილის და ფეხის დაბუჟების კონტროლზე

ხ. მაკირიაინი, ა. კოკალჟი

უმაღლესი მიღწევების ნაციონალური სპორტული ცენტრი, ბუენოს-აირესი, არგენტინა

ტენდინოპათია წარმოადგენს შრომისუნარიანობის დაკარგვის ძირითად მიზეზს სპორტსმენ-ათლეტებში. მკურნალობის ძირითადი მიმართულება ამ შემთხვევაში ტკივილის შემცირება და ფუნქციონირების გაუმჯობესებაა.

წინამდებარე რანდომიზებული, კომპარატორული, კონტროლირებადი სამეცნიერო კვლევის მიზანს წარმოადგენდა ფეხის ტკივილის და დაბუჟების კონტროლის ეფექტურობის შეფასება სპორტსმენ-ათლეტებში აქილევსის მყესის მწვავე ტენდინოპათიის დროს.

რანდომიზებული მეთოდით შერჩეული იყო 56 ათლეტი (საშუალო ასაკი – 37,5±11,0 წელი) ზემოაღნიშნული დაავადებით, მათგან 28-ს ჩაუტარდა მკურნალობა ეტორიკოკსიბით (Etoricoxibum) – 120 მგ per os დღეში ერთხელ; 28 ათლეტს – დიკლოფენაკით 100 მგ per os, ასევე, დღეში ერთჯერადად.

ტკივილის ხარისხი განისაზღვრებოდა 100 მმ ვიზუალური,ანალოგიური სკალით (VAS) - ანალგეტიკური ეფექტი – ტკივილის შემცირების პროცენტული მაჩვენებლით (VAS); კმაყოფილება ტკივილის მკურნალობით (PGART) და ფეხის დაბუჟების ხარისხის (LSR) შეფასება ხდებოდა მკურნალობის დაწყებიდან ერთი კვირის შემდეგ. აღნიშნულ ვადაში მიღწეული იქნა ტკივილის საგრძნობი შემცირება (საშუალო VAS 26.7±2.2 და 56.4±1.8, შესაბამისად, p<0.001); ანალგეტიკური ეფექტურობა შეადგენდა საშუალოდ 53.7±38.1% (ეტორიკოკსიბი – 56.4% და დიკლოფენაკი – 50.6%, p=0.64) პაციენტები აღნიშნავდნენ კმაყოფილებას ანთებისსაწინააღმდეგო მკურნალობის მიმართ (PGART=2.0±1.3). აღინიშნებოდა ფეხის დაბუჟების ხარისხის შემცირება (LSR 0.89±0.1 vs. 0.95±0.1; p=0.038). ეტორიკოკსიბით და დიკლოფენაკით ჩატარებული მკურნალობის შემდეგ PGART და LSR კოეფიციენტებს შორის განსხვავება უმნიშვნელო იყო (p=0.46, და p=0.37, შესაბამისად).

აუცილებელია აღინიშნოს, რომ ეტორიკოკსიბით მკურნალობის შემთხვევაში გამოვლინდა მნიშვნელოვნად ნაკლები გვერდითი ეფექტი, ვიდრე დიკლოფენაკით მკურნალობისას (0% и 14,2%, შესაბამისად, p=0.037).

ჩატარებული კვლევის შედეგად დადგენილია ეტორიკოკსიბის მაღალი კლინიკური ეფექტურება აქილევსის მყესის მწვავე ტენდინოპათიის მკურნალობაში.

სპორტთან დაკავშირებული ჭრილობების შემთხვევაში, ტკივილის და ფეხის დაბუჟების თავიდან აცილების მიზნით, აუცილებელია ტკივილის აქტიური ფაზის ეფექტური კონტროლირება.

DISTRIBUTION OF CANCER STEM CELLS IN DUCTAL INVASIVE CARCINOMA OF BREAST (REVIEW)

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Breast cancer is the most common malignancy in women all over the world, accounting for >40,000 deaths each year in the United States. It is not a single disease but a heterogeneous group of diseases with different pathologies, biological characteristics, and clinical behavior [9,24,30,32,33]. Despite advances in detection and treatment of metastatic breast cancer, mortality from this disease remains high because current therapies are limited by the emergence of therapy-resistant cancer cells [12,28]. As a result, metastatic breast cancer remains an incurable disease by current treatment strategies.

Cancers are believed to arise from a series of sequential mutations that occur as a result of genetic instability and/or environmental factors [7,36]. A better understanding of the consequences of these mutations on the underlying biology of the neoplastic cells may lead to new therapeutic strategies.

The stochastic model predicts that the tumor is relatively homogeneous and the tumorigenic mechanisms (pathways, genetic programs) that underlie the malignancy are operative in all cells. Thus, studying the bulk of the cells that make up the tumor mass can identify the key properties of the tumor. The hierarchy model predicts functional heterogeneity among the cells that comprise the tumor and that the rare tumor initiating cells (T-IC) are different from the vast majority of the cells that make up the tumor. Therefore, tumorigenic pathways may function differentially in distinct tumor subpopulations. This model also predicts that although eradication of the non-T-IC cells may result in a remission, the disease will relapse if the T-IC cells are not eliminated. Resolution of the T-IC problem requires both purification of tumor cells into subfractions and a functional assay to detect cells with the capacity to initiate tumor growth *in vivo*. The stochastic model suggests that it will be impossible to predict which kind of cells become T-IC and that stochastic events will cause T-IC cells to be found in any two sorted cell fractions with equal probabilities. By contrast, the hierarchy model predicts that it should be possible to separate T-IC from non-T-IC.

Research on T-IC is most advanced for the hematological malignancies. Key to these studies is the depth of understanding of normal hematopoietic development that has been gained in the past four decades. Functional *in vitro* and *in vivo* assays are available for all stem and progenitor cell types ranging from the primitive pluripotential stem cells to multipotential and unipotential progenitors [29]. In addition, a rich collection of cell surface differentiation markers enable detailed characterization of normal hematopoietic development, as well as providing insight into how normal differentiation becomes disrupted in human leukemia. It is clear that leukemic tissues, although abnormal, still retain remnants of normal differentiation and developmental programs [20]. Unfortunately, purification of solid tumor T-IC has been difficult because of the paucity of cell surface markers that enable cell sorting. Moreover, T-IC xenograft assays for primary human solid tumor tissue have traditionally been carried out in nudemice. These mice still possess significant residual immune function causing variability in the rate of xenograft initiation and it is possible that host resistance mechanisms will not permit single T-IC to be detected.

For most cancers it is less clear which cells within the tumor clone possess tumor-initiating cell function. In the past decade, many treatments undergoing clinical trials have been developed based on molecular profiles of different cancers [13]. However, one of the most promising therapy targets came with the identification of a pool of cancer cells with stem characteristics - cancer stem cells (CSC). The CSC model proposes that tumors, as normal tissues, are organized in a cellular hierarchy, in which CSC are the only cells with unlimited proliferation and tumorigenic potential; therefore, being capable of riving tumor growth, progression and metastasis due to their stem cell-like characteristics: self renewal and differentiation [7]. Recent evidence has demonstrated that CSC are resistant to various forms of therapies, including radio and chemotherapy [6,8,11,15,17,19,23]. Based on these observations, the CSC model became the foundation for new preventive and therapeutic strategies in cancer.

The idea of cancer stem cells originates from many years ago. Stem cells have three distinctive properties: self-renewal (i.e. at cell division, one or both daughter cells retain the same biological properties as the parental cell); the capacity to generate multiple cell lineages; and the potential for sustained proliferation. Indeed, the attribute of self-renewal is especially notable because its subversion is highly relevant to oncogenesis and malignancy [3,22]. Therefore, malignant cells harboring the three features that define normal stem cells have been termed “cancer stem cells”. Even though, recently, the awareness of this concept in cancer research has greatly expanded, it is not new. In 1855, Rudolf Virchow proposed the embryonic rest hypothesis, stating that cancer arises from the activation of “dominant” cells present in mature tissues that are remaining embryonic cells [35]. This theory, based on the

morphological similarities between developing fetal cells and some cancer cells, was the first description of what we now call the CSC. Later in 1875, this theory was expanded by other pathologists such as Julius Cohnheim, who proposed that misplacement of stem cells during embryonic development could lead later on in life to the development of tumors [10]. The studies went on and on, classification systems have been improved. It brought the development of cancer studies to better understanding of stem-like properties in different cells of different tumors [27].

Finding breast CSCs (BCSCs) has been an important goal for many breast cancer researchers, who were trying to define a universal marker or combination of markers able to specifically identify these cells in breast tumors and ultimately isolate them.

The aim of the review was to assess the current literature published on the breast cancer stem cells.

Reviewing the literature, it appears that the most studied BCSC markers are the transmembrane proteins CD44 and CD24. A subpopulation of tumor cells that strongly express CD44 but not CD24 (the $CD44^+CD24^-$/low phenotype) was identified as CSCs by Al-Hajj and collaborators [3], and this was subsequently confirmed by other authors. CD44 is a transmembrane glycoprotein, of which several isoforms exist, that normally regulates cell–cell adhesion and cell–matrix interactions, as well as cell migration. This glycoprotein binds mainly to hyaluronic acid, as well as to collagen, fibronectin, laminin and chondroitin sulphate - all important components of the ECM. It also binds the cytokine osteopontin [21].

Members of the CD44 family differ in their extracellular domain by the insertion of variable regions through alternative splicing [25]. The gene-encoding CD44 consists of 20 exons. In the standard form (CD44s), 10 of the 20 exons are transcribed. Multiple variant isoforms (CD44v1–v10) arise from alternative messenger RNA (mRNA) splicing of the other ten exons [2]. Many cancer cell types as well as their metastases express high levels of CD44 and/or CD44 variants. Since the blockage of CD44-ligand interaction inhibits local tumor growth and metastatic spread, CD44 may confer a growth advantage to breast cancer cells. In contrast to the standard form of CD44, which is usually ubiquitously expressed on epithelial cells and lymphocytes, CD44 variants exhibit tissue-specific expression.

Some of these variants, in particular splice variant CD44v6, are associated with aggressive tumor behavior in that their expression correlates with poor prognosis in a variety of human malignancies including breast cancer [34].

CD24 is a small, heavily glycosylated mucin-type protein, which is linked to the cell membrane via a GPI anchor. This molecule is involved in the regulation of cell proliferation and cell-cell interactions and it was shown to be expressed by normal pre-B lymphocytes (which lose its expression during the maturation into plasma cells), as well as in various hematological alignancies and solid tumors of some organs [18]. CD24 is the ligand of P-selectin and also an adhesion receptor expressed on activated endothelial cells and platelets, and this has led to the suggestion that it might play an important role in the metastatic process [16]. Using a combination of these two cell surface markers, Al-Hajj and collaborators [3] were the first to distinguish cancer cells that were tumorigenic in immunocompromised (non-obese diabetic/severe combined immunodeficient (NOD/SCID)) mice from non-tumorigenic cells, in small number of breast tumors. In flow sorting experiments, they used the ESA as an epithelial cell marker with $CD44^+/CD24^-$/low as CSC marker combination, after eliminating the non-epithelial cells that were stained for their lineage-specific markers (hematopoietic and endothelial). Since then, many more breast carcinomas have been reported to contain a subpopulation of $CD44^+/CD24^-$/low cancer cells, which are capable of generating tumors in the NOD/SCID mice, even when implanted in very low numbers. In contrast, other cancer cell populations fail to generate tumors, even when implanted in high numbers. These reports therefore established tumorigenicity and self-renewal potential of these cells, in vitro and in vivo [1,31]. Even though some clinical studies confirmed that $CD44^+/CD24^-$/low-expressing tumors have a poor prognosis [5,14,26], controversy remains concerning this issue [1,20]. Shipitsin et al. demonstrated that genes specifically expressed in $CD44^+$ cells, among which many known stem cell markers, identified carcinomas with poor patient survival [30], suggesting that $CD44^+$ expression is prognostically relevant and justifying its consideration as a new therapeutic target for breast cancer. In contrast, Mylona et al. [20] observed that breast cancers with the opposite $CD44^-/CD24^+$ phenotype are associated with poor patient prognosis, in stark contrast with the CSC $CD44^+/CD24^-$/low phenotype. Furthermore, Abraham et al. failed to confirm that the occurrence of $CD44^+/CD24^-$/low tumor cells in breast cancer is associated with worse survival [1]. These contradictory data demanded additional efforts to find other markers that could complement the CSC markers CD44 and CD24, to arrive at an improved correlation with patient survival.

Comprehensive gene expression profiling using DNA microarrays has identified five major molecular subtypes in breast cancer: luminal A, luminal B, $HER2^+$, basal-like, and normal breast-like [9,30,33]. Tumors in different subtypes follow different clinical courses and respond differently to treatment. In general, patients with basal-like tumors have worse prognosis whereas those with luminal A tumors have more favorable outcomes [30,33]. In addition to this intertumor heterogeneity, there is also a high degree of intratumor diversity that is already present in ductal carcinoma in situ (DCIS) [32]. Specifically, a single tumor at any given time can contain tumor cell populations with distinct molecular profiles and biological properties.

Currently prevailing models explaining intratumor heterogeneity include the clonal evolution and cancer stem cell hypotheses. The clonal evolution model emphasizes diversity for heritable traits that in combination with continuous selection allows for the expansion of tumor cells with the most favorable characteristics. According to the cancer stem cell hypothesis, cancer stem cells, defined as a subset of tumor cells with stem cell–like features, have the capacity to self-renew and differentiate, giving rise to a heterogeneous tumor cell population.

As mentioned In breast cancer, $CD44^+/CD24^{-}$/low cells were identified as candidate breast cancer stem cells based on xenotransplant assays in nonobese/severe combined immunodeficient mice. More recently, additional markers including aldehyde dehydrogenase (ALDH) activity, CD133, and ITGA6 have also been proposed as putative markers of mammary epithelial and breast cancer stem cells.

Interestingly, some studies revealed an enrichment of the $CD44^+/CD24^{-}$/low and $CD44^-/CD24^+$ cell populations in basal-like and luminal breast cancer cell lines, respectively, CD44 being positively associated with stem cell-like characteristics and CD24 expression related to differentiated epithelial features. These in-vitro data were later demonstrated in primary breast carcinomas, but the clinical and prognostic impact of these markers in breast cancer remains a controversial issue, demanding additional efforts to find other CSC markers that could better predict breast cancer patient survival.

In contrast, most studies analyzing primary human breast cancer samples have not revealed a significant association between CD44 and CD24 expression and tumor subtypes. Only one recent study reported the enrichment of $CD44^+/CD24^{-}$ cancer cells in basal-like invasive breast tumors. The expression of ALDH1 in invasive breast tumors was also correlated with poor clinical outcome, but the association of this with tumor subtypes was not reported.

The ability of small numbers of $CD44^+CD24^{-}$/lowLineage - tumorigenic cells to give rise to new tumors was reminiscent of the organogenic capacity of normal stem cells. Normal stem cells self-renew and give rise to phenotypically diverse cells with reduced proliferative potential. To test whether tumorigenic breast cancer cells also exhibit these properties in one of the studies, tumors arising from 200 ESA^+CD44^+CD24-/lowLineage- T1 or 1,000 $CD44^+CD24$-/low Lineage- T2 cells were dissociated and analyzed by flow cytometry. The heterogeneous expression patterns of ESA, CD44, or CD24 in the secondary tumors resembled the phenotypic complexity of the tumors from which they were derived. Within these secondary tumors, the $CD44^+CD24^{-}$/lowLineage - cells remained tumorigenic, whereas other populations of Lineage -cancer cells remained nontumorigenic. Thus, tumorigenic cells gave rise to both additional $CD44^+CD24^{-}$/lowLineage - tumorigenic cells as well as to phenotypically diverse nontumorigenic cells that recapitulated the complexity of the primary tumors from which the tumorigenic cells had been derived. These $CD44^+CD24^{-}$/lowLineage-tumorigenic cells from T1, T2, and T3 have now been serially passaged through four rounds of tumor formation in mice, yielding similar results in each passage with no evidence of decreased tumorigeneity. These observations suggest that $CD44^+CD24^{-}$/lowLineage -tumorigenic cancer cells undergo processes analogous to the self-renewal and differentiation of normal stem cells. These results demonstrate that heterogeneous populations of cells in breast cancers consist of a phenotypically distinct tumorigenic population, as well as a much larger population that lacks this tumorigenic potential. It is known that breast cancer cells are genetically unstable, and thus individual breast cancer cells from the tumorigenic population may sometimes be unable to proliferate as a consequence of chromosomal aberrations acquired during mitosis [1,4,14]. Nevertheless, the observation that in eight of nine tumor specimens the tumorigenic subpopulation displayed a common phenotype that allowed for their identification suggests that common pathways may drive this tumorigenic population.

The tumorigenic $CD44^+CD24^{-}$/lowLineage - population shares with normal stem cells the ability to proliferate extensively, and to give rise to diverse cell types with reduced developmental or proliferative potential [26].

The initial reports that only the $CD44^+CD24^{-}$/low subpopulation of human breast cancer cells contains BCSC have been challenged by subsequent studies. Honeth et al. detected a $CD44^+CD24^{-}$/low subpopulation in only 31% of 240 human breast cancer samples analyzed, with a strong association with the basal-like phenotype. Creighton et al. reported that a gene expression signature common to both $CD44^+/CD24^{-}$/low and mammosphere-forming cells was mainly present in breast cancer of the recently identified claudin-low molecular subtype, which is characterized by expression of many epithelial-mesenchymal transition-[EMT-] associated genes. In addition, contrasting results have been reported by different groups in regard to the invasiveness of $CD44^+CD24^+$ compared with $CD44^+CD24^{-}$/low cells. Some researchers have found that CD24 is not a consistent breast cancer stem cell marker. In particular, in a human breast carcinoma model originated from bone marrow micrometastases of a breast cancer patient, they have recently shown that the stem cell growth of $CD24^+$ and $CD24^-$ sorted breast cancer cell subpopulations and their single-cell clones resulted in similar take and growth rates. Single cell-sorted CD24- and CD24 high MA-11 gave rise in vitro to cell populations with heterogeneous CD24 expression. Also, all tumor xenografts derived from $CD24^+$ and CD24- cells expressed CD24 on their cell surface in vivo [4].

In fact most of the results demonstrate a clear variation in the prevalence of $CD44^+/CD24^-$tumor cells between breast tumors of different subtypes. The occurrence of this phenotype is high in basal-like tumors – and especially in BRCA1 hereditary tumors – is lower in tumors of luminal type and is particularly low in the $HER2^+$ tumors, irrespective of ER status. These results emphasize the biological heterogeneity of breast cancer and an enrichment of putative tumor-initiating cells in the aggressive basal-like tumor subtype. Far from all basal-like tumors contain $CD44^+/CD24^-$cells, however, and their scarcity in $HER2^+$ tumors suggests that tumorigenicity may not be confined to cells of this phenotype and that other markers remain to be identified. Moreover, the obvious heterogeneity of cells with various CD44/CD24 expressions within individual tumors may be indicative of a cancer stem cell subpopulation giving rise to more differentiated and committed cell populations. This does by no means exclude the coexistence of cancer cell clones of independent origin, evolution and tumorigenic ability.

The concept of cancer stem cells responsible for tumor origin, maintenance, and resistance to treatment has gained prominence in the field of breast cancer research. The therapeutic targeting of these cells has the potential to eliminate residual disease and may become an important component of a multimodality treatment. Recent improvements in immunotherapy targeting of tumor-associated antigens have advanced the prospect of targeting breast cancer stem cells, an approach that might lead to more meaningful clinical remissions. It's important to get a correct understanding of the role of breast cancer stem cells in disease, and the potential to target these cells.

There are not so many studies, revealing the diversity of cancer stem cells in different types, different sizes and different grade of breast cancers. Often these studies do not contain information about CSC distribution in breast cancer with lymph node involvement and metastasizing cases. Correlation of CSC profile with existence of circulating tumor cells, chemoresistance and frequency of relapses is not clearly studied yet.

So the concept of cancer stem cells in breast cancer is still a topic for discussions. Controversy in data causes confusions and inability to conduct these researches into clinical settings. So more studies are necessary first of all to identify if these markers are truly characteristic for CSC and what is the real phenotype of these cells in breast tumors of different type, size, and stage. This can bring light to better understanding of the pathological process in breast cancer and ways to target it precisely.

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SUMMARY

DISTRIBUTION OF CANCER STEM CELLS IN DUCTAL INVASIVE CARCINOMA OF BREAST (REVIEW)

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Despite advances in detection and treatment of breast cancer, mortality from this disease remains high. According to contemporary point of view the reason for this lies in fact, that in addition to intertumor heterogeneity, there is also a high degree of intratumor diversity in cancer cell population. For most cancers it is less clear which cells within the tumor clone possess tumor-initiating cell function.

During studying oncogenesis and malignиation processes a pool of cancer cells with stem characteristics - cancer stem cells (CSC) was identified. Indeed, the specifications of them let us conclude, that exactly these cells comprise the leading substrate for cancer initiation and self-renewal. Breast carcinomas have been reported to contain a subpopulation of $CD44^+/CD24^-$/low cancer cells, which are capable of generating tumors even when implanted in very low numbers. Exactly these cells are considered to be CSCs in different subtypes of breast cancer and cancers with $CD44^+/CD24^-$/low phenotype are confirmed to have a poor prognosis (but some controversies remain concerning this issue). The aim of the review was to assess the current literature published on the breast cancer stem cells.

There are not so many studies, revealing the diversity of cancer stem cells in different types, different sizes and different grade of breast cancers. CSC distribution in breast cancer with lymph node involvement, metastasizing and chemoresistant cases, existence of circulating tumor cells in not studied precisely.

So the concept of cancer stem cells in breast cancer is still a topic for discussions. This can bring light to a better

understanding of the pathological process in breast cancer and ways to target it.

Keywords: breast cancer, cancer stem cells.

РЕЗЮМЕ

ОСОБЕННОСТИ РАСПРЕДЕЛЕНИЯ СТВОЛОВЫХ КЛЕТОК ИНВАЗИВНОГО ПРОТОКОВОГО РАКА МОЛОЧНОЙ ЖЕЛЕЗЫ (ОБЗОР)

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Несмотря на усовершенствование методов выявления и лечения рака молочной железы, смертность от этой патологии по сей день остаётся высокой. По современным представлениям, кроме разнообразия типов опухоли, велика разница и между популяциями клеток в одной опухоли. В большинстве случаев неизвестно, какая клетка в клоне носит функцию инициатора рака.

Целью данного исследования явилось изучение ретроспективной и текущей научной литературы по вопросу распределения стволовых клеток инвазивного протокового рака молочной железы.

В процессе изучения онкогенеза и малигнизации выявлена группа клеток со свойствами стволовых клеток - стволовых клеток рака. Их характеристики позволяют сделать вывод о том, что именно данные клетки являются ведущими в процессе развития и самообновления опухоли. Исследованиями подтверждено, что рак молочной железы содержит популяцию клеток $CD44^+/CD24^-$/низкая, которые в случае внедрения в организм даже в небольших количествах способствуют развитию рака. Именно эти клетки являются стволовыми клетками различных подтипов рака молочной железы. Рак с экспрессией $CD44^+/CD24^-$/низкая характеризуется худшим прогнозом, хотя по данному вопросу имеются противоречивые данные.

Анализ научной литературы выявил, что исследования, изучающие изменения стволовых клеток рака в опухолях разного типа, размера, степени и дифференциации малочисленны. Недостаточно изучено и распределение стволовых клеток рака в случаях метастазов при химиорезистентности и при выявлении циркулирующих раковых клеток. Следовательно, вопрос о стволовых клетках рака подлежит детальному изучению и обсуждению. Исследования могут способствовать пониманию механизмов развития патологического процесса при раке молочной железы и разработке новых стратегий для превентивной терапии.

რეზიუმე

ღეროვანი უჯრედების განაწილების თავისებურებები ძუძუს დუქტურ ინვაზიურ კარცინომაში (მიმოხილვა)

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ღეროვანი უჯრედების განაწილების თავისებურებების ირგვლივ არსებული რეტროსპექტული და მიმდინარე ლიტერატურის შესწავლამ გამოაშკარავა,რომ ძუძუს კიბოს დეტექციის და დამიზნებითი მკურნალობის მეთოდების დახვეწის მიუხედავად, ამ პათოლოგიით სიკვდილიანობის მაჩვენებელი საკმაოდ მაღალია, რაც არსებული მონაცემებიდან გამომდინარე, გამოწვეულია არა მხოლოდ სიმსივნეთა მრავალფეროვნებით, არამედ თვით სიმსივნის შეგნით უჯრედებს შორის არსებული განსხვავებით. სიმსივნეთა უმრავლესობისთვის არ არის დადგენილი, თუ რომელი უჯრედი ატარებს მაინიცირებელ ფუნქციას სიმსივნურ კლონში.

ონკოგენეზისა და მალიგნიზაციის შესწავლის პროცესში აღმოჩენილ იქნა სიმსივნურ უჯრედთა ჯგუფი ღეროვანი უჯრედების მახასიათებლებით – სიმსივნის ღეროვანი უჯრედები. ამ უჯრედების თავისებურებები საფუძველს იძლევა ვივარაუდოთ, რომ სწორედ ისინი წარმოადგენს სიმსივნის წარმოქმნისა და თვითგანახლების წამყვან სუბსტრატს. კვლევებმა დაადასტურა, რომ ძუძუს კიბო შეიცავს $CD44^+/CD24^-$/დაბალი უჯრედების სუბპოპულაციას, რომელიც ორგანიზმში მცირე რაოდენობით ჩანერგვის შემთხვევაში იწვევს სიმსივნის განვითარებას. სწორედ ეს უჯრედებია მიჩნეული სიმსივნის ღეროვან უჯრედებად ძუძუს სხვადასხვა ქვეტიპის სიმსივნეებში. $CD44^+/CD24^-$/დაბალი ფენოტიპის მაექსპრესირებელი სიმსივნეები უფრო აგრესიული პროგნოზით ხასიათდებიან, თუმცა, ამ საკითხზე მონაცემები ურთიერთსაწინააღმდეგოა.

კვლევათა რაოდენობა,რომლებშიც განხილულია სიმსივნის ღეროვანი უჯრედების ცვლილებები სხვადასხვა ტიპის, ზომის და ავთვისებიანობის ხარისხის ძუძუს კიბოში მცირერიცხოვანია,ხოლო სიმსივნის ღეროვანი უჯრედების განაწილება ძუძუს კიბოს მეტასტაზირებულ შემთხვევებში, ქიმიორეზისტენტობის დროს და მოცირკულირე სიმსივნური უჯრედების გამოვლენისას არ არის დაზუსტებული. სიმსივნის ღეროვანი უჯრედების

საკითხის ირგვლივ ლიტერატურის შესწავლამ ნათელჰყო, რომ დამიზნებითი მკურნალობის ახალი სტრატეგიის შემუშავებისათვის, რაც სადღეისოდ უაღრესად მნიშვნელოვან პრობლემას წარმოადგენს, აუცილებელია ძუძუს კიბოს დროს პათოლოგიური პროცესის მექანიზმების დაზუსტება.

DISTRIBUTION OF CD44/CD24 POSITIVE CELLS IN DUCTAL INVASIVE CARCINOMA OF BREAST OF DIFFERENT GRADE AND MOLECULAR SUBTYPE

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Cancer stem cells which can be responsible for tumor development, formation and treatment resistance are the subject of an extensive and comprehensive study of breast cancer research [14,18,20,21]. For many years the prevailing opinion was, that most of tumor cells have potential of intensive proliferation and metastasis, which must be eliminated by effective treatment, although last studies show that only limited subpopulations with similar features as stem cells have ability of proliferation and progression, for which was established the term "tumor stem cells". Tumor stem cells have potential of self renovation, differentiation in different directions and active proliferation. Suppression of tumor stem cells is important in oncogenesis [5,12,16,17]. Study of tumor stem cells was subject of many researches. Studies were performed for determine universal markers or marker combination for aim of isolation tumor stem cells. During literature review, were found that the most studied breast cancer stem cells express transmembrane proteins CD24 and CD44.

Some studies show that CD24 and CD44 phenotype cells are markers for tumor stem cell, increased amount of above mentioned cells is prediction of breast cancer bad prognosis. For one's part are studies which show that positivity of isolated CD24 and CD44 markers determines tumor aggressivity and bad prognosis [1,4,19].

The CD44 antigen is glycoprotein of cell-surface which in normal and tumor tissue Provides cell – cell interactions, cell adhesion and migration. This glycoprotein connects hyaluronic acid, collagen fibronectin, laminin and chondroitin hydro sulfate with important components of extracellular matrix. CD44 variants Reveals a tissue specific expression. Part of them is associated with tumor aggressivity. Expression of this markers is in correlation with different types of tumors, among them is breast cancer [2,13,15,22]. CD 24 is mucin type protein, which is linked with cell membrane by glycosylphosphatidylinositol, this molecule is Involved in regulation of cell proliferation and cell-cell regulation. CD 24 is P selectin ligand and adhesion receptor in activated endothelial cells and thrombocytes, This indicates that it can play important role in process of metastasis [3,9,10].

The purpose of our research was to study distribution of CD44/CD24 positive cells in ductal invasive carcinoma of breast of different grade and molecular subtype.

Material and methods. For research was used 1324 post-operative breast cancer samples, from which were selected 393 patients with invasive ductal carcinoma samples, examined 2008-2012 in laboratory of Pathgeo Union of Pathologists and N. Kipshidze Central University Hospital. The age range was between 23-73 year. The degree of malignancy in HE (hematoxylin/eosin) samples were estimated according to system of Scarf-Bloom-Richardson. For all cases were performed immunohistochemical study using ER, PR, Her2, Ki67, CK5- molecular markers. Immunohistochemical study was performed by using mononuclear antibodies from Leica Microsystems. For mononuclear antibody visualisation was also used visualisation system from Leica Microsystems Novolinc Polymer Detection System. For purpose of Reveal tumor stem cells was used mononuclear antibodies CD44 and CD24 (BIOCARE MEDICAL, CD44 – Clone 156-3C11; CD24 - Clone SN3b). Figure N1,2. CD44 expression was estimated in the following way: negative, which include negative-0 and low expression (1-10%), intermediate expression 11-50%, high expression >51% [6,7,11]. Classification of breast cancer is based on determination of expression their genes in luminal A and B, basal type, HER2+, Normal breast-like subtypes. These subtypes are associated with tumor features and results of disease. Luminal type is linked with estrogen receptor positivity, While the basal and normal types almost always are estrogen receptor negative, as well as HER2+ tumors. Multiple studies show that basal type has exceptionally bad prognosis [1,12,17], though it's unclear how much worse clinically significant has basal like tumor compared to different ER negative tumors [8].

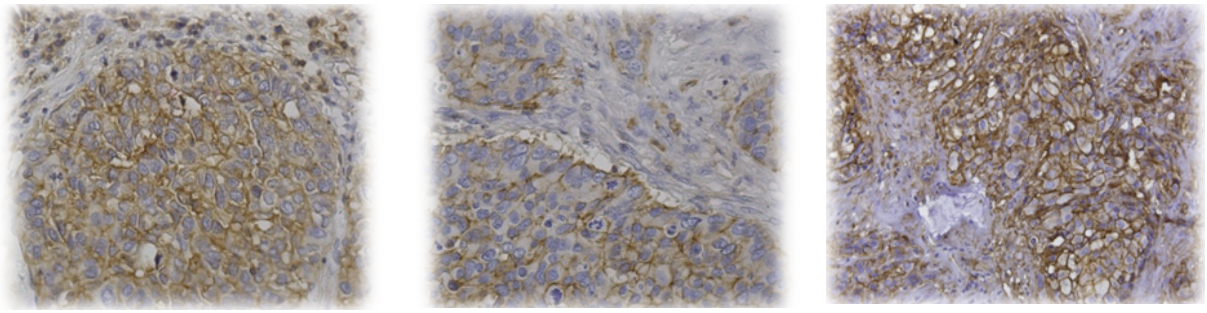

Fig. 1. Immunohistochemistry stain, CD 44 (X200)

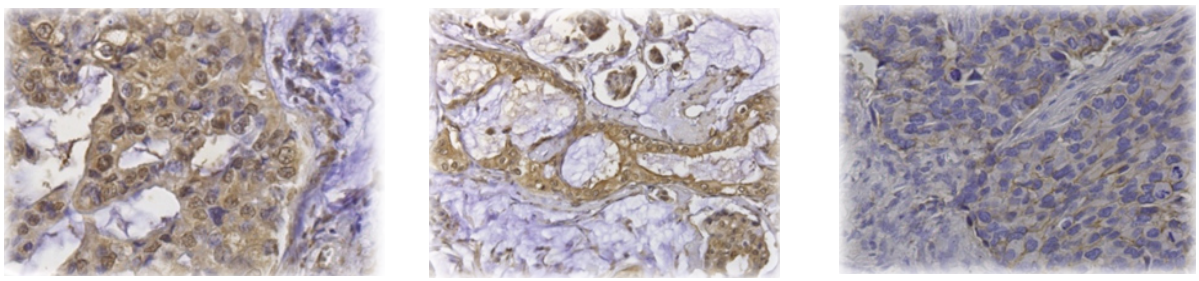

Fig. 2. CD 24 Antibody Immunohistochemistry stain (X200)

Association of CD44/CD24 expression in different subtypes of cells, between clinic-pathological parameter and different biological characteristics were performed by Pirson correlation and using X2 tests. Obtained quantitative statistical analyses were performed by using SPSS V.19.0 program. Statistically significant were Considered 95 % of confidence interval.

Results and their discussion. Results of detection CD44 expression are shown on table 1.

For comparative analysis of characteristics CD44 expression we shifted absolute numbers to a percentage. Results are shown on diagram 1.

Table 1. Characteristics of tumor stem cell markers in Different grade and molecular subtype

	Total positive	negative	low	intermediate	high	P Value
Histological grade	**175**	**218**	**22**	**53**	**100**	**<0.001**
Grade I	26	66	2	12	12	
Grade II	86	130	10	24	52	
Grade III	63	22	10	17	36	
Molecular subtypes	**175**	**218**	**22**	**53**	**100**	**<0.001**
Luminal A	59	189		19	40	
Luminal B	46	6	8	6	32	
Her2	13	10	1	4	8	
Bazal Like	57	13	13	24	20	

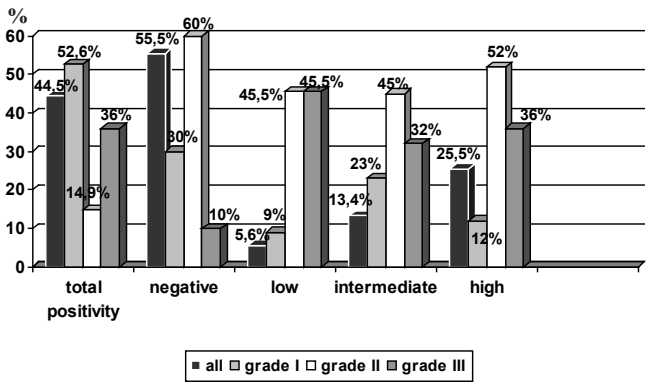

Diagram 1. CD44 expression percentage in different grade in Invasive Ductal Carcinoma

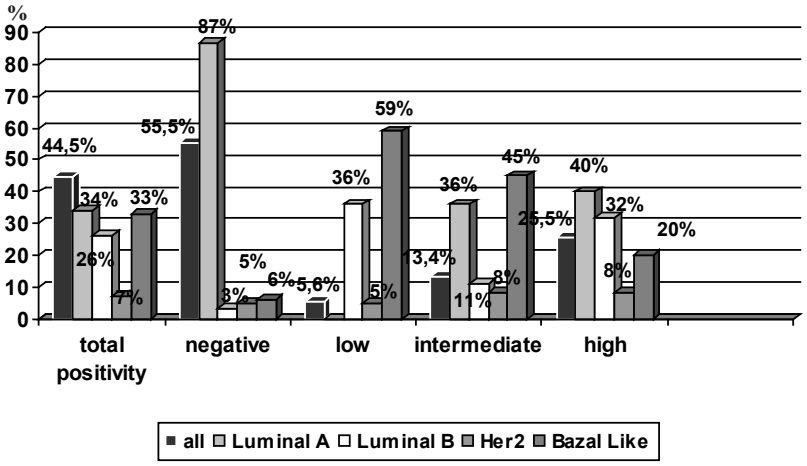

Diagram 2. Features of CD44 expression according to molecular subtype

Study of CD44 expression has shown that positive cases are 1,2 times less than negative cases. At I grade positive cases were 1.2 times less than the negative. For G2 tumors 1,2 times less and for G3 tumors 3,6 times more than negative cases.

As the data show to the direction G1-G3 CD44 positive cases increased by an average of 2 times.

Estimation intensity of CD44 positive expression show that CD44 with low expression is equal in G2-G3 tumors and 5 times less in G1 tumors. CD 44 with moderate expression is slightly different in G2-G3 tumors and approximately 2 times less in G1 tumors. High expression of antigen was observed in II degree of malignancy, its 1,4 times less in G3 tumors and 4,3 times less in I degree of malignancy. In G1 tumors CD44 with high degree of malignancy amounts 46 %, in G2 60 % and in G3 57%. Which indicate that cases with CD44 high expression are steadily higher and slightly different despite degree. CD 44 expression dynamics of a mild grade is almost the same as high expression. Different dynamics shows a low expression of the CD44 which are Almost 5.6% of the positive cases. Features of CD44 expression according to molecular subtype is shown on diagram 2.

Data show, that CD44 positive cases are evenly distributed between Luminal A, Luminal B, HER2+ triple negative/basal like subtypes and is 4,8 times less for HER2 cases. Maximal amount of CD44 negative cases observed during a Luminal A case. For Luminal A CD44 positive cases are 2,6% less compare with negative cases, whereas for Lumina B Her2 positive and basal like subtypes 8,6, 14 and 5,5 times less. Special recognition deserves HER2+ subtype, in which CD44 positive cases by average are 4,8 times less than other subtypes. Nevertheless in Luminal A positive cases are less than negative, The vast majority of them is presented by high antigen expression. The same trend is maintained for Luminal B, for Her2+ subtypes CD44 high expression cases amounts 69% and 61% and for basal like subtypes CD 44 high and moderate expression is presented as equal amounts, accordingly 42% and 35%. As data show in Luminal A Subtype negative cases are very high, which can be reason of reliable process and high sensitivity for chemotherapy. For one's part such aggressive subtypes of breast cancer as Luminal B and basal like type are characterized by CD 44 positivity and antigen high expression, which can be basis of tumor aggressive behavior and Chemoresistance. As regards the HER2+ tumors which are in list of aggressive tumors. CD44 low expression in this tumor Indicates that they have different base of carcinogenesis. Well-known that bases of her2+ tumor carcinogenesis, is HER2 gene amplification or stem cell mutation. CD24 positive cases are shown on table 2, and percentage of it are shown on diagram 3.

Table 2. Features of CD 24 according the aggressivity and molecular subtyp

	Total positive	**Neg/low**	**intermediate**	**High**	**P Value**
Histological grade	**102**	**291**	**47**	**55**	**0.01**
Grade I	30	62	14	16	
Grade II	43	173	17	26	
Grade III	29	56	16	13	
Molecular subtypes	**102**	**291**	**47**	**55**	**<0.001**
Luminal A	57	191	17	40	
Luminal B	11	41	5	6	
Her2	20	3	13	7	
Bazal Like	14	56	12	2	

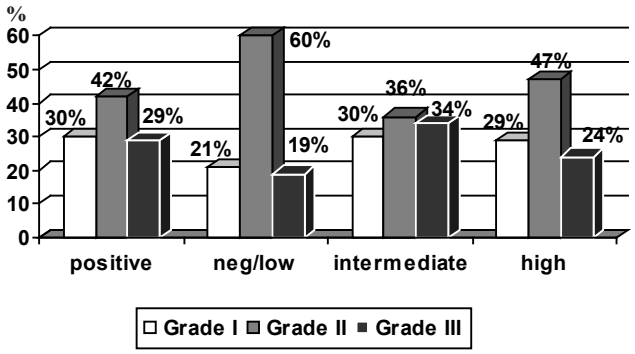

Diagram 3. Shows percentage of CD24 expression in different grade of Ductal Invasive Carcinoma

For most authors examination of (-) CD24 negative and low expression cases are unified in same group (-). Amount of CD24 positive cases are similar for G1 and G3 tumors and is by average 1,4 times more than negative cases, while for G2 tumors its 1,4 times less than negative cases. As well as cases with CD24 inermediate and high expression are similar in G2 and G3 tumors. As amount of CD24 positive cases according the malignancy degree, also features of antigen expression do not show any type of correlation between malignancy type and CD24 positive cases or CD 24 expression features. CD24 expression features according the tumor molecular subtype are shown on diagram N4, which clearly shows that in all tumor subtypes CD24 negative cases are 2,8 times more than positive. Positive cases are most often in Luminal A type, which are 5,1; 2,8 and 4 times more than Luminal B and HER2 + cases. Especially interesting are HER2+ cases which in 87% are CD 24 positive.

Besides CD24 and CD44 expression study, we performed Quantitative Assessment of CD24 and CD44 co-expression. For research we Separated 4 Group: CD24+/ CD44+; CD24+/ CD44-; CD24-/ CD44+; CD24-/ CD44-.CD24/ CD44 co-expression results are shown on table 3.

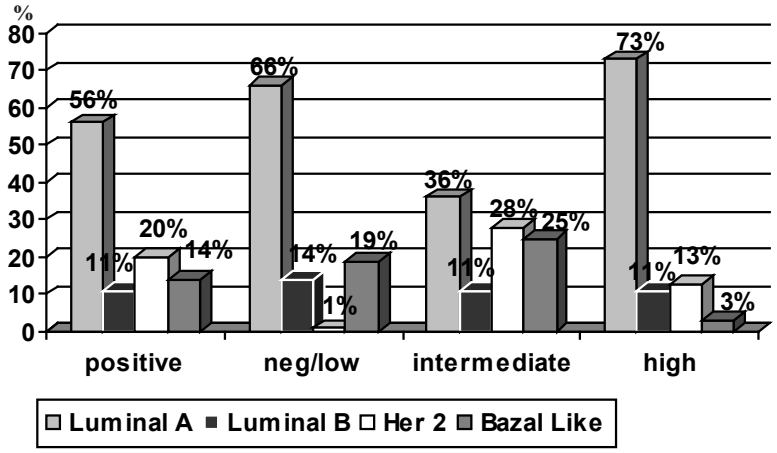

Diagram 4. CD24 expression features according tumor molecular subtype

Table 3. CD24/CD44 co-expression result according to tumor grade and molecular subtype

	total	CD44 +/ CD24+	CD44 +/ CD24-	CD44 -/ CD24+	CD44 -/ CD24-	P Value
Histological grade	**393**	**60**	**115**	**42**	**176**	**< 0.001**
Grade I	92	13	13	17	49	
Grade II	216	21	65	22	108	
Grade III	85	26	37	3	19	
Molecular subtypes	**393**	**60**	**115**	**42**	**176**	**< 0.001**
Luminal A	248	30	29	27	162	
Luminal B	52	8	38	3	3	
Her2	23	10	3	10		
Bazal Like	70	12	45	2	11	

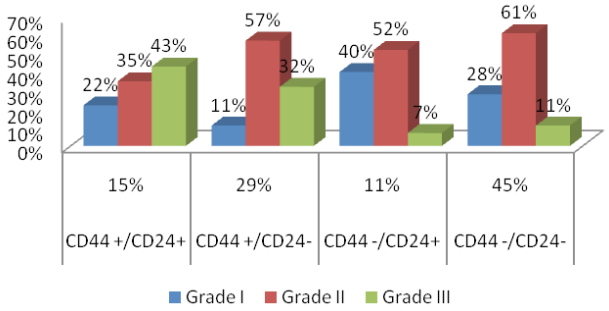

Diagram 5. CD24/CD44 co-expression features according to tumor grade

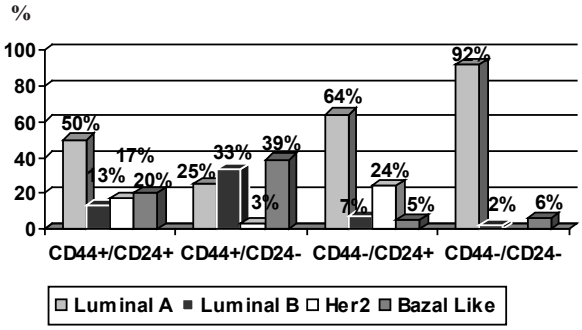

Diagram 6. CD24/CD44 co-expression according the molecular subtype

Percentage of CD24/CD44 co-expression features according to tumor grade are shown on diagram 5.

As shown on diagram: 45% (the most amount) are CD44-/ CD24- cases , CD44+/ CD24- cases are 1,5 times less than CD44-/ CD24- cases. CD44+/ CD24+ cases are 3 times less and CD44-/ CD24+ cases 4 times less. According the data of some group of authors, for phenotype of stem cells are considered CD44+/ CD24- group. According to percentage distribution of grade above mentioned groups. Increased 5 times in G1-G2 tumors , though in G3 group its 1,8 times less than G2, but almost 5 times more than G3 tumor group. Results of CD24/CD44 co-expression according the molecular subtype are shown on diagram 6.

Diagram 6 clearly shows that CD44+/ CD24- phenotype in Luminal A subtype is 25%, in Luminal B molecular subtype tumors with same phenotype are 33 %, in HER 2 positive group - 3%, and in basal like group 39 %. as result, as higher is the malignancy degree, chemoresistance and bad prognosis of molecular subtype, as more is CD44+/ CD24- tumor phenotype present, which means presence of stem cells, that can be reason of above mentioned chemoresistance and bad prognosis. Exceptions are HER2 positive tumors, because they have different base of carcinogenesis.

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SUMMARY

DISTRIBUTION OF CD44/CD24 POSITIVE CELLS IN DUCTAL INVASIVE CARCINOMA OF BREAST OF DIFFERENT GRADE AND MOLECULAR SUBTYPE

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The purpose of our study was to learn the distribution characteristics of cancer stem cell markers (CD24, CD44) in invasive carcinomas with different grade and molecular subtype. For research was used 1324 postoperative breast cancer samples, from which were selected 393 patient with invasive ductal carcinoma samples examined 2008-2012 in Laboratory of "Pathgeo Union of Pathologist" is and N.Kipshidze Central University Hospital. The age range is between 23-73 year. For all cases were performed immunohistochemical study using ER, PR, Her2, Ki67, CK5- molecular markers (Leica Microsystems). For identify cancer stem cells mononuclear antibodies CD24 (BIOCARE MEDICAL, CD44 - Clone 156-3C11; CD24 - Clone SN3b) were used. Association of CD44/CD24 expression in different subtypes of cells, between clinicopathological parameters and different biological characteristics were performed by Pearson correlation and usind X^2 tests. Obtained quantitative statistical analyses were performed by using SPSS V.19.0 program. Statistically significant were considered 95% of confidence interval.

The data shows, that towards G1-G3, amount of CD44 positive cases increased twice. CD44 positive cases are evenly distributed between Luminal A, Luminal B, HER2+, triple negative basal like cell subtypes and in significantly less (4,8 times) in Her2+ cases. Maximum amount of CD44 negative cases is shown in Luminal A subtype, which could be possible cause of better prognosis and high sensitivity for chemotherapy. For one's part such aggressive subtypes of breast cancer as Luminal B and basal like cell type, are characterized by CD44 positive and antigen high expression, which can be reason of aggressive nature of this types and also reason of chemotherapy resistance. As well as amount of CD24 positive cases according to malignancy degree, also antigen expression features does not show any type of correlation between malignancy degree and CD24 positivity or with CD24 expression features, or presence of stem cells. That can be the reason of tumor aggressivity and chemoresistance. exceptions are Her2 positive tumors because they have different base of carcinogenesis.

Keywords: breast cancer, cancer stem cells.

РЕЗЮМЕ

ОСОБЕННОСТИ ЭКСПРЕССИИ МАРКЕРОВ CD24/CD44 В ПРОТОКОВЫХ ИНВАЗИВНЫХ КАРЦИНОМАХ МОЛОЧНОЙ ЖЕЛЕЗЫ С РАЗЛИЧНОЙ СТЕПЕНЬЮ И ФЕНОТИПОМ ЗЛОКАЧЕСТВЕННОСТИ

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Стволовые раковые клетки, которые, по всей вероятности, ответственны за развитие опухоли, резистентности к формированию и лечению, являются предметом широкого и разностороннего исследования при раке молочной железы.

Цель исследования - выявление и оценка особенностей распределения маркеров (CD24, CD44) стволовых опухолевых клеток в различных молекулярных подтипах инвазивных протоковых карцином разной степени злокачественности.

Исследован материал 1324 постоперационных молочных желез. Исследование проведено в лаборатории Центральной университетской клиники имени акад. Н. Кипшидзе и в Объединении «Pathgeo» за 2008-2012 гг. Отобрано 393 случая протоковой инвазивной карциномы, возрастной диапазон - 23-73 лет. Во всех случаях осуществлено иммуногистохимическое исследование с использованием ER, PR, Her2, Ki67, CK5-молекулярных маркеров (Leica Microsystems). С

целью выявления стволовых клеток использованы моноклонные антитела CD44 и CD24 (BIOCARE MEDICAL, CD44 - Clone 156-3C11; CD24 - Clone SN3b). Корреляция экспрессии маркеров CD44/CD24 в различных молекулярных подтипах с клиникопатологическими параметрами и между различными биологическими характеристиками проводилась с использованием коэффициента Пирсона и теста X^2. Статистический анализ проведен с применением программы SPSS V.19.0. при 95% интервале достоверности.

Анализ результатов исследования выявил, что в направлении G1-G3 количество позитивных случаев CD44 увеличивается, в среднем, в 2 раза. CD44 позитивные случаи равномерно распределены между Luminal A, Luminal B, трипл негативными подтипами рака молочной железы, однако, гораздо в меньшем количестве (в 4,8 раз) в случаях HER2+. Максимальное количество CD44 негативных случаев отмечается при подтипе Luminal A, что, возможно является причиной благоприятного течения и высокой чувствительности к химиотерапии данного типа опухоли. С другой стороны, такие агрессивные подтипы рака молочной железы как Luminal B и базальные подтипы опухоли, характеризуются позитивностью CD44 и высокой экспрессией антигена, что, по всей вероятности, является одной из причин агрессивного их поведения и химиорезистентности. Между показателями количества CD24 позитивных случаев по степени злокачественности и особенности экспрессии антигена какой-либо корреляции не выявлено. Чем более агрессивным течением и химиорезистентностью характеризуется молекулярный подтип опухоли, тем выше наличие в нем опухолей фенотипа $CD44^+/CD24^-$, т.е. стволовых клеток. Исключение составляют HER2+ опухоли, так как опухоли данной группы имеют другие причины канцерогенеза.

რეზიუმე

CD24/CD44 მარკერების ექსპრესიის თავისებურება ავთვისებიანობის სხვადასხვა ხარისხისა და ფენოტიპის ძუძუს ინვაზიურ კარცინომებში

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კიბოს ღეროვანი უჯრედი,რომელიც სავარაუდოა, რომ პასუხისმგებელია სიმსივნის განვითარებაზე, ფორმირებაზე და მკურნალობის მიმართ რეზისტენტობაზე,ფართო და მრავალმხრივი შესწავლის საგანია ძუძუს კიბოს შემთხვევებში. კვლევის მიზანს წარმოადგენდა სიმსივნის ღეროვანი უჯრედების მარკერების (CD24, CD44) განაწილების თავისებურებათა შეფასება სხვადასხვა ხარისხის ავთვისებიანობისა და მოლეკულური ქვეტიპის ინვაზიურ დუქტურ კარცინომებში. საკვლევად გამოყენებულ იქნა აკადემიკოს ნ. ყიფშიძის სახ. ცენტრალური საუნივერსიტეტო კლინიკისა და შპს „პათჯეო-პათოლოგანატომთა გაერთიანების" ლაბორატორიაში 2008-2012 წლებში გამოკვლეული 1324 პოსტოპერაციული ძუძუს მასალა, რომლიდანაც შერჩეულ იქნა 393 პაციენტის დუქტური ინვაზიური კარცინომის შემთხვევა. ასაკობრივი დიაპაზონი - 23-73 წელი. ყველა შემთხვევაში განხორციელდა იმუნოჰისტოქიმიური გამოკვლევა ER, PR, Her2, Ki67, CK5-მოლეკულური მარკერების გამოყენებით (Leica Microsystems). ღეროვანი უჯრედების იდენტიფიკაციის მიზნით გამოყენებულ იქნა მონოკლონური ანტისხეულები CD44 და CD24 (BIOCARE MEDICAL, CD44 – Clone 156-3C11; CD24 - Clone SN3b). CD44/CD24 მარკერების ექსპრესიის ასოციაცია განსხვავებულ მოლეკულურ ქვეტიპებში, კლინიკოპათოლოგიურ პარამეტრებსა და სხვადასხვა ბიოლოგიურ მახასიათებელს შორის განხორციელდა პირსონის კორელაციით და X^2 ტესტის გამოყენებით. მიღებული რაოდენობრივი მონაცემების სტატისტიკური ანალიზი ჩატარდა SPSS V.19.0 პროგრამის გამოყენებით. სტატისტიკურად სარწმუნოდ მიჩნეულ იქნა 95%-იანი სარწმუნოობის ინტერვალი.

G1-G3 მიმართულებით მიღებული მონაცემების მიხედვით CD44 პოზიტიური შემთხვევების რაოდენობა იზრდება საშუალოდ 2-ჯერ. CD44 პოზიტიური შემთხვევები თანაბრად არის განაწილებული Luminal A, Luminal B, ტრიპლ ნეგატიური ქვეტიპებს შორის და მკვეთრად (4,8-ჯერ) ნაკლებია HER2+შემთხვევებში. რაოდენობრივად CD44 ნეგატიური შემთხვევების მაქსიმალური რაოდენობა აღინიშნება Luminal A ქვეტიპის შემთხვევაში,რაც, სავარაუდოა, რომ წარმოადგენს ამ უკანასკნელის კეთილსაიმედო მიმდინარეობისა და ქიმიოთერაპიისადმი მაღალი მგრძნობელობის შესაძლო მიზეზს. თავის მხრივ,ძუძუს კიბოს ისეთი აგრესიული ქვეტიპები, როგორიცაა Luminal B და ბაზალურის მსგავსი ქვეტიპები, ხასიათდება CD44 პოზიტიურობით და ანტიგენის მაღალი ექსპრესიით,რაც, შესაძლოა,წარმოადგენდეს მათი აგრესიული ქცევისა და ქიმიორეზისტენტობის ერთ-ერთ საფუძველს. როგორც CD24 პოზიტიური შემთხვევების რაოდენობა ავთვისებიანობის ხარისხის მიხედვით, ასევე ანტიგენის ექსპრესიის თავისებურებები არ ავლენს კორელაციას ავთვისებიანობის ხარისხსა და CD24 პოზიტიურ შემთხვევებს ან CD24-ის ექსპრესიის თავისებურებებს შორის. რაც უფრო აგრესიული მიმდინარეობით, ქიმიორეზისტენტობით და არაკეთილსაიმედო პროგნოზით ხასიათდება

მოლეკულური ქვეტიპი, მით უფრო მაღალია მასში CD44+/ CD24-/დაბალი ფენოტიპის სიმსივნეების არსებობა, ანუ ღეროვან უჯრედოვანი სიმსივნეების არსებობა. გამონაკლისს წარმოადგენს HER2+ სიმსივნეები, რომელთაც კანცეროგენეზის სხვა მიზეზი გააჩნია.

THE NOVEL HYPOTHESIS OF CARCINOGENESIS AND ANTI-CANCER TREATMENT PERSPECTIVES – HYDROXYETHYLTHIAMINE DIPHOSPHATE

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According to the World Health Organization the cancer is a leading cause of death worldwide. It accounted for 7.4 million deaths (around 13% of all deaths) in 2004 (statistics published in 2009). Deaths from cancer worldwide are projected to continue rising, with an estimated 12 million deaths in 2030. Carcinogenesis or tumorogenesis is literally the creation of cancer. It is a process by which normal cells are transformed into cancer cells. Cancer is fundamentally a disease of regulation of tissue growth. In order for a normal cell to transform into a cancer cell, genes that regulate cell growth and differentiation must be altered [6]. Genetic changes can occur at many levels, from gain or loss of entire chromosomes to a mutation affecting a single DNA nucleotide. At present, a number of theories of carcinogenesis and cancer treatment have been suggested. E.g. Boveri's somatic mutation theory of cancer. He believed that cancer was caused by abnormal chromosomes. It was now known that many things lead to the development of cancer, but genetic instability is at the root of cancer [4]; Chance mutation theory of Labb; theory of early chromosomal instability of Lengauer and Vogelstein [10]; theory of aneuploidy of Duesberg [8]; Cohnheim's embryonal theory [5]; the chronic irritation theory of Virchow; theory of chemical carcinogenesis of Yamagiwa and Ichikawa; Virus theory of Borrel and Rous; Mitochondrial theory of O. Warburg; theory of Connective-Tissue cells of Vasiliev [2], etc. Summarizing, at least five coherent models of carcinogenesis have been proposed in the history of cancer research in the last century. Model 1 is mainly centered on mutations induced by chemical environment, radiation and viruses. Model 2 - genome instability focuses on familiality. Model 3 is based on non-genotoxic mechanisms, and clonal expansion and epigenetics are its main features. Model 4 which can encompass the previous three, based on the concept of a "Darwinian" cell selection. Finally, a Model 5 is based on the concept of "tissue organization" [9,12,16].

According to the majority of theories, nuclear structures have been considered as the basic targets for majority of carcinogens. For instance, effects of carcinogens such as cyclic hydrocarbon benzpyrene, nitrozoamines, certain alkylating and acylating agents has been explained by their influence on cell DNA eventually leading to disorders of gene structure and induction of malignant growth. Mechanism of cancer growth developed after exposure to ionizing radiation has been explained by radiolysis of water and produced H^+ and OH^- toxic free radicals effecting on cell DNA, etc. We are not going to discuss in details all theories of carcinogenesis existing at present. We offer our hypothesis of carcinogenesis developed after exposure to chemical carcinogens, in particular, cyclic hydrocarbons - benzene, and physical – ionizing radiation.

Chemical carcinogenesis - Let's discuss chemical carcinogenesis on a benzene example. Benzene is an organic chemical compound with the molecular formula C_6H_6. Its molecule is composed of 6 carbon atoms joined in a ring with 1 hydrogen atom attached to each carbon atom. Each carbon atom is bound with hydrogen and other carbon atoms owing to 3 σ-bonds (Fig. 1).

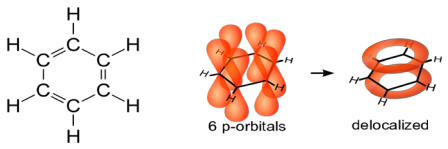


Fig. 1. Benzene structure and its electron cloud

Because benzene molecules contain only carbon and hydrogen atoms, it is classed as a hydrocarbon (cyclic hydrocarbon), aromatic hydrocarbon [3,11]. In carbon atoms sp^2-hybridization of electron cloud occurs. Remaining 4th

electron cloud of each carbon atom does not undergo hybridization and electrons remain unpaired. All of these 4th unpaired electrons of each 6 carbon atom form junctions – π-bonds that do not fix in Benzene molecule. This bond is six-centered (not two-centered). Such type of junction is called as delocalized. Analogous delocalized π-bonds have been detected in other cyclic hydrocarbons also, e.g. benzpyrene - carcinogenic hydrocarbon, etc. [3,11]. Supposedly, at the expense of these multi-centered π-bonds composed of unpaired electrons of carbon atoms the attachment of H^+ (protons) is possible via formation of π-complex, as it happens with cations in reaction of electrophylic substitution (S_E). I.e., π-electrons of benzene attract electrophylic particle forming π-complex. This process proceeds slowly. Thereafter, the second stage involves formation of σ-complex, where electrophyl takes 2 electrons from benzene ring and forms σ-bond with carbon atom. This second stage is related with proton removal and its return in solution to anion that has been remained after removal of initial electrophylic particle from the latter.

Benzene molecule can penetrate in any organelle of cell, but according to our hypothesis, malignant transformation of cells will start if benzene molecule penetrate into mitochondrion (not in nucleus). It is known that during biological oxidation (oxidative phosphorylation) on inner membrane of mitochondrion, i.e. at electron transport from substrates to O_2, the first of dehydrogenases that enters in this process is nicotinamide adenine dinucleotide (NAD^+). Moreover, it accepts H molecule from substrate in such a way that one H atom completely joins with NAD^+, and from the second H atom, only electron joins. The free H^+ ion remains in solution (medium). Inner membrane of mitochondrion usually is not permeable for certain molecules, but taking into consideration the fact that benzene belongs to hydrocarbons, most of them having lipophylic features respectively, they can penetrate and enter into inner membrane. So, at benzene penetration in mitochondrion, remaining free H^+ ion can be easily attracted by benzene electron cloud leading to formation of bond similar to those of developed at reaction of electrophylic substitution, however without formation of σ-complex and proton detachment from aromatic ring, as far as in oxidative phosphorylation anion doesn't follow proton with which H^+ ion of benzene molecule could combine. At such condition, carcinogenesis or malignant transformation of normal cells after exposure to the above-mentioned hydrocarbons could be explained as follows. Benzene invading the cell, if enters in mitochondrion, it will attract H^+ protons from solution with delocalized electron cloud. These H^+ protons from dehydrogenases, precisely from their coenzymes e.g. $NADH_2$, $FADH_2$ and ubichynon – coenzyme QH_2, via passing through solution should bind with O_2^- producing H_2O during biological oxidation [1]. But now, in our opinion, at such abnormal condition, when certain amount of protons has been bound with electron cloud of carcinogen as nucleaphylic substrate, in mitochondrion the remaining H^+ ions will not be enough for production of H_2O, and as a result, instead of reaction: $2H^+ + O^{2-} \rightarrow H_2O$ the following occurs: $H^+ + O^{2-} \rightarrow OH^-$, eventually leading to accumulation of OH^- radicals in mitochondrion. Supposedly, for neutralization of OH^- radicals the cell buffer system activates. E.g. OH^- radicals combine with H_2CO_3.

$$OH^- + H_2CO_3 = H_2O + HCO_3^-$$

Thereafter, Cl^- replaces HCO_3^- like that, as it occurs in erythrocytes at gas exchange process, and as a result, Cl^- ions accumulate in cells.

Before we continue explanation, it would be reasonable to discuss how the junction between sulfur of methionine and Fe^{3+} atom in cytochrome develops in norm.

Ferrum and sulfur atoms are bound covalently. Methionine's sulfur atom with 2 unpaired electrons on the 3-d orbital binds with 2 carbon atoms. The sulfuric atom S on outer shell has 2 more pairs of electrons. The 1 pair remains with sulfuric atom (lower pair on sulfuric atom) (Fig. 2), and from the second pair of electrons, one electron goes to certain anion (supposedly to chlorine producing ion Cl^-). The sulfuric atom S converts into ion S^+ with one more unpaired electron.

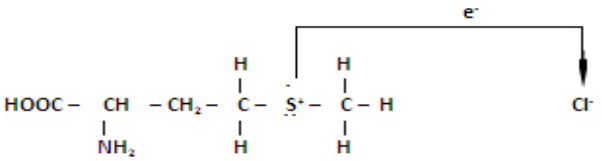


Fig. 2. Formation of S^+-methionine

As for ferrum - the electrons of Fe^{3+} on the orbitals allocate in the following order (Fig. 3).

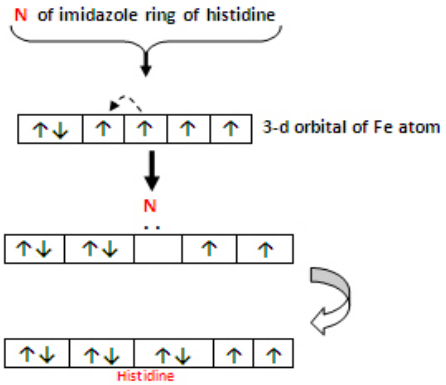


Fig. 3. Allocation of electrons on 3-d orbitals of ferrum atom in heme and binding of heme Ferrum with Nitrogen of imidazole ring of histidine

So, the last unpaired electron of sulfur forms the one covalent bond with the one unpaired electron of Fe^{3+} (Fig. 4,5).

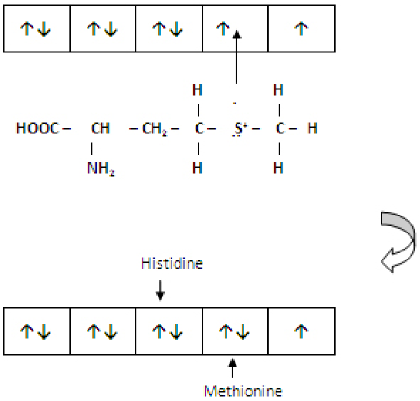


Fig. 4. Formation of bond between ferrum and S^+-methionine

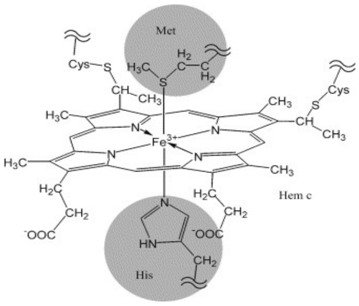


Fig. 5. Heme structure in cytochrome

The second unpaired electron of Fe^{3+} atom remains without pair and participates in electron transport from $NADH_2$ to oxygen atom ½O_2 forming ion O^{2-}. Cl^- supposedly binds with H^+ (existing in solution) producing HCl. Now, let's come back to the solution, where Cl^- ions has been accumulated. In our opinion, increase in Cl^- ion concentration in mitochondrion will support penetration of few of them in protein pocket of cytochrome and re-binding one of them with S^+-methionine (as it was mentioned above, sulfur gave one of its electrons to chlorine atom producing Cl^-) leading to formation of the following complex (Fig. 6).

Formation of ionic bond between S^+-methionine and Cl^-.

$$\left[\begin{array}{l} HOOC - \underset{\displaystyle NH_2}{\underset{|}{CH}} - CH_2 - CH_2 - S^+ - CH_3 \end{array} \right] Cl^-$$

Fig. 6. Complex of S^+-methionine with Cl^-

Therefore, covalent bond between S^+-methionine and Fe^{3+} will brake and the new – ionic junction between S^+-methionine and Cl^- will develop. Fe^{3+} atom will remain with 2 unpaired electrons (Fig. 7).

Fig. 7. Unpaired electrons on 3-d orbitals of ferrum

Should be mentioned that in inner parts of the protein toward the heme or prosthetic group of cytochrome, only hydrophobic radicals of amino acids of protein are directed, while their hydrophilic groups - OH^-, NH_2 are directed outside [1]. So, as far as in closed protein pocket of cytochrome neither H_2O nor OH^- radicals are able to penetrate, we think that on the last two 3-d orbitals of Fe^{3+}, i.e. in two last cells, one unpaired electrons in each cell will remain. At such condition, owing to these 2 unpaired electrons in respiratory chain an intense electron flow will start, and cell will be adapted for increased consumption of glucose.

Due to exaggerated glucose consumption, the electron flow through cytochrome system will increase twice, concentration gradient of H^+ ions on inner membrane of mitochondrion will increase, ATP synthesis will increase also leading to accumulation of the latter in cells, which in turn will support intense phosphorilation of amino acids and protein synthesis. As a result, it may lead to exaggerated cell division, or high mitotic rate, hyperplasia of tissues will occur and process will persist until OH^- radical enters the protein pocket of the cytochrome.

Naturally raises question. How the OH^- radical can penetrate the protein pocket of the cytochrome? We suggest the following explanation. If carcinogen persists on cell, little by little concentration of OH^- radicals will increase extremely in mitochondria, because buffer system now is unable to bind OH^- radicals, that's why normal physiologic pH in mitochondria will shift toward alkalinity. So, mitochondrial pH will be alkaline. Supposedly, due to pH alterations in mitochondria, or at presence of increased alkalinity, opening of the "pocket" of the protein of cytochrome occurs in a similar way that takes place in hemoglobin at oxygen transport. It is well known that in the molecules of hemoglobin and myoglobine the side groups of distal remnants of the histidine and closely adjacent arginine form the certain kind of "door". Opening of this "door" lets oxygen to enter in heme. Opening and closure of this "door" that occur in norm in lungs and tissues on periphery, depends on certain factors, such as: pH level of medium, O_2 and CO_2 partial pressure in lungs and tissues, concentration of 2,3 diphosphoglicerate etc. (Bohr effect). In lungs, increase in pH level affects the globin "pocket", opens it and facilitates oxygen transport to get to the heme. Only just after oxygenation, the medium becomes acidic. In tissues, on periphery, the opposite reaction takes place – the medium is more acidic with high concentration of CO_2, which in turn supports release of oxygen from "pocket" and entry of H_2O molecules in it [1]. Thus, coming back

to the cytochrome, supposedly, the protein "pocket" of the cytochrome will open due to high alkalinity of the medium, and OH^- radicals will easily penetrate within the "pocket". Just after penetration, the OH^- radicals will form the coordination bond (not covalent) with Fe^{3+} atom. Thereby, OH^- radical will result in forced pairing of the two last electrons on the 3-d orbitals of Fe^{3+} atom as a stronger ligand than thio-ligand, and will convert the Fe^{3+} atom into Fe^{2+} atom.

In chemistry and biochemistry, a ligand (from the Latin ligandum, binding) is a substance (usually ions, or a small molecules), directly bound with complexing agent via nonionogenic bonds. At one and the same central ion and complexes with same configurations, the disconnection of d-orbital energy level is as more as stronger the field created by ligands [3,11]. Therefore, ligands according to their power are disposed in the following order:
1) strong – $CO \approx NO \approx CN^- >$
2) average – En (ethilendiamine) > $NH_3 > H^- \approx H_2O > OH^- > F^- >$
3) weak – $Cl^- \approx SCN^- > Br^- > J^-$.

The stronger is ligand the forced pairing of electrons occurs simultaneously with splitting of energetic levels of d-orbitals (deviation from the Hund rule). It has been considered that OH^- radical is stronger that thio-ligand. That's why OH^- radical can form the coordination bond with ferrum atom via forced pairing of electrons, while thio-ligand forms the covalent bond with ferrum atom without forced pairing of electrons of the latter (Fig. 8).

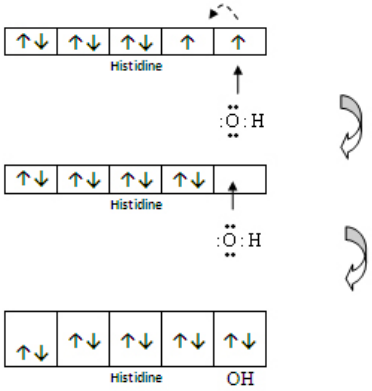


Fig. 8. Formation of coordination bond between ferrum atom and OH^- radical

Thus, due to formation of the coordination bond between OH^- radical and Fe^{2+}, the electron transport through such type of cytochrome will be altered leading to disorders of mitochondrial respiratory chain. The same time, new substrates – H_2 donors, particularly glucose molecules, fatty acids will be delivered again in cells causing NAD^+ and FAD reduction up to $NADH_2$ and $FADH_2$. $NADH_2$ and $FADH_2$ will accumulate and in case of excessive delivery of glucose, despite of free access of O_2 within cells, glucose will be hydrolyzed only up to pyruvic acid because, at cessation of tissue respiration and continues delivery of substrates - H_2 donors, the cell activates binding of excess molecules of the hydrogen (H_2) expending great amount of NAD^+ and FAD. As a result increases concentration of $NADH_2$ and $FADH_2$ in cells. Exaggerated consumption and resultant depletion of all resources of NAD^+ and FAD, also accumulation of great amount of $NADH_2$ and $FADH_2$ in cells will lead to shifting of the reaction towards formation of lactic acid:

lactic acid + $NAD^+ \leftrightarrow$ pyruvic acid + $NADH_2$
CH_3–CHOH–COOH + $NAD^+ \leftrightarrow$ CH_3–CO–COOH + $NADH_2$

We suppose that pyruvic acid will be used for discharging of cells and removal of excess amount of $NADH_2$ and $FADH_2$ via oxidation of them up to NAD^+ and FAD. However, must be mentioned that accumulation of pyruvic acid and cessation of its involvement and participation in aerobic glycolysis, supposedly occurs not only due to the aforesaid reason. It is known that in normal cells thiamine diphosphate (TDP - thiamine diphosphate, or thiamine pyrophosphate), and lipoic acid amide which is coenzyme of enzyme E_2-Lipoat-acetyltransferase, are the major components in oxidative decarboxylation of pyruvate. i.e. carbon (C) of pyruvic acid that has been combined with E_1-TDP, initially binds with S-amide lipoic acid producing acetyl-lipoat (Fig. 9).

Only just after this reaction it combines with HS-CoA, producing acetyl-CoA, i.e. CH_3-CO-S-CoA [1]. But as far as in alkaline medium TDP (thiamine pyrophosphate) activity decreases, according to our hypothesis, the first reaction - binding of pyruvate with E_1-TDP drops out of oxidative decarboxylation of pyruvic acid, and the Krebs cycle stops along with cessation of fatty acids' breakdown, and cell starts using of anaerobic glycolysis. Thus, for the mentioned reason aerobic glycolysis ceases and pyruvic acid accumulates in cells, which is followed by accumulation of lactic acid (due to excess of reduced form of dehydrogenase – $NADH_2$).

In contrast, lipoic acid activity does not change. At least, carbon skeleton of dithiol ring of amide lipoic acid is more resistant toward pH alterations, than TDP, which in neutral and alkaline media has unlocked thiazole ring. Optimal pH for lipoic acid is 7-7.5, and for thiamine – 6.5. Therefore, in our opinion, lipoic acid amide could be used by the cell in reaction of S-adenosyl methionine formation.

Raises question. How does it occur? We think that at the moment when the bond between S^+-methionine and Fe^{3+} brakes down, ATP can bind with S^+-methionine forming S^+-adenosyl methionine, or SAM (Fig. 10).

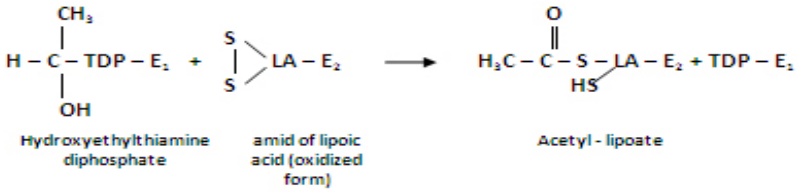


Fig. 9. The second stage of oxidative decarboxilation of pyruvic acid. Formation of C-S bond between Hydroxyethylthiamine diphosphate and amid of lipoic acid

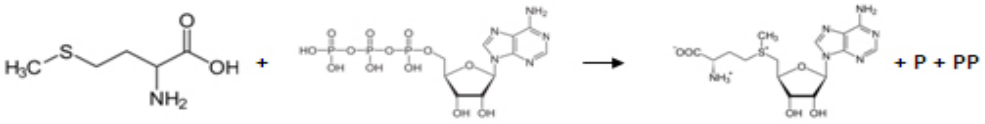
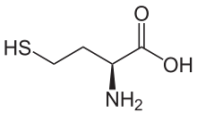


Fig. 10. Formation of S-adenosyl methionine. Formation of C-S bond between adenosine and S^+-methionine

SAM synthesis generally occurs in hepatocytes at the presence of enzyme adenosyl-methionine-transferase [1]. If SAM synthesis mainly occurs in hepatocytes, it is interesting, how could be produced S-adenosyl methionine in all other cells? Supposedly, enzyme lipoate E_2-acetyl-transferase with its Co-enzyme (lypoic acid amide) can successfully catalyze this reaction instead of adenosyl-methionine-transferase. It is well known that enzyme adenosyl-methionine-transferase (synthetase) belongs to class of enzymes E.C.2 (transferase) and denoted as E.C.2.5.1.6. The Lipoat-acethyltransferase also belongs to class of enzymes E.C.2 (transferase) and denoted as E.C.2.3.1.12. Thus, both of them belong to one and the same class of transferase, and probably, lead to formation of bonds between carbon and sulfur atoms (C-S). On the other hand, enzymes forming bonds between carbon, nitrogen and sulfur atoms, or C-C, C-N, C-S etc., belong to class of synthetases or ligases and are denoted as E.C.6. Those enzymes, i.e. ligases, that form a bond between carbon and sulfur atoms (C-S), are denoted as E.C.6.2.

Usually, formation of C-S bonds is catalysed by enzymes that are involved in the synthesis of acyl derivatives of coenzyme A (CoA, i.e. acyl-CoA, or acetyl-CoA). In any case, do they (adenosyl-methionine-transferase and Lipoat-acethyltransferase) belong or not to class of transferases or synthetases, it is not excluded that in the reaction of S-adenosyl methionine formation from ATP and S^+-methionine, enzyme adenosyl-methionine-transferase (synthetase) could be replaced with enzyme Lipoat-acethyltransferase in such cell, where it is not involved in oxidative decarboxilation of pyruvic acid. The more so that Lipoat-acethyltransferase does not belong to specific enzymes [1].

Thus, for formation of S-adenosyl methionine (combination of ATP with methionine), carbon of adenosyl initially must bind with sulfur of lipoic acid amide (as ligase or synthetase) like that it occurs in the reaction of oxidative decarboxylation of pyruvate. The reaction is catalyzed by the enzyme lipoate E_2-acetyl-transferase. Only after this, the adenosyl-lipoate binds with methionine forming adenosylmethionine and lipoic acid amide. Further, produced S-adenosyl methionine will enter in cytoplasm and will lead to methylation of nitrogen bases – adenosine and uracyl with further formation of other nucleotides -thimidin, minor nitrogen bases. Thereafter, SAM gives methyl group to substrate producing S-adenosyl homocystein, which in turn brakes down on adenosine and homocystein (Fig. 11).



S-adenosyl-methionin → S-adenosyl-homocystein → adenosine + homocystein
Fig. 11. Homocystein

In our opinion, Homocysteine, accumulated in cell cytoplasm, and further penetrating in nucleus, will start affecting on DNA nucleotides. It will lead to demethylation of adenosyl or other methylated nucleotides of normal DNA. Such demethylated nucleotides, now easily drop out of DNA strands producing mutation, and further - cell atypism. Thus, DNA structure changes and from such altered cells after mitosis the atypical cells are produced, and simultaneously, at the presence of disorders in mitochondria (described above), induces malignant growth. As for Fe^{2+} produced during the above discussed processes, owing to citrates will be washed out from cytochromes and deposited in ferritin, which concentration, as is well known, dramatically increases in cancer cells. Thus, cell with altered DNA starts division producing two atypical daughter cells with mutations in DNA, which in turn will synthesize proteins with abnormal structures.

According to our hypothesis, level of malignant cell differentiation depends on concentration, or duration of exposure of carcinogens on cell. If the effect of carcinogen is not strong, and buffer system is able to manage situation, the mitochondrial pH of daughter cells changes slightly,

oxidized decarboxilation of pyruvate in cells and tissues alters, but does not stop, Fe^{3+} enters in cytochromes completely. As far as in newly generated daughter cells protein synthesis is slightly altered (due to DNA mutation in parent cell), these cells after mitotic division will produce new generation of atypical cells that would not be distinguished markedly from normal cells. However, proliferation of these cells will go relatively faster than normal cells at prolongation of exposure of carcinogen, even with small doses, because it will lead to accumulation of Cl^- ions in mitochondria again, leading to disorders of covalent bond between S^+-methionine and ferrum of cytochrome, and as a result electron stream will increase through cytochrom system. All of these reactions will result in production of malignant cells with low-grade malignization, or high-grade differentiation (Grade 1).

Let's discuss another situation, when effect of carcinogen is prolonged and stronger (high concentration) than in previously discussed example. The pH level in cells will shift towards alkalinity more than in the first case. Perhaps, in such cells E_1-TDP would not be active and oxidative decarboxylation of pyruvate will stop. Amide of lipoic acid will combine adenosil from ATP with methionine producing SAM. The latter will dissociate giving adenosine and homocysteine. Homocysteine will lead to demethylation of DNA nucleotides of daughter cells. These cells after mitotic division will be with more abnormal DNA producing abnormal proteins respectively, i.e. cells will be more malignant, or less differentiated than in first case. It will be 2nd or 3rd level of differentiation (Grade 2 or Grade 3). And at last, the third condition, when effect of carcinogen is more prolonged and extremely strong, and as a result pH level in mitochondria is strongly alkaline. At such situation, not only oxidative decarboxylation of pyruvate will be ceased, but Fe^{3+} will not be involved in cytochromes also, as far as at very high pH level Fe^{2+} does not undergo oxidation to produce Fe^{3+}. As a result, the respiratory system definitely will stop functioning. Such type of cell will be undifferentiated. So, it will be 4th level of differentiation, or Grade 4. It is important to emphasize that the more alkaline is environment of the cells, the poor is the functioning of most of enzymes, especially oxidases (FADs). I.e., oxidative deamination of amino acids (the main type of deamination in animals, plants and in majority of aerobic microorganisms) decreases and amino acids mainly are used for protein synthesis. That's why cells are less differentiated and more malignant. In higher alkaline environment, malignant cells with less degree of differentiation grow faster than the malignant cells with a high degree of differentiation, because as it was mentioned above, in less alkaline medium, functioning of oxidases is still maintained supporting amino acids deamination, thereby interfering with the malignant growth and resisting cancer development. Although, at the beginning mechanism of carcinogenesis initiated by the different carcinogens are different, we still strongly believe that the common denominators in carcinogenesis, or the main steps in mechanism of carcinogenesis that are same and common for all types of cancers developed after influence of different types of carcinogens are:

1. Disorders of bond between ferrum and sulfur atoms (Fe^{3+} and S^+-methionine);
2. Blockage of the last 3-d orbital of ferrum by the certain ligand and formation of 6th coordinated bond between them, leading to Fe^{3+} reduction up to Fe^{2+} and cessation of tissue respiration;
3. Increase of pH in mitochondria and further in cell leading to decrease in activity of most of enzymes – E_1-TDF, oxidases etc.;
4. Formation of S-adenosyl-methionine with its further dissociation and production of adenosine and homocysteine;
5. Homocysteine effects on DNA structures, homocysteine-induced demethylation of certain nitrogen basis and their extrusion from strands of DNA leading to DNA mutations and cellular atypism.

At the beginning, as was mentioned above, carcinogenesis after influence of different carcinogens on cell might be different. For instance, ionizing radiation can initiate cancers as following. Ionizing radiation is the stream of different particles (electrons, protons, etc.). Malignization of cells, more likely occurs due to effects of electrons (β^- - dissipated by excited atoms). In our opinion, electrons of ionizing radiation supposedly knock out electrons from covalent bonds between ferrum and nitrogen atoms of pyrrole ring and let the electron stream through the cytochromes (as occurs during photosynthesis), or directly can fill in last cell of Fe^{3+} atoms. Such Fe^{3+} atoms unable to accept electrons from $NADH_2$, $FADH_2$, and $CoQH_2$. So, their oxidation will stop. Oxygen delivery continues. Oxygen atoms $½O_2$ will start accept electrons from Fe^{2+}, i.e. ferrum atoms that has been reduced owing to electrons of ionizing radiation, leading to their oxidation up to Fe^{3+}. Thus, oxygen atoms in blood will be reduced not at the expense of H_2 electrons (not in the process of $NADH_2$ oxidation), but at the expense of electrons of ionizing radiation:

$$½O_2 + 2e^- \rightarrow O^{2-}$$

Great amount of reduced $NADH_2$, $FADH_2$ and O^{2-} will accumulate in cells. O^{2-}, as a high reactive ion interacts with reduced forms of dehydrogenases – $NADH_2$, leading to formation of OH^- - radicals and H^- ions:

$$O^{2-} + NADH_2 \rightarrow OH^- + H^- + NAD^+$$

H^- ions will enter the protein pocket and as an anion, instead of Cl^- ion, will combine with S^+-methionine, leading to disjunction of S^+-methionine and Fe^{3+}. Thereafter, according to the above-described mechanism of effects of carcinogens on cells, the exaggerated (doubled) electron stream through the system of cytochrome will start leading to: tissue hyperplasia, and hereafter, S^+-methionine detachment from

protein, opening of protein pocket, entry of OH^--radicals (accumulated in cells as a result of $NADH_2$ oxidation with O^{2-}) in these pockets, formation of coordinated bonds between OH^- -radicals and ferrum atom, leading to Fe^{3+} reduction up to Fe^{2+} , and disorders of respiratory chain. Owing to these reactions the process of tissue hyperplasia will turns into the process of malignization.

It must be mentioned that H^- and OH^- radicals belong to ligands of medium forces. Both of them can function as anion or ligand. If they are active as anion, among one of them and S^+-methionine the ionic bond will form, but if they are in the form of ligands – the coordinated bond could be formed between one of them and ferrum atom. Moreover, only H^- ion supposedly will be enough for formation of both types of junctions the same time.

In our opinion, ionizing radiation especially affects such cells, which are rich with pigment, with melanin and retinal (retinal pigment epithelium - RPE) in particular. The pigment in this case, may function as light collector system (photo attractant), like chlorophyll, as it is in plants. So, melanin, simply attract the rays and all other goes as it was described above.

Rises question. What is the way out of the situation? How we could support such damaged and atypically changed cell, or tissue to regain its normal functioning? According to our hypothesis, in order to stop the process of cell malignization, it is essential to administrate in organism such solutions that could be able to shift cell pH (exactly mitochondrial) towards acidity in an attempt to increase activity of enzymes - E_1-TDP, support entry of pyruvic acid in Krebs cycle, and activate oxygenases, which in turn will provide amino acid deamination and thereby will stop protein synthesis in atypical cells. Another suggestion is binding of lipoic acid amid. We are not going to pay attention on substrates having potential of cell pH alterations as far as they would not produce the local effects. This manipulation will be practically hard to do, and would not have desired effect. Better to go back to the second suggestion and let's discuss how to produce binding of lipoic acid amid.

We consider that for this purpose it is reasonable to administrate hydroxyethylthiamine diphosphate – complex of pyruvic acid with E_1-TDP that will lead to binding of carbon atom from this complex with lipoic acid amid. When it happen, lipoic acid amid would not be able to bind with adenosyl from ATP, and the latter would not combine with methionine. Homocysteine would not be formed, which in turn would not damage the cell DNA.

If the aforesaid is correct, the process of malignization must be stopped and cell must regain its previous and normal functioning. Let's discuss in details, to what kind of biochemical transformations may lead administration of hydroxyethylthiamine diphosphate in organism with cancer.

Hydroxyethylthiamine diphosphate will enter in reaction with lipoic acid amid, oxidative decarboxylation of pyruvic acid will be restored, and acetyl-CoA will be produced. For starting of Krebs cycle, acetyl-CoA must bind with oxalacetic acid, but due to presence of great amount of reduced forms of $NADH_2$ oxalacetic acid is converted into Malic acid (malates), that's why it is not in mitochondria and Krebs cycle does not function yet. But oxalacetic acid could be produced from pyruvic acid at presence of acetyl-CoA, which have been synthesized already and present in cells. Thus, obtained acetyl-CoA initially will go not into Krebs cycle, but will be utilized for synthesis of oxalacetic acid. Only just after when both components - acetyl-CoA and oxalacetic acid present in mitochondria, the Krebs cycle will start normal functioning. In Krebs cycle, owing to the first reaction Citric acid, and thereafter, Isocitric acid will be produced. However, Isocitric acid unable to convert into α-Ketoglutaric acid, because it requires oxidized forms of NAD^+. As far as respiratory system does not function yet, only reduced form of $NADH_2$ is available in mitochondria. So, it is important synthesis of great amount of Citric and Isocitric acids in mitochondria. After the mentioned, supposedly, pH in mitochondrial will shift towards acidity, I.e., mitochondrial pH will be restored and oxygenases will start functioning. They will lead to oxidative deamination of amino acids, NH_3 will be produced which in turn, at the presence of H^+ in medium, will transform into NH_4^+ . The latter will penetrate in protein pocket of cytochrome, will attract OH^- -radical from 6^{th}–orbital of Fe^{2+} leading to formation of NH_4OH. Electrons on 3-d orbital of ferrum will be unpaired, S^+-methionine again will form covalent bond with Fe^{3+} and electron stream will flow through system of cytochromes. The respiratory chain will start functioning, $NADH_2$ and $FADH_2$ will oxidize producing NAD^+ and FAD. At the presence of oxidized forms of dehydrogenases the Isocitric acid will convert into α-Ketoglutaric acid and Krebs cycle will go ahead. As a result the respiratory chain will be restored completely.

Considering all of the aforesaid, we have conducted an experiment in order to check up and state correctness of our hypothesis. We were aimed to capture lipoic acid amide (LAA) with Hydroxiethyl-thiamine diphosphate (HTD - the product of the reaction pyruvic acid with E_1-TDP).

Material and methods. For this purpose, as far as we had no ready for use HTD, we took 98 % solution of pyruvic acid and thiamine bromide (as is known the thiamine bromide in organism converts into thiamin diphosphate at the expense of phosphorilation with ATP) [7] in hope that we could obtain Hydroxyethylthiamine diphosphate (according to our hypothesis, only HTD and not these two substances are essential for LAA capture). Animal studies were carried out on 30 C57BL/6J male mice 2-3 months old and with body weight of 18-20 g. All animals were fed standard laboratory chow and given free access to water. The care and use of the

animals complied with the Georgian regulations on protection of animals, with Guidelines prepared by the Ethics Committee of the Institutional Animal Care and with the National Institutes of Health Guide for the Care and Use of Laboratory animals. For creation of cancer model, all experimental animals were subjected to subcutaneous inoculations with Ehrlich carcinoma ($1x10^6$ tumor cells). Thereafter, they were randomly divided into 2 groups. The Group -1 was control, and the Group – 2 was experimental, where mice were subjected to intraperitoneal injections of the mixture of pyruvic acid and thiamine bromide with the following regimen: for 1 mouse we took 0.0002 ml of 98 % pyruvic acid and 1 mg Thiamine bromide. i.e. 0.1 ml of 98 % pyruvic acid were diluted with distilled water 1000 times, and 5 mg Thiamine bromide was added. Out of the obtained solution, 0,2 ml was injected in each experimental mice. Initially, during first 10 days injections were carried out every other day, and then - every day, during 2 weeks. Treatment efficacy was evaluated by calculating: i) the volume of cancer tissue (mm^3) with the use of formula $V = \pi/6$ (AxBxC), where π=3,14, (A) is the length, (B) is the width, and (C) is the height of the tumor tissue; ii) cancer growth inhibition percent – $V_{contr.}$-$V_{exp.}$/$V_{contr.}$ x100 (%); iii) average rate of cancer growth V_1-V_2/N (N – is the number of days). Morphology of cancer tissue was studied using hematoxylin-eosin staining method. Samples were collected intact and fixed in 9% neutral buffered formalin and processed for histological analysis. Serial transverse sections were processed for light microscopy. Paraffin embedded sections were cut at 5 μm, stained with hematoxylin and eosin. Indices of cancer growth were measured every 3rd day of cancer growth. Obtained data were analyzed statistically with the use of SPSS 16.0 for Windows. Differences between tumor control and treated animals were determined by using the Student's *t* test. The criterion for significance was set to $p < 0.05$.

Results and their discussion. Results of experiments are presented on Fig. 12- 14. Investigations have shown that in experimental animals cancer growth was inhibited compared to control group animals. In control (untreated) group mice cancer growth average rate was 141,2 mm^3/day, and in experimental group mice treated with HTD the cancer growth average rate was 37,5 mm^3/day on average. At the beginning of treatment, the cancer growth inhibition percent in experimental group mice treated with HTD was 36,3% on average, and later – cancer growth was inhibited by 71,2% on average. Cancer growth inhibition percent was more when injections were carried out every day.

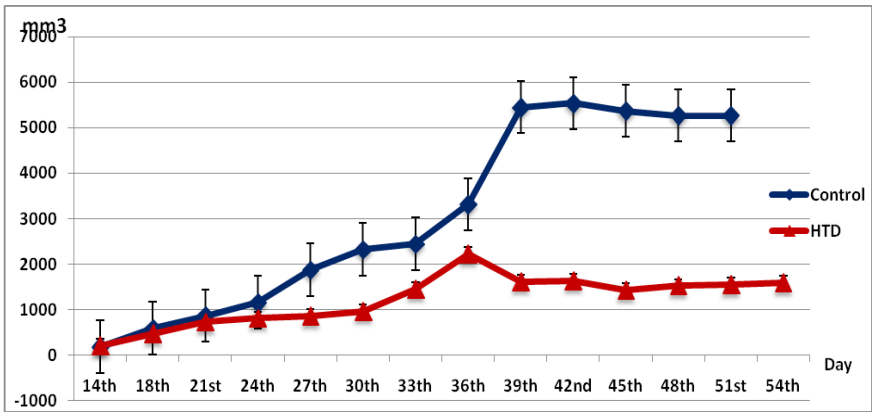


Fig. 12. Volume of cancer tissue in control (untreated) and experimental mice treated with Hydroxyethylthiamine diphosphate

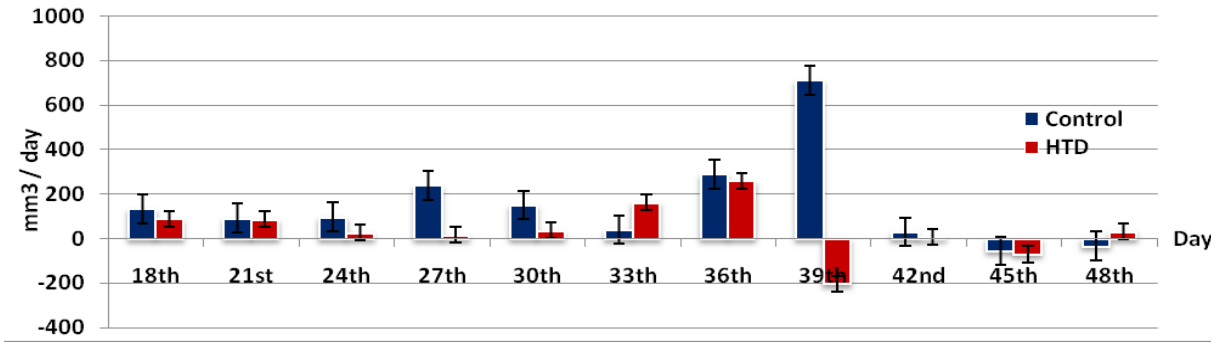


Fig. 13. Cancer growth average rate in control (untreated) and experimental mice treated with Hydroxyethylthiamine diphosphate

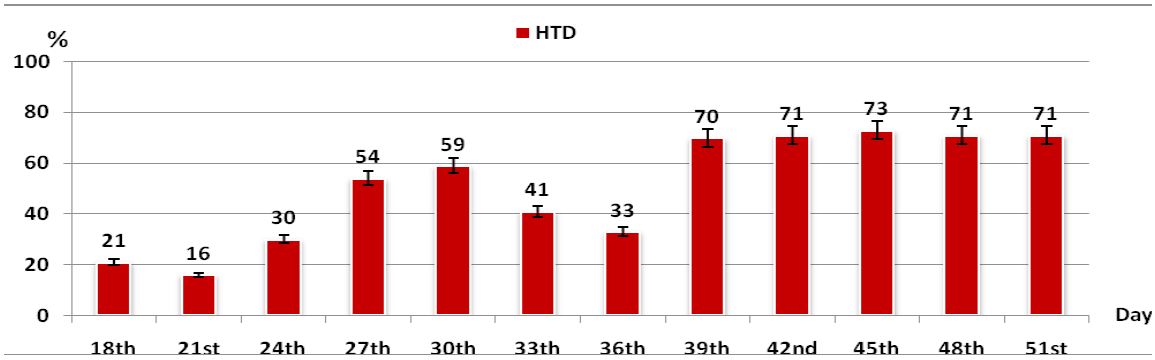
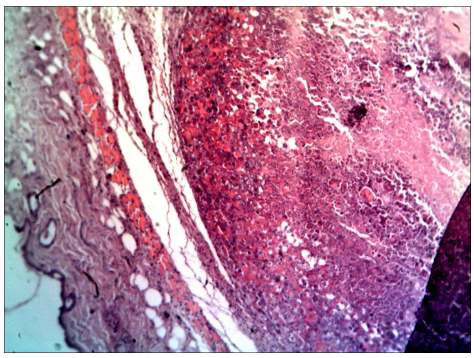
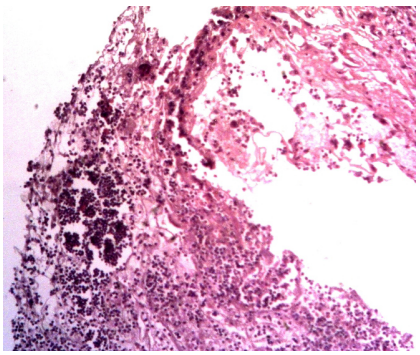


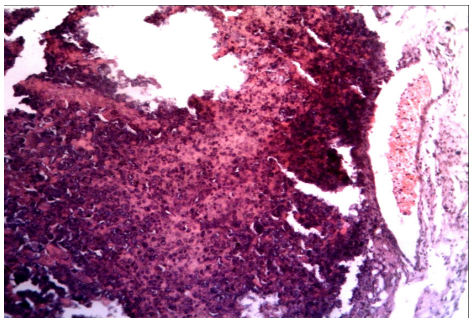
Fig. 14. Cancer growth inhibition percent in mice treated with Hydroxyethylthiamine diphosphate



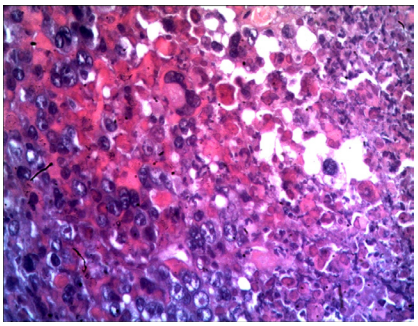
Pic. 1. Cancer tissue (magnification - x10). Fibrotic capsule, necrotic zones. Xanthome cells. Inflammatory leukocytic infiltration



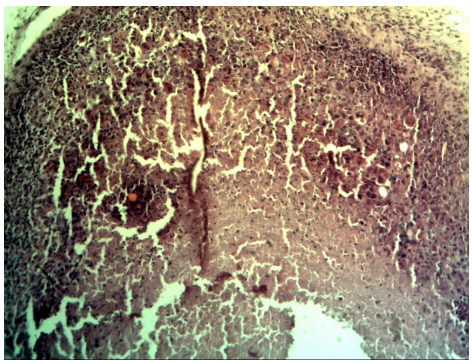
Pic. 2. Cancer tissue (magnification - x10). Lymphadenoid follicular hyperplasia



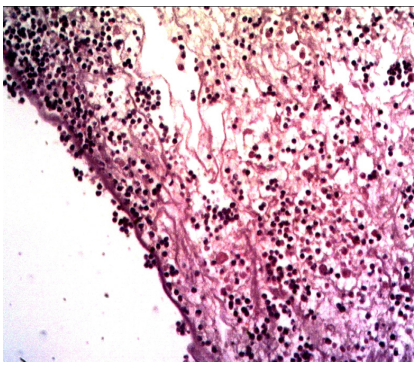
Pic. 3. Cancer tissue (magnification - x10). Dilated blood vessel, erythrocytic extravasation, perivascular inflammatory infiltrations



Pic. 4. Cancer tissue (magnification - x40). Must cells and segmento-nuclear leukocytes



Pic. 5. Cancer tissue (magnification - x10).Central necrosis with adjacent inflammatory mono- and polymorph infiltrations



Pic. 6. Cancer tissue (magnification - x40). Lymphoplasmocytic infiltration of connective tissue

Morphological investigations of cancer tissue after treatment with HTD have shown necrotic zones, inflammatory infiltrations, central necrosis with adjacent inflammatory mono- and polymorph infiltrations, must cells, segmento-nuclear leukocytes, lymphadenoid follicular hyperplasia (Pic. 1-6).

According to preliminary studies and obtained results we think that carried out experiments more or less approved our hypothesis that encouraged us to continue investigations for elaboration of technology for obtaining of preparation and treatment regimen. This result supports the need of a further detailed investigation of HTD anticancer properties with the final aim of its possible use as therapeutic agent.

Let's come back to the literature and data that are somehow similar to our hypothesis. We have found information about experiments carried out in different countries referring on biological reactions proceeding in mitochondria. For instance, E. Michelakis (Canada, 2006) used dichloroacetate (DCA) in the clinic against glioblastoma [14]. Young H. Ko, Barbara L. Smith et all (USA, 2004) tasted the 3-bromopyruvate acid in experiments on rabbits with liver cancer [18], etc. Their theories mainly relay on theory of Otto Heinrich Warburg. In 1924, Warburg hypothesized that malignant growth is caused by tumor cells mainly generating energy (ATP) by nonoxidative breakdown of glucose (a process called glycolysis) and the subsequent recycling of the metabolite NADH back to its oxidized form, for reuse in the glycolytic cycle to complete the process (known as fermentation, or anaerobic respiration). This is in contrast to "healthy" cells, which mainly generate energy from oxidative breakdown of pyruvate. Pyruvate is an end product of glycolysis, and is oxidized within the mitochondria. Hence, and according to Warburg, cancer should be interpreted as a mitochondrial dysfunction. Summarized in a few words, the prime cause of cancer is the replacement of the respiration of oxygen in normal body cells by a fermentation of sugar [17]. Moreover, in 2009 experiments carried out in Boston College and Medical school at Washington University researchers managed to prove Warburg's hypothesis. They detected certain alterations (anomaly) in cardiolipin of mitochondrial membranes. They assert that cardiolipin present in all types of malignant cells and are closely related with energy production. We think that although hypothesis of carcinogenesis suggested by us completely is based on biochemical processes, and the anaerobic mechanism represents one of its constituent parts, it still differs from the above-discussed theories.

Differences are following:
1. Warburg's hypothesis (mitochondrial theory) refers to disorders only in mitochondria. Our hypothesis is "mitochondrial-nuclear", or "respiratory-mutation". I.e.disorders started in respiratory system, in mitochondria, results in mutations in gene apparatus i.e. in nucleus, and all of these alterations in complex can initiate malignant transformation of cell and support the cancer growth;

2. In our hypothesis we provide more detailed explanation of biochemical reactions, thereby giving answers to many questions, and clarifying some questionable points of view concerning carcinogenesis.
Namely, according to our hypothesis the direct cause of malignant transformation of cell is the disorder of cytochrome structure due to effects of different carcinogenic substrates. Cytochrome disorders in one case could be caused by increased pH in mitochondria, and in the other case – pH alterations in mitochondria is the result of disorders of electron-transport chain (its block), as far as the latter will stop the Krebs cycle. Cessation of Krebs cycle means cessation of production of 8 acids such as: oxaloacetic acid, citric acid, cis-aconitic acid, isocitric acid, α-ketoglutaric acid, succinic acid, fumaric acid, and malic acid. Although, in Krebs cycle they interconvert, in our opinion, their complex exactly, provide normal mitochondrial pH.

Secondly – reason for uncontrolled malignant growth is the decreased activity of oxidases as a result of increased pH in mitochondria and thereafter entire in cell, which in turn will lead to disorders (cessation) of deamination of amino acids supporting their accumulation in malignant cells and their further use for protein synthesis. Another reason of uncontrolled malignant growth supposedly could be the intense synthesis of methionil–tRNA. Methionil–tRNA is the first, or initiator aminoacyl-tRNA providing inclusion of N-terminal amino acid in protein synthesis. So, initiating translation. In malignant cells for methionil–tRNA synthesis there are both, the methionine and the tRNA.

Thirdly – Disorders in enzyme specter in malignant cells are in close relation with cell pH. I.e., it is the ability of malignant cell to synthesize specific enzymes in more differentiated cells due to presence of relative normal pH, and synthesis of other, abnormal enzymes, in less differentiated cells due to high pH level.

Fourthly – difference between mechanisms of hyperplasia at pre-cancers, and hyperplasia in regenerating tissues we explain by disorders (duplication) of electron stream through cytochrome system and increased ATP synthesis in the first case (hyperplasia at pre-cancers), while in normal tissues, any proliferation develops owing to cell mitotic division, or without disorders in electron transport chain.

Fifthly – the malignant growth after removal-, or cessation of effects of carcinogen goes owing to decreased activity of oxidases and increased synthesis of methionil–tRNA.

Sixthly – the reverse of malignant process, or involution could be explained by normalization of cell pH and activation of oxidases leading to deamination of amino acids and brake down of abnormal (cancerous) proteins.
3. At last – Suggested substrate Hydroxyethylthiamine diphosphate (HTD) for cessation of malignant growth, also differs from preparations (dichloroacetate, and

3-bromopyruvate acid) used by E. Michelakis, Young H. Ko, Barbara L. Smith and others. Moreover, information about administration of HTD as an anti-cancer treatment agent has not been found up today in the available modern literature.

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SUMMARY

THE NOVEL HYPOTHESIS OF CARCINOGENESIS AND ANTI-CANCER TREATMENT PERSPECTIVES – HYDROXYETHYLTHIAMINE DIPHOSPHATE

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In presented article, the novel hypothesis of carcinogenesis, and thorough discussion of some essential biochemical mechanisms which might be responsible for the malignant transformation of cells (bond disorders between Fe^{3+} and S^{+}-methionine in cytochrome; blockage of the last 3-d orbital of Fe by the certain ligand and formation of 6th coordinated bond leading to Fe^{3+} reduction up to Fe^{2+} and cessation of tissue respiration; increase in mitochondrial pH and further in cell, leading to decreased activity of most of enzymes (thiamine diphosphate, oxidases); formation of S-adenosyl-methionine with its further dissociation and production of adenosine and homocysteine; effects of homocysteine on DNA structures, homocysteine-induced dimethylation of certain nitrogen basis and their extrusion from strands of DNA leading to mutations and cellular atypism) has been suggested. Along with theoretical discussions, article provides results of preliminary investigations carried out on C57Bl/6J mice with Ehrlich carcinoma aimed to capture lipoic acid amide with Hydroxyethylthiamine diphosphate, and study effects of Hydroxyethylthiamine diphosphate against malignant transformation of cells. Experiments have shown inhibition of cancer growth in treated animals. Morphological investigations of cancer tissue revealed necrotic zones, inflammatory infiltrations, central necrosis with adjacent inflammatory mono- and polymorph infiltrations, must cells, segmento-nuclear leukocytes, lymphadenoid follicular hyperplasia. According to the novel hypothesis of carcinogenesis and results of experiments the new approaches and perspectives of anti-cancer treatment with the use of Hydroxiethyl-thiamine diphosphate has been suggested.

Keywords: Carcinogenesis, pH, Hydroxyethylthiamine diphosphate, lipoic acid amide, Homocystein.

РЕЗЮМЕ

НОВАЯ ГИПОТЕЗА КАНЦЕРОГЕНЕЗА И ПЕРСПЕКТИВЫ ПРОТИВООПУХОЛЕВОГО ЛЕЧЕНИЯ – ГИДРОКСИЭТИЛТИАМИНДИФОСФАТ

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В статье рассматривается новая гипотеза канцерогенеза. Обсуждаются некоторые биохимические меха-

низмы, которые, по всей вероятности, ответственны за злокачественную трансформацию клетки. Согласно предложенной гипотезе, принципиальными моментами, которые играют решающую роль в канцерогенезе, являются: 1) нарушение связи между атомом железа и атомом серы (Fe^{3+} и S^{+}-метионина) в цитохромах; 2) блокада последней 3-d орбитали атома железа каким-либо лигандом и образование между ними 6-ой координационной связи, приводящей к восстановлению железа до Fe^{2+} и прекращение тканевого дыхания; 3) повышение pH митохондрий, что может привести к падению активности многих ферментов – E_1-ТДФ, оксидаз, и других; 4) образование S-аденозил-метионина и последующего его распада на аденозин и гомоцистеин; 5) воздействие гомоцистеина на структуры ДНК, диметилирование гомоцистеином некоторых азотистых оснований и их выпадение из нитей ДНК с образованием мутаций в структуре ДНК с возможным развитием атипии клетки. Для лечения опухолей предлагается гидроксиэтилтиаминдифосфат. В статье одновременно с теоретическими предположениями представлены результаты предварительного исследования, проведенного на мышах C57B1/6J с карциномой Эрлиха с применением для лечения гидроксиэтилтиаминдифосфата с целью связывания амид липоевой кислоты и изучения эффекта воздействия гидроксиэтилтиаминдифосфата на злокачественно трансформированные клетки. В ходе эксперимента выявлена задержка роста опухоли у животных. Морфологическое исследование опухолевой ткани выявило некротические зоны, воспалительные инфильтрации, центральные некрозы со смежными воспалительными моно- и полиморфными инфильтрациями, мышечные клетки, сегментонуклеарные лейкоциты, лимфаденоидную фолликулярную гиперплазию.

რეზიუმე

კანცეროგენეზის ახალი ჰიპოთეზა და სიმსივნისსაწინააღმდეგო მკურნალობის პერსპექტივები – ჰიდროქსიეთილთიამინ დიფოსფატი.

ლ. გევორქიანი, ქ. ღამბაშიძე

ონკოლოგიის ნაციონალური ცენტრი. თბილისის სახ. სამედიცინო უნივერსიტეტი, საქართველო

სტატიაში შემოთავაზებულია კანცეროგენეზის ახალი ჰიპოთეზა და განხილულია ის ბიოქიმიური პროცესები, რომლებიც, სავარაუდოდ, მნიშვნელოვან როლს ასრულებენ ნორმალური უჯრედების ავთვისებიან ტრანსფორმაციაში. კერძოდ, 1) Fe^{3+} და S^{+}-მეთიონინს შორის კავშირის დარღვევა ციტოქრომებში; 2) Fe-ის 3-d ორბიტალის ბლოკადა გარკვეული ლიგანდით და მათ შორის მე-6 კოორდინირებული კავშირის წარმოქმნა, Fe^{3+}-ის აღდგენა Fe^{2+}-მდე და ქსოვილოვანი სუნთქვის შეწყვეტა; 3) მიტოქონდრიული pH–ის მომატება და ფერმენტების ინაქტივაცია (თიამინდიფოსფატი, ოქსიდაზები); 4) S-ადენოზილმეთიონინის წარმოქმნა და მისი შემდგომი დისოციაციით ადენოზინის და ჰომოცისტეინის წარმოქმნა; 5) ჰომოცისტეინის გავლენა დნმ-ის სტრუქტურაზე, ჰომოცისტეინით ინდუცირებული ნიტროგენული ბაზისის დიმეთილირება და მისი გამოძევება დნმ-ის სპირალიდან შემდგომი მუტაციების და უჯრედული ატიპიზმის განვითარებით.

თეორიული განხილვების პარალელურად სტატიაში წარმოდგენილია ერლიხის კარცინომიან C57Bl/6J თაგვებზე ჩატარებული კვლევის მასალები. ექსპერიმენტში, რომლის მიზანიც იყო ჰიდროქსიეთილთიამინდიფოსფატით (ჰეთდ) ლიპოის მჟავის ამიდის შებოჭვა და ჰეთდ-ს გავლენის შესწავლა სიმსივნურად ტრანსფორმირებულ უჯრედებზე, გამოვლინდა სიმსივნური ზრდის შეფერხება. სიმსივნურ ქსოვილში მორფოლოგიურად დადგინდა ნეკროზული უბნები, ანთებითი მონო- და პოლიმორფული ინფილტრაციები, პოხიერი უჯრედები, სეგმენტბირთვიანი ლეიკოციტები, ლიმფადენოიდური ფოლიკულური ჰიპერპლაზია.

ИЗУЧЕНЕ АНТИОКСИДАНТНОЙ АКТИВНОСТИ ФЕНОЛЬНЫХ СОЕДИНЕНИЙ НЕКОТОРЫХ ВИДОВ ФЛОРЫ ГРУЗИИ

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Создание лекарственных средств природного происхождения является основным направлением фитохимиков, изучающих химический состав, биологическую и фармакологическую активность веществ растительного происхождения. Среди биологически активных растительных веществ с точки зрения практического применения особое место занимают фенольные соединения, которые характеризуются широким спектром терапевтического действия. Поэтому изучение антиоксидантной активности местного растительного сырья, содержащего фенольные соединения, и создание на их основе лекарственных средств и пищевых добавок весьма актуально [2,8,9].

Целью исследования явилось изучение антиоксидантной активности фенольных соединений некоторых растений флоры Грузии.

Материал и методы. Объектом исследования явились надземные части растений, собранные в фазу цветения в различных районах Грузии. Проводился анализ спиртово-водных и водных экстрактов отдельных частей растений: *Hamamelis virginiana L., Astragalus caucasicus Pall.*, *Astragalus micricephalus Willd., Vitis vinifera L., Rhododendron ponticum L.*, *Rhododendron Ungernii Trautv., Ginkgo biloba L., Salvia officinalis L.*, *Querqus iberica Stev., Maclura aurantiaca Nutt., Cotinus coggygria Ledeb.*, *Fraxinus ornus L.*, *Urtica dioica L., Rhododendron caucasicum Pall., Pueraria hirsuta Matsum.*, *Geranium pusillum L.*, *Astragalus Tanae Sosn.*, *Pinus silvestris L.* Посредством качественных реакций и анализов бумажной хроматографии в различных системах растворителей устанавливалось наличие фенольных соединений в анализируемых объектах. Они, в основном, представлены флавонами, флавонолами, антоцианами, изофлавонами, танинами, а в ряде случаев, и циклоартанами. Структуру отдельных компонентов устанавливали на основании изучения физико-химических свойств как самых веществ, так и продуктов их химического превращения, а также данными УФ, ИК, ^{1}H и ^{13}C ЯМР, HSQC, HMBC, DEPT, COSY [3].

Антиоксидантную активность изучали в опытах *in vitro* и оценивали определением промежуточного липидно-пероксидного процесса – малондиальдегида (МДА). Липидно-перекисное инициирование производили под влиянием двухвалентных ионов железа. МДА определяли тестом тиобарбитуровой кислоты спектрофотометрическим методом [4,6].

Результаты и их обсуждение. В процессе химического изучения исследуемых видов растений были выделены и охарактеризованы следующие соединения: лютеолин - 5,7,3’,4’-тетраоксифлавон; цинарозид -лютеолин-7-О-β-D– глюкозид; лютеолин-7-О-β-D-глюкуронид; виценин 2 – апигенин-6,8-ди–С-β-D-глюкопиранозид и розмариновая кислота - [3,4-диоксифенил-α-(3’,4’- дигидроксицинамоил) пропионовая кислота] - *Salvia officinalis L.*; кемпферол - 3, 5, 7, 4’-тетрагидроксифлавон; кверцетин - 3,5,7,3’,4’-пентагидроксифлавон, трифолин – кемпферол-3-О-β-D-галактозид; кемпферол-3-О-β-D-глюкуронид; гиперин - кверцетин-3-О-β-D-галактозид, кверцитурон - кверцетин-3-О-β-D-глюкорунид; (+)-катехин - 3,5,7,3’,4’-пентагидроксифдаван; цианидин - 3,5,7,3’,4’-пентагидроксифлавил, дельфинидин - 3,5,7,3’,4’,5 –гексагидроксифлавил; хлорогеновая кислота - 3-кофеил-D-хинон; гамамелитанин - 2’,5-ди-галлоил-гамамелоза; галловая кислота - 3,4,5-тригидроксибензойная кислота - *Hamamelis virginiana L.*; галловая кислота, метилгалат, диметилгалат, триметилгалат, тетра- и пента-галлоилглюкозы; мирицетин-3-О-β-D-галактопиранозид; мирицетин-3-О-α-L-рамнопиранозид - *Cotinus cjggygria Scop.*; никотифлорин - кемпферол-3-О-β-D-рутинозид, кемпферол-глюко-галакто-рамнозид, робинин - кемпферол-3-О-β-D-робинобиозид-7-О-α-L-рамнопиранозид, асказид - кемпферол-3-О-β-D-галактопиранозид-3’’,4’’-О-α-L-дирамнопиранозид - *Astragalus caucasicus*; (+)-катехин, (-)–эпикатехин, (+)-галлокатехин, кверцитрин - кверцетин-3-О-α-L-рамнозид, изокверцитрин - кверцетин-3-О-β-D-глюкопиранозид, цианидин, дельфинидин - *Rhododendron ponticum L.*; кверцетин, кверцитрин – кверцетин-3-О-α-L-рамнозид, изокверцитрин, гиперин, рутин - кверцетин-3-О-β-D-рутинозид, (+)-катехин, (-)-эпикатехин, (-)-галокатехин, лейкоцианидин - *Rhododendron ungernii Trautv.* [1,5,7,10].

Результаты изучения антиоксидантной активности экстрактов, содержащих суммы фенольных соединений, приведены в таблице и на рис.

Таблица. Антиоксидантная активность суммы фенольных соединений в некоторых видах растений флоры Грузии

№	Объекты исследования	Изученная часть растений	Относительная антиоксидантная активность в %
1	*Hamamelis virginiana L.*	Листья	139
2	*Astragalus caucasicus Pall.*	Листья	144
3	*Astragalus microcephalus Willd.*	Надземная часть	90
4	*Vitis vinifera L.*	Косточки	128
5	*Rhododendron ponticum L.*	Листья	140
6	*Rhododendron Ungernii Trautv.*	Листья	118
7	*Ginkgo biloba L.*	Перикарпии плодов	122
8	*Salvia officinalis L.*	Листья (водный экстракт)	200
9	*Querqus iberica Stev.*	Кора	141
10	*Maclura aurantiaca Nutt.*	Плоды	140
11	*Cotinus coggygria Ledeb.*	Листья	147
12	*Fraxinus ornus L.*	Листья	127
13	*Salvia officinalis L.*	Листья (спиртовый экстракт)	175
14	*Urtica dioica L.*	Стебли	157
15	*Rhododendron caucasicum Pall.*	Листья	147
16	*Pueraria hirsuta Matsum.*	Листья	136
17	*Geranium pusillum L.*	Листья	133
18	*Astragalus Tanae Sosn.*	Листья	120
19	*Maclura aurantiaca Nutt.*	Корны	103
20	*Pinus silvestris L.*	Иглицы	102
21	Этилендиаминтетраацетат (ЭДТА)	-	90
22	α-токоферол	-	97

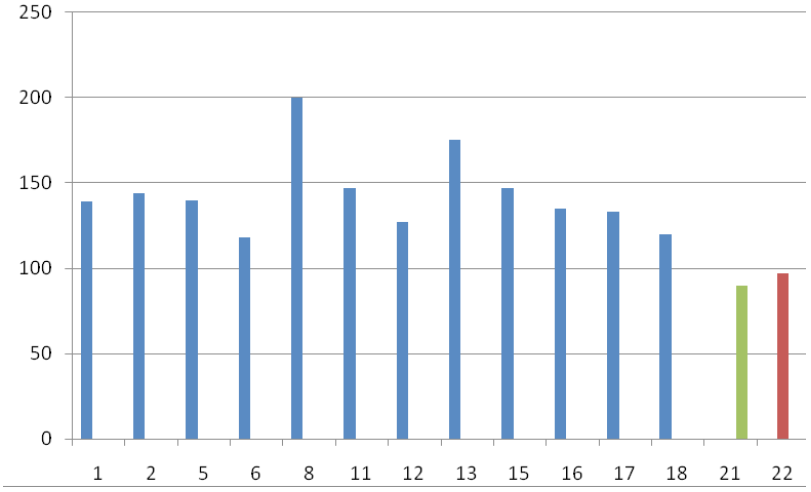

Рис. Диаграмма сравнительной антиоксидантной активности экстрактов листьев исследуемых растений (нумерацию объектов см. в таблице)

По антиоксидантной активности, образцы, полученные из листьев исследуемых растений, можно расположить в следующем порядке (рис.): *Salvia officinalis* – водный экстракт (200%) → *Salvia officinalis* - спиртовый экстракт (175%) → *Cotinus coggigrya* (147%) →*Rhododendron caucasicum* (147%) → *Astragalus caucasicus* (144%) → *Rhododendron ponticum* (140 %) → *Hamamelis virginiana* (139%) → *Pueraria hirsuta* (136%) → *Geranium pusillum* (133%) →*Fraxinus ornus* (127%) → *Astragalus Tanae* (120%) → *Rhododendron ungernii* (118%) → *Pinus silvestris* (102%).

Как следует из данных, приведенных в таблице, наиболее активным оказался водный экстракт *Salvia*

officinalis, химическое изучение которого показало, что, наряду с низкомолекулярными фенолами, основными его компонентами являются также конденсированные танины [10].

В результате проведенных исследований из *Salvia officinalis L.* – шалфея лекарственного разработана биологически активная пищевая добавка «Салбин» в виде капсул для профилактики атеросклероза [1].

Дальнейшее изучение активных экстрактов предполагает выявить соединения или группу веществ, ответственных за биологический эффект, а также позволит установить взаимосвязь между структурой вещества и биологической активностью.

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SUMMARY

STUDY OF ANTIOXIDANT ACTIVITY OF PHENOLIC COMPOUNDS FROM SOME SPECIES OF GEORGIAN FLORA

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The antioxidant activity of extracts obtained from different parts of Georgian flora species *Hamamelis virginiana L., Astragalus caucasicus Pall.*, *Astragalus microcephalus Willd., Vitis vinifera L., Rhododendron ponticum L.*, *Rhododendron Ungernii Trautv., Ginkgo biloba L., Salvia officinalis L.*, *Querqus iberica Stev., Maclura aurantiaca Nutt., Cotinus coggygria Ledeb.*, *Fraxinus ornus L.*, *Urtica dioica L., Rhododendron caucasicum Pall., Pueraria hirsuta Matsum.*, *Geranium pusillum L.*, *Astragalus Tanae Sosn.*, *Pinus silvestris L*. has been studied. Comparison with ethylentetraacetate and α-tocopherole revealed high efficacy of all extracts studied.

45 individual phenolic compounds were isolated and described by chemical examination of biologically active objects.

Common sage (*Salvia officinalis*) extract turned out as the most active (200 %). The chemical study revealed the dominant content of condensed tannins and low molecular phenolic compounds, which may be attributed to the high antioxidant activity.

Biologically active antiatherosclerotic food additive “Salbin” was developed on the basis of Common sage - *Salvia officinalis L*. phenolic compounds.

Keywords: antioxidant activity, phenolic compounds, *Salvia officinalis*.

РЕЗЮМЕ

ИЗУЧЕНИЕ АНТИОКСИДАНТНОЙ АКТИВНОСТИ ФЕНОЛЬНЫХ СОЕДИНЕНИЙ НЕКОТОРЫХ ВИДОВ ФЛОРЫ ГРУЗИИ

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Изучена атнтиоксидантная активность экстрактов различных частей растений флоры Грузии: *Hamamelis*

virginiana L., Astragalus caucasicus Pall., *Astragalus microcephalus Willd., Vitis vinifera L., Rhododendron ponticum L.*, *Rhododendron Ungernii Trautv., Ginkgo biloba L., Salvia officinalis L.*, *Querqus iberica Stev., Maclura aurantiaca Nutt., Cotinus coggygria Ledeb.*, *Fraxinus ornus L.*, *Urtica dioica L., Rhododendron caucasicum Pall., Pueraria hirsuta Matsum.*, *Geranium pusillum L.*, *Astragalus Tanae Sosn.*, *Pinus silvestris L.* Установлена их высокая эффективность по сравнению с этилендиаминтетраацетатом и α-токоферолом.

Химическим исследованием активных объектов выделены и охарактеризованы 45 индивидуальных веществ фенольной природы.

Наиболее активной оказалась сумма экстрактивных веществ из сухого водного экстракта *Salvia officinalis* (200%). Химическое изучение шалфея лекарственного в качестве основных компонентов выявило содержание низкомолекулярных фенольных соединений наряду с конденсированными танинами, чем, по всей вероятности, обусловлено его высокое антиокислительное действие.

В результате проведенных исследований на основе суммы фенолов листьев *Salvia officinalis L.* – шальфея лекарственного разработана биологически активная пищевая добавка «Салбин» в виде капсул для профилактики атеросклероза.

რეზიუმე

ფენოლური ნაერთების ანტიოქსიდანტური აქტივობის შესწავლა საქართველოს ფლორის ზოგიერთ სახეობაში

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თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი,
იოველ ქუთათელაძის ფარმაკოქიმიის ინსტიტუტი, თბილისი, საქართველო

შესწავლილია საქართველოს ფლორის მცენარეთა *Hamamelis virginiana L., Astragalus caucasicus Pall.*, *Astragalus microcephalus Willd., Vitis vinifera L., Rhododendron ponticum L.*, *Rhododendron Ungernii Trautv., Ginkgo biloba L., Salvia officinalis L.*, *Querqus iberica Stev., Maclura aurantiaca Nutt., Cotinus coggygria Ledeb.*, *Fraxinus ornus L.*, *Urtica dioica L., Rhododendron caucasicum Pall., Pueraria hirsuta Matsum.*, *Geranium pusillum L.*, *Astragalus Tanae Sosn.*, *Pinus silvestris L.* სხვადასხვა ნაწილებიდან მიღებული ექსტრაქტების ანტიოქსიდანტური აქტივობა. დადგენილია მათი მაღალი ეფექტურობა ეთილენდიამინტეტრაძმარმჟავასა და α-ტოკოფეროლთან შედარებით. ბიოლოგიურად აქტიური ობიექტების ქიმიური შესწავლის შედეგად მათგან გამოყოფილია და იდენტიფიცირებულია 45 ინდივიდუალური ფენოლური ბუნების ნივთიერება. ყველაზე აქტიური აღმოჩნდა სამკურნალო სალბიდან (*Salvia officinalis*) მიღებული ექსტრაქტულ ნივთიერებათა ჯამი (200%),რომლის ქიმიურმა შესწავლამ გამოავლინა დაბალმოლეკულური ფენოლური ნაერთებისა და კონდენსირებული ტანინების დომინანტური შემცველობა, რითაც შესაძლოა განპირობებულია მაღალი ანტიოქსიდანტური მოქმედება. ჩატარებული კვლევების შედეგად სამკურნალო სალბის - *Salvia officinalis L.* –ის ფოთლების ფენოლების ჯამის საფუძველზე კაფსულირებული სახით შემუშავებულია ანტიათეროსკლეროზული აქტივობის ბიოლოგიურად აქტიური კვებითი დანამატი “სალბინი”.

SECTION – ADOLESCENT'S INTEGRAL HEALTH: A MULTIDISCIPLINARY APPROACH

With an Educational Project of Private Accredited Hospital Quisisana, Ferrara (Italy) and an Educational Grant of Novartis

Guest Editors:
Karaman Pagava, Ashraf Soliman, Vincenzo De Sanctis

სექცია – მოზარდთა ინტეგრალური ჯანმრთელობა: მულტიდისციპლინური მიდგომა

კვისისანას კერძო ჰოსპიტლის, ფერარა (იტალია)
და კომპანია "Novartis" მხარდაჭერით

მოწვეული რედაქტორები:
ყარამან ფაღავა, აშრაფ სოლიმანი, ვინჩენცო დე სანკტისი

СЕКЦИЯ – ИНТЕГРАЛЬНОЕ ЗДОРОВЬЕ ПОДРОСТКОВ: МУЛЬТИДИСЦИПЛИНАРНЫЙ ПОДХОД

При поддержке частного Квисисанского госпиталя, Феррара (Италия) и компании "Novartis"

Приглашенные редакторы:
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ACANTHOSIS NIGRICANS IN ADOLESCENTS: A PRACTICAL APPROACH

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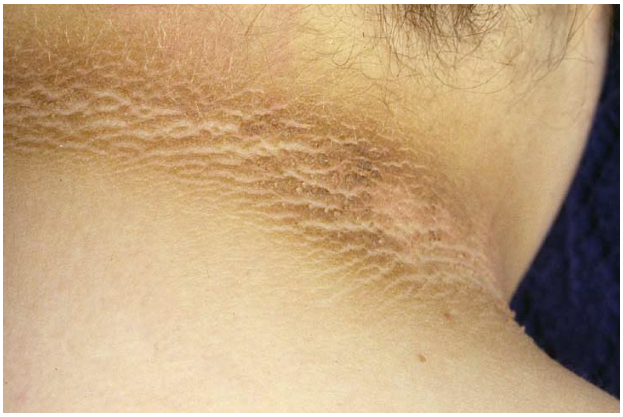
Acanthosis nigricans (AN) is the term proposed by Unna [18] to indicate a disorder characterized clinically by thickening and darkening velvety plaques symmetrically distributed on the sides of the neck, axillae and groin. These lesions may be skin-coloured or brownish and may vary between 1 mm and 1 cm [9]. The neck is involved 93% to 99% of the time [4,23,24]. Less often it affects eyelids, palms, soles of the feet, nipples and phalanges and rarely the mucosa of the mouth, respiratory mucosa and genital region [4,23,24] (Figs. 1, 2).



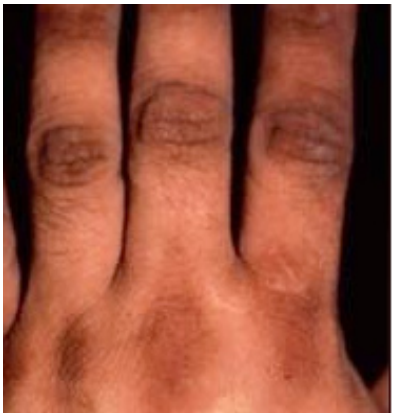


Fig. 1, 2. Features of acanthosis nigricans, affecting neck and knuckles of the fingers (From: Soliman A., personal observations)

Prevalence

The prevalence of AN varies from 7% in unselected populations to 74% in obese people [23,24]. The prevalence also varies in different racial groups. For example, African Americans are 25 times more likely to have AN than patients of European descent [23,24]. A study from the USA reports that the prevalence of AN is about 3% among Caucasians, 19% in Hispanics and 28% in American Indians [23,24].

Pathogenesis of AN

Biochemical mechanisms for developing this hyperplastic lesion are unclear, but likely involve local cutaneous growth factors. Insulin and insulin-like growth factor-I, and their receptors on keratinocytes are involved in the complex regulations leading to the peculiar epidermal hyperplasia [19]. At high concentrations, insulin may exert potent proliferative effects via high-affinity binding to IGF-1 receptors. In addition, free IGF-1 levels may be elevated in obese patients with hyper-insulinemia, leading to accelerated cell growth and differentiation [10].

Histologically there is papillomatosis, hyperkeratosis and acanthosis with minimal or no hyperpigmentation of the basal layer.

Table 1. A quantitative scale of AN developed by Burke et al. (modified)

Location and score	Description
Neck severity	
0	Not detectable on close inspection.
1	Clearly present on close visual inspection, not visible to the casual observer, extent not measurable.
2	Mild: limited to the base of the skull, does not extend to the lateral margins of the neck
3	Moderate: extending to the lateral margins of the neck (posterior border of the sternocleidomastoid)
4	Severe: extending anteriorly, visible when the participant is viewed from the front.

Assessment

A quantitative scale of AN has been developed by Burke et al. [4]. (Table 1). This scale takes into consideration the severity of AN in the neck and axilla, neck texture and the presence or absence of AN in knuckles, elbows and knees. The scale is easy to use and correlates well with fasting insulin and body mass index (BMI). Neck AN severity is strongly associated with elevated fasting insulin and BMI in both diabetic and non-diabetic subjects, elevated fasting glucose, systolic blood pressure, diastolic blood pressure and with decreased high density lipoprotein (HDL) in non-diabetic subjects. Therefore, the observation of AN would help to diagnose insulin resistance (IR).

Table 2. AN and related medical conditions

A. Type 1: Hereditary benign acanthosis nigricans
    1. Idiopathic onset during childhood or puberty
B. Type 2: Benign acanthosis due to insulin resistance
    1. Obesity resulting in insulin resistance
    2. Diabetes mellitus
    3. Androgen excess (hyperandrogenism)
        a. Cushing's syndrome
        b. HAIR-AN syndrome
    4. Hypogonadism
    5. Addison's disease
    6. Hypothyroidism
C. Type 3: Pseudo-acanthosis nigricans
    1. Seen in patients with darker pigmentation
D. Type 4: Drug-induced acanthosis nigricans
    1. Glucocorticoids
    2. Niacin
    3. Protease inhibitors
    4. Oral Contraceptive
E. Type 5: Malignant acanthosis nigricans
    1. Causes
        a. Paraneoplastic tumors (adenocarcinoma)
        b. Lymphoma

Types of AN

Currently, 8 types of AN have been identified, according to Schwartz [19] (Table 2):

1. Obesity-associated AN: This is the most common type of AN. Lesions may appear at any age but are more common in adulthood. The dermatosis is weight dependent and lesions may completely regress with weight reduction. Insulin resistance is often present in these patients; however, it is not universal.

Other endocrine disturbances associated with AN are Cushing's disease, polycystic ovary syndrome (PCOS), thyroidopathies, hirsutism, Addison's disease and acromegaly. Some of these disorders occur along with insulin resistance [17].

2. Unilateral AN: Lesions are unilateral in distribution and may become evident during infancy, childhood or adulthood. Lesions tend to enlarge gradually before stabilizing or regressing. It is believed to be inherited as an autosomal dominant trait

3. Familial AN: The lesions typically begin during early childhood but may manifest at any age and the condition often progresses until puberty, at which time it stabilizes or regresses. It seems to be genetically transmitted.

4. Syndromic AN is the name given to AN that is associated with a syndrome. Familial and syndromic forms have been subdivided into insulin-resistance syndromes and fibroblast growth factor defects.

Insulin-resistance syndromes include those with mutations in the insulin receptors (i.e., leprechaunism, Rabson-Mendenhall syndrome), peroxisome proliferator-activated receptor gamma (i.e., type 1 diabetes with acanthosis nigricans and hypertension), 1-acylglycerol-3-phosphate O-acyl transferase-2 or seipin (Berardinelli-Seip syndrome), lamin A/C (Dunnigan syndrome), and Alstrom syndrome gene [10,19,20].

Fibroblast growth factor defects include activating mutations in FGFR2 (Beare-Stevenson syndrome), FGFR3 (Crouzon syndrome with acanthosis nigricans, thanatophoric dysplasia, severe achondroplasia with developmental delay, and acanthosis nigricans). Familial cases of acanthosis nigricans with no other syndromic findings have also been linked to FGFR mutations [3,21].

Type A syndrome: Also termed HAIR-AN syndrome (**h**yper**a**ndrogenemia, **i**nsulin **r**esistance and **AN**. The lesions of AN may arise during infancy and progress rapidly during puberty. This syndrome is often familial, affecting primarily young women (especially black women). It is associated with polycystic ovaries or signs of virilization (e.g. hirsutism, clitoral hypertrophy). High plasma testosterone levels are common. Early detection, diagnosis, and treatment can help reduce further morbidity, improve self-esteem, and have a positive impact on the quality of life of these patients.

Type B syndrome: Generally occurs in women who have uncontrolled diabetes mellitus, ovarian hyperandrogenism, or an autoimmune disease such as systemic lupus erythematosus, scleroderma, Sjögren syndrome, or Hashimoto thyroiditis. Circulating antibodies to the insulin receptor may be present. In these patients, the lesions of AN are of varying severity.

Besides these variants there are other numerous syndromes, the most well known of which are Hirschowitz syndrome, which is familial, characterized by early onset, deafness and gastrointestinal disorders; and Lawrence-Seip syndrome [15,20] with lipodystrophy associated with AN.

Skeletal dysplasias: AN has been reported in association with severe skeletal dysplasias due to activating mutations in FGFR3. The development of AN in patients with skeletal dysplasias is not due to insulin insensitivity or treatment with recombinant human growth hormone. Whether the AN

is due to altered melanocyte function in these individuals remains to be elucidated [2].

5. Acral AN: The hyperkeratotic velvety lesions are most prominent over the dorsal aspects of the hands and feet. It occurs in patients who are in otherwise good health. Acral AN is most common in dark-skinned individuals, especially those of African American descent.
6. Drug-induced AN, although uncommon, may be induced by several medications, including nicotinic acid, insulin, systemic corticosteroids and oral contraceptives. The lesions of drug-induced AN may regress following the discontinuation of the offending medication.
7. Malignant AN is associated w76
ith internal malignancy, particularly gastric adenocarcinoma. It is characterized by sudden onset and it is sometimes associated to other cutaneous markers of malignancy such as eruptive seborrheic warts, florid cutaneous papillomatosis and hyperkeratosis of the palms and soles. In the case of malignant AN, multiple growth factors including transforming growth factor α, insulin-like growth factor, and fibroblast growth factor have been implicated. These factors most likely act by exerting an insulin-like effect on keratinocytes and dermal fibroblasts.

In approximately one third of cases of malignant acanthosis nigricans, patients present with skin changes before any signs of cancer. In another one third of cases, the lesions of acanthosis nigricans arise simultaneously with the neoplasm. In the remaining one third of cases, the skin findings manifest sometime after the diagnosis of cancer [10].

Warning flags that should trigger a careful evaluation for malignancy in patients presenting with acanthosis nigricans include unintentional weight loss and rapid onset of extensive AN [19]. Mucosal AN is more common in patients who have AN in association with a malignancy, as are tripe palms, florid cutaneous papillomatosis, and the sign of Leser-Trélat [19].

8. Mixed-type AN refers to those situations in which a patient with one of the above types of AN develops new lesions of a different etiology. An example of this would be an overweight patient with obesity-associated AN who subsequently develops malignant AN.

Laboratory assessment
The following investigations are recommended in subjects with AN:
1) serum glucose and insulin
2) lipid profile
3) glycosylated hemoglobin level, and in selected cases oral glucose tolerance test.

These data are useful tools for baseline screening and follow-up of subjects at risk for type 2 diabetes mellitus, hypercholesterolemia and hypertension.

In 2000, the American Diabetes Association established acanthosis nigricans as a formal risk factor for the development of diabetes in children [22].

Insulin resistance (IR) is a metabolic disorder in which target cells fail to respond to normal levels of circulating insulin, which results in compensatory hyperinsulinemia in an attempt to obtain an appropriate physiological response [1,12].

In order to assess insulin resistance, insulin secretion and insulin sensitivity, methods based on the hyperglycaemic clamp have been validated both in obese and normal adults [5,25]. In children, the euglycemic clamp procedure is cumbersome, time consuming and technically difficult to perform especially on a large group.

HOmeostasis **M**odel **A**ssessment of IR index (**HOMA**-IR: fasting plasma insulin in mU/l x FPG in mmol/l/22.5) [10], HOMA of percent b-cell function (HOMA-b%:20 x fasting insulin in mU/l)/(fasting glucose in mmol/l – 3.5) (16) and **QU**antitative **I**nsulin-sensitivity **C**hec**K** **I**ndex (QUICKI: 1/(log10 fasting plasma insulin in mU/l + log10 glucose in mg/dl) [13] indices calculated on fasting samples have the advantage of being quicker, simpler, less expensive and cumbersome than those based on minimal models, making them more acceptable to children, and ideal for large and longitudinal studies. As a measure of insulin resistance in obese youths, HOMA-IR seems more reliable than the fasting glucose/insulin ratio and QUICKI [14].

Normal percentiles values (from the 2.5th to the 97.5th) of HOMA-IR, HOMA-b% and QUICKI for the adolescent Italian population are available in the literature [8].

One hundred and forty-nine overweight and obese children were screened for AN by Dubnov-Raz et al. [7]. Twenty-two (14.8%) children had AN. Children with AN had greater height, weight, BMI, waist circumference, waist-to-height-ratio, triceps skinfold thickness, and total and truncal body fat percentage, compared to those without AN. After adjustment for age and BMI, no adiposity measure was increased in children with AN. The authors concluded that overweight and obese children with AN basically have greater overall and central adiposity than those without it.

A large-scale screening of fifth-grade students in West Virginia explored the prevalence of metabolic syndrome among 676 male and female participants who had mild to severe AN. Children with AN who were classified as obese or morbidly obese were at significantly increased odds of having metabolic syndrome [11].

Treatment
Treatment is directed towards management of the underlying cause that includes either weight reduction, discon-

tinuation of offending drugs, correction of the endocrine abnormality or therapy of the underlying malignancy.

Emollients, keratolytics, calcipotriol, systemic retinoids, CO2 laser ablation, and long-pulsed laser may improve appearance. In the setting of AN with obesity, weight loss with appropriate dietary and lifestyle modifications should be encouraged [10]. Oral metformin is a first choice drug in the treatment of AN associated with obesity and insulin resistance [6]. Follow up care should be coordinated with the patient's primary care physician and should include periodic measurements of body weight and blood insulin levels.

Conclusions

Acanthosis nigricans is a lesion affecting localized areas of the skin in persons with obesity and/or hyperinsulinemia. Biochemical mechanisms for developing this hyperplastic lesion are unclear, but likely involve local cutaneous growth factors. Clinicians should recognize acanthosis nigricans because it heralds disorders ranging from endocrinologic disturbances to malignancy. AN in association with high BMI (at or above the 85th percentile) is a sensitive screening tool for identifying children and youth with IR who are at increased risk for developing type 2 diabetes and other features of the metabolic syndrome [11].

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SUMMARY

ACANTHOSIS NIGRICANS IN ADOLESCENTS: A PRACTICAL APPROACH

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Acanthosis nigricans (AN) is a lesion affecting localized areas of the skin in persons with obesity and/or hyperinsulinemia. Biochemical mechanisms responsible for developing this hyperplastic lesion are unclear, but likely involve local cutaneous growth factors. It is associated with obesity, endocrinopathies (insulin resistance, diabetes mellitus, Cushing disease and acromegaly) and visceral malignancies. Clinicians should recognize AN because it may herald disorders ranging from endocrine disturbances to malignancy. Early recognition of these conditions is essential to identify children who are at highest risk for developing type 2 diabetes and further metabolic abnormalities.

Keywords: Acanthosis nigricans, assessment, adolescents.

РЕЗЮМЕ

АКАНТОКЕРАТОДЕРМИЯ У ПОДРОСТКОВ: ПРАКТИЧЕСКИЙ ПОДХОД

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Акантокератодермия (Acanthosis nigricans – AN) – поражение локализованных участков кожи чаще встречается у лиц с ожирением и/или гиперинсулинемией. Биохимические механизмы этих гиперпластических изменений неясны, возможно, определенную роль играют факторы роста кожи. AN ассоциируется с ожирением, эндокринопатиями (резистентность к инсулину, сахарный диабет, болезнь Кушинга и акромегалия), а также с злокачественными опухолями внутренних органов. Клиницисты должны уметь распознавать AN, так как она может предвещать ряд нарушений – от эндокринопатий до опухолей. Раннее распознавание этого состояния весьма важно для идентификации детей с высоким риском развития сахарного диабета типа 2 и дальнейших метаболических нарушений.

რეზიუმე

აკანტოკერატოდერმია მოზარდებში: პრაქტიკული მიდგომა

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აკანტოკერატოდერმია (Acanthosis nigricans – AN) კანის ლოკალური დაზიანებაა, რომელიც გვხვდება პაციენტებში სიმსუქნით და/ან ჰიპერინსულინემიით. ამ ჰიპერპლასტიური დარღვევის ბიოქიმიური მექანიზმები სადღეისოდ გარკვეული არ არის; შესაძლებელია, კანის ზრდის ფაქტორები გარკვეულ როლს ასრულებს. AN ასოცირებულია სიმსუქნესთან, ენდოკრინოპათიებთან (ინსულინისადმი რეზისტენტობა, შაქრიანი დიაბეტი, კუშინგის დაავადება და აკრომეგალია) და, ასევე, შინაგანი ორგანოების ავთვისებიან სიმსივნეებთან. კლინიცისტებს უნდა შეეძლოთ AN იდენტიფიცირება, ვინაიდან იგი შეიძლება მოასწავებდეს მთელ რიგ დარღვევებს - ენდოკრინოპათიებიდან სიმსივნე-ებამდე. ამ მდგომარეობის ადრეული დიაგნოსტიკა მნიშვნელოვანია შაქრიანი დიაბეტის ტიპი 2-ის და შემდგომი მეტაბოლური დარღვევების განვითარების მაღალი რისკის მქონე ბავშვების იდენტიფიკაციისათვის.

GROWTH HORMONE DEFICIENCY IN ADULTS WITH THALASSEMIA: AN OVERVIEW AND THE I-CET RECOMMENDATIONS

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Although growth hormone (GH) is secreted throughout life, its role in adulthood has been mainly studied in the last two decades.

GH is an important growth-promoting factor that has been shown to have metabolic, inflammatory and immunologic importance [15,33]. Adults with GH deficiency (GHD) experience lack of positive wellbeing, depressed mood, feelings of social isolation, decreased energy, and an overall poorer quality of life when compared with controls [4,33]. Their bone mineral density (BMD) is also reduced, resulting in increase in bone fracture rate [40,43,49,52].

Moreover, experimental studies suggest that GH and IGF-1 have stimulatory effects on myocardial contractility, possibly mediated by changes in intracellular calcium handling [21].

Short-term GH replacement therapy in adults has been associated with beneficial effects on body composition, fat distribution, cardiac function, BMD and quality of life [11]. It remains to be determined, however, whether or not chronic GH replacement therapy can indeed have beneficial effects on morbidity and mortality [57].

Significant barriers to treatment of adults who have GHD exist. These include a perceived difficulty in making a secure diagnosis, non standardized strategies for dosing and treatment monitoring, a lack of awareness or acceptance of data supporting efficacy, concern regarding the relationship of GH to side effects or complications, cost and need for daily injections.

This review paper provides a summary of the current state of knowledge regarding GHD in adults and gives some recommendations for the diagnosis and treatment of GHD in adult patients with thalassaemia major (TM).

GHD in adult TM patients

Adults with TM have several clinical manifestations comparable to those with GHD: muscle wasting, weakness and cramps, neuromuscular abnormalities, cardiomyopathy and cardiac complications, decreased bone density and osteoporosis associated with an increased risk of fracture, decreased muscle mass, abnormal fat metabolism, increased prevalence of impaired glucose tolerance or diabetes, decreased insulin sensitivity and premature senescence of cells (lymphocytes) [25,35,43,44,54].

Although anemia and chronic iron overload are important etiologic factors, other complications such as chronic liver diseases and endocrinopathies (hypothyroidism, hypoparathyroidism, insulin dependent diabetes, GHD and insulin like growth factor 1 (IGF-1) deficiency) may actively contribute to these clinical manifestations [28].

GHD or neurosecretory GH dysfunction, as well as reduced IGF-1 production, have been reported in children and adults with TM [16,50,51]. The documented GH-IGF-1 alterations in adult TM patients could be a continuation of the childhood GHD state or a newly developed deficiency with age [42,44,45,48,50-52].

In adult TM patients, the prevalence of GHD and /or IGF-I deficiency is relatively high and varies from 8% to 44% in different centres (Table). De Sanctis et al. [19] sent a questionnaire to 29 centres treating a total of 3817 TM patients. Thirty-six per cent of patients were over the age of 16 years. Short stature was present in 31.1% of males and 30.5% of females, and the prevalence of GHD was on average 7.9% in males and 8.8% in females. Analysis of these results indicate that GHD in TM patients probably is an age-related phenomenon and the risk of pituitary and/or hypothalamic dysfunction remains high in adult TM patients, despite improvements in the care of hematologic problems and treatment of iron overload.

These functional abnormalities of the GH/IGF-1 axis, in patients with TM, are accompanied by structural abnormalities of the pituitary gland (pituitary siderosis and atrophy) and its stalk [1,2].

The presence of hepatic siderosis with cirrhosis and the high incidence of chronic liver disease due to chronic hepatitis can explain in part the defective synthesis of IGF-1 in the liver [51,52].

Table. Prevalence of GH and IGF-1 deficiency in adults with thalassemia in different studies

Author and reference	Number of patients	GH provocative test	GHD %	IGF-1 %	Comments
Poggi et al (38)	28	Arginine + GHRH	32%	Low	The group affected by GHD showed a worse bone profile
Cavallo et al (7)	29	Clonidine and ITT	45%	Low	The reduction of GH reserve is more frequently due to a hypothalamic than to a pituitary dysfunction
Scacchi et al (42)	94	GHRH + arginine	41.4%	Low	80% of patients with normal GH reserve had low IGF-1 SDS. The reduced liver synthetic activity and low GH secretion are major determinant of low IGF-1 production. Biosynthetic GH replacement therapy in GHD thalassaemic adults is worth considering
Scacchi et al (43)	64	GHRH + arginine	42.1%	*Low	Analysis pointed to both GH peak and IGF-1 SDS as predictors of femoral T-score. Mean femoral T-score was significantly lower in patients with severe GHD than in those with normal GH
Pincelli et al (37)	25	GHRH + arginine	8%	72%	Patients with hepatitis C virus infection showed lower IGF-1 concentrations than uninfected subjects despite a normal GH reserve, suggesting partial GH insensitivity at the post-receptor level
La Rosa et al (30)	16	GHRH + arginine	19%	low	GH status should be retested in adult thalassaemic patients with childhood-onset GHD. If the diagnosis of adult GHD is established, GH treatment may be considered as it could contribute to improved heart function and BMD
Vidergor et al (58)	16	GHRH + arginine	25%	69%	The clinical benefits of GH therapy need to be determined. GHD alone does not account for the high prevalence of reduced IGF-1 in adult β thalassemia
De Sanctis et al (16)	33	Glucagon (GST)	44%	Low	86.6% of patients with normal GH response to GST had low IGF-1 level, indicative of a relative resistance to GH. 9% (n =3) of patients with GHD and normal T2* were found to have reduced LVEF

**IGF-1 < -1.88 SDS (54.6%)*

The relevance of the defective GH-IGF-I axis to the pathogenesis of cardiovascular disease in thalassemia

Cardiomyopathy remains the leading cause of death in patients with TM [15,33]. Despite regular transfusions, these patients have larger ventricular volumes, higher cardiac outputs and lower total vascular resistances. These hemodynamic findings may be related to chronic anemia and/or severe iron overload [39,47]. However, endocrinopathies like GH-IGF-1 deficiency, hypothyroidism and hypoparathyroidism may contribute to the heart pathology [55].

The role of GH and IGF-1 as modulators of myocardial structure and function are well established [6]. Receptors for both GH and IGF-1 are expressed in cardiac muscles [21]. Therefore, GH may act directly on the heart or via the induction of local or systemic IGF-1. In patients with GHD, GH administration dramatically improves cardiac function.The first case report of reversible severe heart failure associated with GHD due to primary pituitary failure improved within days on daily subcutaneous GH [14]. Furthermore, in a GHD patient with severe dilated cardiomyopathy, and reduced left ventricular ejection fraction (LVEF), intramuscular treatment with 4 IU of GH daily resulted in a marked improvement in myofibrillar content of myocardiocytes [24].

Limited information are available in the literature of TM patients with GHD and cardiac dysfunction.

A 21-year-old woman with TM developed end-stage heart failure within 3 months after withdrawal of GH. Intensive treatment with digoxin, angiotensin-converting enzyme inhibitor, diuretics and intensive iron chelation therapy with desferrioxamine did not improve her progressive heart failure. A myocardial biopsy excluded myocarditis and showed moderate iron deposit in the heart. GH was restarted and her heart failure reversed. One year later her cardiac function normalized [22].

The second case was an Italian 23-year-old man with GHD, insulin-dependent diabetes mellitus, hypogonadism and enlarged left ventricle. He was treated with GH and six months later physical examination, laboratory and cardiologic evaluation showed a reduction in abdominal waist adiposity, an amelioration of gluco-metabolic control, a reduction of LVEF and an improvement of ventricular motility [46].

In summary, the fact that untreated GHD, in the context of varying degrees of hypopituitarism, is associated with an adverse cardiovascular risk profile provides circumstantial evidence for a causative role of GHD in mediating increased rates of a cardiac dysfunction [36].

Therefore, clinicians taking care of TM patient should consider GHD as a potential risk factor for cardiac failure and should evaluate the potential favorable effect on cardiac performance of GH treatment in TM patients.

The relevance of the defective GH-IGF-1 axis to the pathogenesis of bone disease in thalassemia

Decreased bone mineral density (BMD) is a recognized phenomenon in adult hypopituitary patients and is associated with an increased fracture risk [15,33]. Measurements of markers of bone formation and bone resorption are consistent with a low bone turnover state in GHD. Deficits in bone mineral content and density are more striking in adults with childhood-onset GHD. Failure to achieve peak bone mass has important implications for the future development of osteoporosis and fracture risk.

Despite the extraordinary improvements in diagnostic and therapeutic management of thalassemia major osteoporosis in TM still represents a prominent cause of morbidity [43].

The pathogenesis of bone disease in TM is multifactorial and complex [29,56]. Peak bone mass is achieved shortly after completion of puberty and normally remains stable until age-related bone mass begins.

GH and sex steroids play a crucial role in bone remodeling and in the maintenance of skeletal architecture during adult life. GH and insulin like growth factors have anabolic effect in bone formation. Sex steroids act probably by increasing the expression of RANKL by osteoblastic cells and alterations in the RANK/RANKL/OPG system in favor of osteoclasts [56].

In TM patients, like in panhypopituitrism, impaired GH secretion and lack of sex steroids due to pituitary damage contribute to failure of achieving optimal peak bone mass [20]. In addition, available evidence indicates that qualitatively similar changes in BMD are found in adult-onset isolated GHD as in panhypopituitrism, therefore supporting a role for GHD in the pathogenesis. Furthermore, these abnormalities in bone metabolism and bone density are favorably influenced by GH replacement therapy [8].

Considering the relatively high prevalence of GHD among children and adult TM patients, the possible role of GH-IGF-I abnormalities in the pathogenesis of the osteopenia/osteoporosis of this disease is raised and investigated in children with TM. In two different studies on 30 and 29 TM children the BMD at femoral neck and lumbar vertebrae was highly correlated with the circulating concentrations of IGF-1 and IGFBP-3, as well as with the auxological parameters [31,49,52].

A study on the relation between GH secretion and BMD in 61 adult TM subjects confirmed the high prevalence of both osteopenia/osteoporosis and GH-IGF-1 deficiency in these patients and indicated that defective GH secretion and diminished serum IGF-1 levels may contribute to femoral demineralization [43]. Another study showed progressive decrease of BMD with progression of age in TM patients (osteopenia increased from 20% in age group 17-20 years; to 40% in age group 21-25 years and 100% in age group 26-30 years) [29].

In summary, the relative high frequency of bone disease associated with GH-IGF-1 axis abnormalities and the evidence of a positive effect of GH on bone accretion in adult GHD endorse GH therapy as a preventive and therapeutic tool for low BMD. However, it is important to note that the effects on fractures are not yet fully proven. Therefore, randomized trials that compare homogenous groups of GHD patients are still needed.

The relevance of the defective GH-IGF-1 axis to the pathogenesis of impaired glucose tolerance in thalassemia

GH reduces insulin sensitivity (IS), whereas IGF-1 increases it. IGF-1 seems to be critical for the development of the β-cells, and impaired IS has been reported in GHD. The frequency of changes in insulin sensitivity varies considerably and is associated with age and abdominal adiposity. GH replacement therapy has often been proposed to restore IS in these patients [33,53].

The relation between GH-IGF-I deficiency and glucose homeostasis in adults with TM and GHD and the effect of GH therapy on glucose metabolism have not been studied.

One of our peripubertal patients developed an impaired glucose tolerance (IGT) [18] and one patient reported by Gallisai (unpublished data, 1994) developed IDDM during treatment with GH. Therefore, the possibility of IGT should be excluded before initiating GH treatment and blood glucose levels should be regularly monitored in order to exclude any adverse influence of treatment on glucose homeostasis.

Growth hormone diagnosis in adults

International consensus guidelines have focused on insulin tolerance test (ITT) and growth-hormone releasing hormone (GHRH) + arginine test as the best available tests for the diagnosis of GHD in adults [11,26,27].

When ITT is contraindicated or GHRH is not available or a hypothalamic GH deficiency is suspected, glucagon stimulation test (GST) is an alternative option [30].

The GST can also provide co-assessment of ACTH reserve. Although the GST is safe, with almost no contraindications, it causes nausea and sometimes vomiting in 15-20% of subjects [26,27].

The presence of obesity (especially abdominal), which increases with advancing age throughout adulthood, creates significant difficulties in diagnosing GHD as the obese state is associated with poor GH responsiveness to secretagogues. Corneli et al. have defined BMI-specific cut-off points for diagnosing adult onset GHD using GHRH + arginine=11.5 ng/mL for those with BMI <25 kg/m^2; 8.0 ng/mL, for BMI 25-30 kg/m^2; 4.2 ng/mL for those with BMI >30 kg/m^2 [12].

Many studies support the notion that IGF-1 levels of 1 SD below the mean for men and women at various ages in their life can be used in the context of a patient's total clinical assessment as an indication of GHD. However, a normal IGF-1 value does not exclude the diagnosis of GHD, as a significant overlap of IGF-1 levels between normal subjects and GHD patients has been noted [11,26].

Diagnosis of GHD in adult thalassemic patients and the I-CET recommendations

Confirmation of persisting GHD at the time of completion of linear growth or an acquisition of GHD in adult TM patients is important for a number of reasons reported above.

Because GH treatment requires analysis of many factors, including efficacy of treatment on cardiac function, metabolic parameters, psychosocial functioning, safety, ethical considerations, financial cost and other burdens of therapy, stringent diagnostic criteria are needed. The following recommendations are suggested by the International Study Group of Endocrine Complications in Thalassaemia (I-CET) [17] for adult TM patients:

1. Selecting thalassemic patients for GH testing and possible therapy appears to be difficult because of the occurrence of many symptoms and signs in thalassemia that overlap with those for adult GHD.
2. Clinical and laboratory parameters that favour performing GH stimulation test include: short stature (HtSDS <-2.5), severe and/or prolonged iron overload, presence of severe osteoporosis and/or serum IGF-I level <-2SDS for age and sex (Soliman, submitted for publication).
3. In consideration of all controversy over the best method to diagnose GHD, we believe that two stimulating tests are necessary and required for an accurate diagnosis of GHD in adult TM patients [37].
4. The ITT test has been the stimulation test used by most endocrinologists to diagnose GHD in adults. Insulin-induced hypoglycaemia, however, may pose significant risks in the setting of any known cardiovascular disease that is quite common in TM patients [39].Therefore, alternative GH stimulation tests must be used. The co-administration of arginine and GH-RH is a powerful stimulus for GH release and glucagon stimulation test is a reliable and safe alternative choice to ITT [26,34]. Glucagon is administered intramuscularly and serum samples are taken up to 240 minutes.
5. Adults who have GHD may have normal responses to GHRH + arginine in the setting of hypothalamic GHD [50,51].
6. Adjustment of peak GH cut-off values for assay differences has been recommended by International Consensus Guidelines for the diagnosis of adult GHD [6,9,10,13]. Reference Preparation (98/574) is used in all GH assays. It should be specified if GH isoforms (e.g. 20 and 22kDa GH) or GH binding protein might interfere [9]. A GH response, to provocative stimulation test <3 ng/mL, when measured by polyclonal antibody (RIA), or less than 2.5 ng/mL, when measured by monoclonal antibody (IRMA), is suggestive of severe GHD [3,26].
7. GHD during the transition period: proposed intervals between cessation of GH treatment and re-testing of the GH axis generally range from 1 to 3 months. During this phase a cut-off level of <5 ng/L is considered as compatible with a diagnosis of GHD. We also recommend optimization of all other hormone therapies, BMI and pubertal status of patient (recognizing that the greatest amount of GH is produced during puberty) for an accurate interpretation of GH test.
8. If provocative testing demonstrates severe GHD, consideration of GH therapy can be raised and discussed in detail with the patient (the pros and cons).
9. GH stimulation testing is not strictly required in TM patients with cardiac failure in presence of a normal MRI cardiac T2* and/or a personal history of childhood-onset GHD.
10. In chronic liver disease, IGF-1 levels are decreased, and the circulating levels correlate to the extent of hepatocellular dysfunction.

11. Very low IGF-1 levels, especially in those patients with childhood-onset GHD, in the presence of pituitary iron deposition and/or atrophy are suggestive of GHD [5].

Potential benefits and adverse effects of GH therapy in adults with GHD including thalassemics

Potential benefits of GH therapy include [9,32,34]:
1. Improvement of bone mineral accretion and possible prevention and/or amelioration of osteopenia.
2. Inotropic effect on the heart and possible prevention and/or amelioration of cardiac function.
3 Anabolic effect on skeletal muscles, lean body mass and exercise capacity.
4. Improvement of physical fitness.
5. Improvement of psychological well-being.

Potential adverse effects of GH therapy include [9,32,34]:
1. Arthralgia, myalgia, fluid retention and carpal tunnel syndrome.
2. Benign intracranial hypertension.
3. Possible increased risk of neoplasia due to mitogenic effects of IGF-1.
4. Impaired glucose tolerance and increased insulin resistance.
5. GH replacement can lower FT4 levels and also inhibit 11- beta hydroxysteroid dehydrogenase resulting in an increase in cortisone production and subsequent decreases in cortisol production. These changes are small and typically not clinically significant. However, it is possible that secondary hypothyroidism and hypoadrenalism may be unmasked in susceptible patients. Those patients already on thyroid and cortisol replacement due to pituitary insufficiency will need to be monitored closely for signs and symptoms indicating a need for increased dosages of these hormones.
6. GH treatment increases cytochrome P450 (CP450) and may alter the clearance of compounds known to be metabolized by CP450 liver enzymes (e.g., corticosteroids, sex steroids, anticonvulsants).

Treatment of GH deficiency in adults with thalassemia
Patient selection
All TM patients with documented severe GHD are eligible for GH replacement. The goal for GH replacement in adults is to correct the abnormalities associated with GHD [9,10,13,23,34].

Proper blood transfusion and iron chelation as well as correction of all nutritional deficits and adequate hormone replacements for other deficiencies (sex steroids, thyroxin, and insulin) are essential prerequisites before starting GH therapy [19].

Each clinician must base treatment decisions for each patient on the clinician's independent judgment, knowledge of the patient's circumstances, and continuing developments in the field.

Basal evaluation, before GH treatment, should include clinical (height, weight, BMI, waist circumference, pubertal status), laboratory, instrumental and radiologic evaluation. Biochemical parameters include measurement of serum ferritin and fructosamine levels, oral glucose tolerance test, liver function tests, hepatitis screening and antitransglutaminase antibodies. Endocrine evaluation includes: measurement of serum IGF-1, TSH, FT4, cortisol and sex steroids. Cardiac evaluation includes: echocardiography and T2* MRI of the cardiac iron overload. BMD and muscular strength, endurance and flexibility are important to assess. Evaluation of quality of life using adult GHD assessment questionnaire and energy status questionnaire is recommended [32,41].

Dose selection
The objective of treatment is to maximize benefit and minimize side-effects. Experience has shown that sensitivity to GH treatment varies considerably between individuals, with elderly individuals being the most sensitive. However, some degree of GH resistance has been reported in TM patients [17,19,48,50].

Dosing of GH replacement therapy in all patients should be individualized. It is recommended that therapy should start with a low dose (0.15-0.30 mg/day; 0.45-0.90 IU/day). The dosage should be increased gradually every one to two months, on the basis of clinical and biochemical responses. The target is achieving an optimal clinical response without any side effects. Clinically significant effects may not be seen before few months of treatment. Biochemically IGF-1 levels is adjusted to be in the upper half of the age-adjusted reference range [9,10,13,34]. Therefore, a high degree of methodological consistency in the assay and a specific reference ranges for IGF1 are essential for the interpretation of results. Furthermore, careful consideration of factors influencing IGF1 levels in adult TM patients is crucially important for correct interpretation of IGF1 levels.

The maintenance dose may vary considerably from person to person and seldom exceeds 1.0 mg/day (3 IU/day) [9,10,13,23,34]. Clinical experience has demonstrated that the variability in subcutaneous absorption and individual responsiveness to GH make dose determination based on body weight or body surface area less helpful than anticipated. Besides, some adult patients experience side-effects even with a low dose [9,10,13,23,32,34].

In accordance with the clinical practice of treating GHD children, we recommend that GH be administered as daily SC self-injections in the evening.

During puberty, adolescents with GHD typically receive GH in a wide range of doses (usually between 1.25 and

2.5 mg/day). Whereas in the transition period, patients should reduce their GH dose. If GH is already discontinued, patients can restart GH therapy at a dose of 0.2-0.5 mg/day [34].

GHD and multiple hormone deficiencies
Standard hormonal replacement therapy should be monitored closely when GH therapy is administered. Testosterone appears to stimulate IGF-1 production [39]. In contrast, oral administration of estrogen increases GH secretion and decreases serum IGF-1 concentration. Therefore, estrogen replacement blunts the IGF-1 response to GH replacement in women, whereas in men, androgen replacement increases IGF-I responsiveness over time.

In patients with GH, central (secondary) hypothyroidism may first become evident or worsen during GH treatment. Therefore, patients treated with GH should have periodic thyroid function tests and thyroid hormone replacement therapy should be initiated or appropriately adjusted when indicated [9,10,13,23,32,34,41].

Follow-up
It is recommended that TM patients with GHD receiving GH replacement should remain under long term surveillance by an endocrinologist to monitor their response as well as to detect possible long-term side-effects early. Patients may need to be seen initially by the endocrinologist as often as monthly. Once treatment is stabilized, two –three visits per year will suffice.

GH therapy in adults with GHD and cancer risk:
The growth promoting effects of GH and IGF-1 provide a plausible theoretical basis by which GH treatment could increase cancer risk. Overall, the published data so far do not fully suggest that GH therapy is associated with causing or accelerating recurrences of tumors. However, it is that some adverse events may become evident over time and, therefore, continued surveillance remains mandatory in patients on GH therapy [11].

Conclusions
GHD is now well-recognized in many adult patients with TM. This clinical syndrome can be corrected by proper GH replacement. Investigating TM patients within the appropriate clinical context is important to identify those who may be eligible for treatment. Each doctor must base treatment decisions for each patient on the clinician's independent judgment, knowledge of the patient's circumstances, and continuing developments in the field to provide maximal safety to the patient. Dynamic tests for investigating GHD should only be performed in patients in whom there is high clinical suspicion and therapy should be limited to those with biochemically proven GHD. The pros and cons of GH treatment must be discussed with each patient, after which GH doses should be individualized and titrated to maximum efficacy with minimal side effects. Prospective studies to monitor potential benefits versus possible side-effects will enable endocrinologists to define recommendations on dosage and the long term effects, particularly on cardiovascular and bone status of GH therapy in adult TM patients.

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SUMMARY

GROWTH HORMONE DEFICIENCY IN ADULTS WITH THALASSEMIA: AN OVERVIEW AND THE I-CET RECOMMENDATIONS

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This review paper provides a summary of the current state of knowledge regarding GHD provides recommendations for the diagnosis and treatment of GHD in adult patients with thalassaemia major (TM). The reported prevalence of

adult GHD and /or IGF-I deficiency in TM patients varies from 8% to 44 % in different centers. Because GH treatment requires analysis of many factors, including the effect of treatment on cardiac functions, metabolic parameters and psychosocial functioning, along with safety, ethical considerations, financial cost and other burdens of therapy, stringent diagnostic criteria are needed. The authors report the diagnostic recommendations of the International Study Group of Endocrine Complications in Thalassemia (I-CET) for adult TM patients.The pros and cons of GH treatment must be discussed with each patient, after which GH doses should be individualized and titrated to maximum efficacy with minimal side effects. Prospective studies to monitor potential benefits versus possible side-effects will enable endocrinologists to define recommendations on dosage and the long term effects, particularly on cardiovascular and bone status of GH therapy in adult TM patients.

Keywords: Thalassemia major, adults, growth hormone (GH), insulin like growth factor-1 (IGF-1), GH deficiency, IGF-I deficiency, diagnosis, therapy.

РЕЗЮМЕ

ДЕФИЦИТ ГОРМОНА РОСТА У ВЗРОСЛЫХ БОЛЬНЫХ ТАЛАССЕМИЕЙ: ОБЗОР ЛИТЕРАТУРЫ И РЕКОМЕНДАЦИИ МЕЖДУНАРОДНОЙ ИССЛЕДОВАТЕЛЬСКОЙ ГРУППЫ ПО ИЗУЧЕНИЮ ЭНДОКРИННЫХ НАРУШЕНИЙ ПРИ ТАЛАССЕМИИ (I-CET)

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В обзорной статье представлено современное состояние вопроса относительно дефицита гормона роста (ДГР), даны рекомендации по диагностике и лечению ДГР у взрослых пациентов с большой талассемией (БТ). По данным различных авторов, частота ДГР и/или дефицита инсулиноподобного фактора роста 1 у больных БТ варьирует в пределах от 8 до 44%. При лечении гормоном роста (ГР) требуется анализ многочисленных факторов, включая действие ГР на функции сердца, метаболические параметры, психо-социальные функции; следует учитывать безопасность лечения, этические соображения, цену и другие трудности терапии. Поэтому необходимы точные диагностические критерии. Авторы представляют диагностические рекомендации Международной исследовательской группы по изучению эндокринных нарушений при талассемии (I-CET) для взрослых пациентов с БТ. Аргументы за и против лечения ГР должны быть обсуждены с каждым пациентом, после чего доза препарата должна быть индивидуализирована и откорректирована для достижения максимальной эффективности при минимуме побочных действий. Проспективные исследования с целью мониторинга потенциальных пользы и вреда (развития побочных эффектов) лечения позволят уточнить рекомендации по дозировке и прогнозированию долгосрочных результатов лечения ГР, в частности, его действия на состояние кардиоваскулярной системы и костей у взрослых пациентов с БТ.

რეზიუმე

ზრდის ჰორმონის დეფიციტი თალასემიის მქონე მოზრდილ პაციენტებში: ლიტერატურის მიმოხილვა და თალასემიის დროს ენდოკრინული დარღვევების შემსწავლელი საერთაშორისო ჯგუფის (I-CET) რეკომენდაციები

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მიმოხილვით სტატიაში მოცემულია ცოდნის თანამედროვე მდგომარეობა ზრდის ჰორმონის დეფიციტის (ზჰდ) შესახებ, წარმოდგენილია დიდი თალასემიის (დთ) მქონე მოზრდილ პაციენტებში ზჰდ დიაგნოსტიკისა და მკურნალობის რეკომენდაციები. სხვადასხვა ცენტრის მონაცემებით,

დთ მქონე პაციენტებში ზჰდ და/ან ინსულინის მსგავსი ზრდის ფაქტორი 1-ის სიხშირე 8-44%-ია. იმის გათვალისწინებით, რომ ზრდის ჰორმონით (ზჰ) მკურნალობა მრავალი ფაქტორის გათვალისწინებას მოითხოვს (მკურნალობის ეფექტი გულის ფუნქციაზე, მეტაბოლურ პარამეტრებზე, ფსიქოსოციალურ ფუნქციებზე, უსაფრთხოება, ეთიკური მოსაზრებები, ფასი და მკურნალობის სხვა სიძნელეები), საჭიროა ზუსტი სადიაგნოსტიკო კრიტერიუმების შემუშავება. სტატიაში მოცემულია თალასემიის დროს ენდოკრინული დარღვევების შემსწავლელი საერთაშორისო ჯგუფის (I-CET) სადიაგნოსტიკო რეკომენდაციები დაავადებული მოზრდილი პაციენტებისათვის. არგუმენტები მკურნალობის დასანიშნად და მის საწინააღმდეგოდ თითოეულ პაციენტთან მიმართებით უნდა იყოს განხილული. ზჰ-ის დოზა უნდა იყოს ინდივიდუალიზებული და მისადაგებული იმდაგვარად, რომ მიღწეულ იქნას მაქსიმალური კლინიკური ეფექტი გვერდითი შედეგების მინიმალიზებით. აუცილებელია პროსპექტული კვლევების ჩატარება ზჰ მკურნალობის პოტენციური სარგებლისა და მავნეობის (გვერდითი ეფექტების) მონიტორინგისათვის. ეს შესაძლებელს გახდის დაიხვეწოს დოზირებისა და მკურნალობის გრძელვადიანი ეფექტების პროგნოზული რეკომენდაციები, განსაკუთრებით - ზჰ თერაპიის ზეგავლენის შესახებ გულ-სისხლძარღვთა სისტემის და ძვლების მდგომარეობაზე დთ დაავადებულ მოზრდილ პაციენტებში.

HIGH PREVALENCE OF CENTRAL HYPOTHYROIDISM IN ADULT PATIENTS WITH B-THALASSEMIA MAJOR

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Over the course of the past 2-3 decades, hypertransfusion regimens and iron chelation therapy have significantly increased the life expectancy and improved quality of life of patients with thalassaemia major (TM) (22). On the other hand, frequent blood transfusions leading to iron overload and the chronic nature of the disease have contributed to a whole new spectrum of complications in adolescents and young adults suffering from TM [24].

In 1995, our Italian Working Group on Endocrine Complications in TM reported delayed puberty in 47% of females and 51% of males, arrested puberty in 12.6% of females and 15.7% of males, secondary amenorrhea in 25% of adult females, diabetes mellitus in 16.8%, impaired glucose tolerance in 13%, hypothyroidism in 15.9% and hypoparathyroidism in 6.3% in a large cohort of thalassemic patients [21]. These complication are mainly attributed to iron overload and chronic liver diseases [4,6,9,21,26].

The commonest form of thyroid dysfunction seen in subjects with TM is primary hypothyroidism due to abnormalities of the thyroid gland which leads to insufficient production of the thyroid hormones [21,26]. Thyroid failure is expected to be more prevalent in older patients (as seen in other endocrine deficits). However, in developing countries it may occur at younger ages as reported by Rindang et al. [25] and Malik et al. [19].

Central hypothyroidism (CH) seems to be an uncommon clinical entity [7,10,16] although the anterior pituitary gland is particularly sensitive to free radical oxidative stresses and MRI shows that even a modest amount of iron deposition within the anterior pituitary can interfere with its function [4]. The diagnosis of CH in TM is not easy because most of the symptoms are nonspecific and frequently are attributed to anaemia or other associated complications [2,12,18].

Therefore, we explored the prevalence of CH in a large group of TM subjects of different ages, followed in our Centres or Outpatient Clinics.

Material and methods. 339 TM patients who were regularly blood transfused to maintain a mean haemoglobin level of 10 g/dl were screened between June 2011 and September 2012,. Chelation therapy with desferrioxamine (DFX) or deferiprome was given to all TM patients, from

the prepubertal age or peripubertal age, in order to reduce the entity of iron overload. The diagnosis of TM was based on the usual haematological criteria, Hb electrophoresis and the need for regular blood transfusions [21].

The following clinical and laboratory data were registered: age, sex, age at first transfusion, age at start of regular chelation therapy , duration of iron chelation therapy , compliance to treatment, serum ferritin level in the last year, hepatitis C virus seropositivity (HCVab and HCV-RNA positivity), free thyroxine (FT4) and thyrotropin (TSH) , anti-thyroglobulin and anti-microsomal antibodies, and the presence of associated growth and endocrine complications (height <3rd percentile, hypogonadism, diabetes insulin-dependent and hypoparathyroidism).

The following exclusion criteria were used:
1. Patients with a diagnosis of thalassaemia intermedia.
2. Patients with acute illness and/or euthyroid sick syndrome. The laboratory parameters of this syndrome includes low serum levels of triiodothyronine (T3 /FT3) and a high level of reverse T3, with normal or low levels of thyroxine (T4/FT4) and normal or low levels of TSH [12].
3. Negative anti-thyroglobulin and anti-microsomal antibodies.
4. The use of pharmacologic agents that may affect TSH or interfere with thyroid function, metabolism and transport [2,11,12,17].

Hormonal data (FT4 and TSH) were compared to those of a group of normal 20 control subjects (31±4 years) and 20 TM patients (mean age 24±3 years) with normal thyroid function (FT4 and TSH in the normal range).

The diagnosis of CH, was based on the following findings: a low free thyroxine level (FT4 below normal reference range) with a low or "inappropriately normal" basal thyrotropin concentration (TSH) as verified at least in two consecutive measures [1,2,11,12].

Serum ferritin level was arbitrarily categorized as good, fair and poor if the levels were <1000 ng/ml, from 1000 to 2000 ng/ml and >2000 ng/ml, respectively (normal level <150 ng/ml).

Ethical approval and informed consent, according to the Declaration of Helsinki, were obtained from each patient and healthy control who participated to the study.

Blood samples were drawn in the morning after an overnight fasting and at least 2-3 weeks after the last blood transfusion.

Samples for TSH and FT4 were assessed with an electrochemoluminescence (ECLIA) assay with a normal range of 0.8-1.8 ng/dL for the FT4 and 0.5-4.6 mIU/l for the TSH. The minimal detectable levels of FT4 and TSH were 0.2 ng/dL, and 0.1 mIU/L, respectively. The inter-assay and intra-assay coefficients of variations of FT4 varied from 5.8% to 6.26%, and from 2.6% to 2.9%, respectively, and those of TSH were from 5.1% to 5.7%, and from 2.2% to 2.9%, respectively.

Characteristics of the studied patients are reported as mean ± standard deviation (SD), median, number and range. Statistical significance of the differences between variables was assessed using the unpaired two-tailed Student's t test or Wilcoxon test using a software package program. A p value < 0.05 was considered as significant. The analysis of frequency distributions for age and sex were analyzed using chi-square test while the multiple regression analysis was conducted using the multiple linear fitting with least squares method.

Results and discussion. Of the 339 cases of TM, 164 (48.3%) were males and 180 (53%) females, with an age range of 1- 48 years (85.2% patients were older than 21 years).

Twenty five were prepubertal (<11 years;13 males), 9 were in peri-pubertal age (between 11 and 16 years; 3 males) and 305 were pubertal (>16 years ; 164 males).

Central hypothyroidism was diagnosed in 26 (7.6%) of patients. Their mean age was 29.9±8.4 years (median 29.1 years). The mean age of TM patients with CH was 30.8±5.8 in males and 28.8±10.9 years (p=NS). The prevalence of CH was 0 % in young patients below 11 years, 22% in peri-pubertal patients and 7.8% in those above 16 years. Twenty two patients (78.5%) were HCV antibody positive and 11 patients (42.3%) HCV –RNA positive

Among the 26 TM patients with CH, 14 (53.8%) were males and 12 (46.1%) were females, with no significant gender difference in the frequency of CH (p=NS). However, the 3 youngest TM patients with CH were females (aged 14, 15 and 18 years, respectively).

Mean FT4 level in TM patients with CH was 0.74±0.08 ng/dL significantly lower when compared to euthyroid TM patients 1.16±0.17 ng/dL and normal controls 1.19±0.17 ng/dL (p<0.01 and p<0.01, respectively).

No specific clinical signs or symptoms of hypothyroidism were reported by the patients.

Serum ferritin levels were "good" in 11 (42%) TM patients with CH and in 5 (25%) TM patient with normal thyroid function, fair in 6 (23%) TM patients with CH and in 8 (40%) TM patient with normal thyroid function and poor in 8 (30.7%) TM patients with CH and in 7(35%) TM patient with normal thyroid function.

Serum ferritin level did not differ significantly between TM patients with CH (1790.7±1872.8 ng/ml) and the euthyroid TM patients (2142.1±2075.2 ng/ml) (p=0.08).

Multiple correlation analysis between FT4, TSH, serum ferritin, serum alanine transferase (ALT) and age, was not significant (F=0.9; p=NS).

Twelve TM patients (46%) with CH had an associated hypogonadotrophic hypogonadism (7 males) , four (15.3 %) had short stature (height < 3rd centile; 1 male) , two (12.5%) had insulin dependent diabetes mellitus (1 male and 1 female) and one patient (3.8%) had hypoparathyroidism (1 female).

Nine TM patients (34.6%) with CH were on treatment with L-thyroxine (mean daily dose 40 mcg; range from 12,5 to 75 mcg/ daily).

Central hypothyroidism (CH) has been reported as an uncommon clinical entity in TM patients although the anterior pituitary gland is particularly sensitive to free radicals of oxidative stresses [4,7,13,31]. The presence of CH in TM patients is reported infrequently in the literature, with a prevalence between 2.3% to 8% [10,16].

Clinically, CH in TM is not easy to diagnose because most of the symptoms (fatigue, apathy, weight gain, dry skin, cold intolerance) are non- specific and frequently are attributed to anaemia or other associated complications [2,12,18]. The diagnosis of CH is usually made on a biochemical basis (low FT4 associated to inappropriately low or normal TSH) [1,2,12,18].

We performed a cross-sectional analysis using a large database from the clinical records of our TM patients aiming to look at the prevalence of CH in prepubertal (<11 years: 25 patients; 13 males) peri-pubertal (between 11 and 16 years: 9 patients; 3 males), and pubertal TM subjects (> 16 years: 305 patients; 164 males).

CH was present in 26 (7.6%) of TM patients; 14 (53.8%) were males and 12 (46.1%) were females. Their mean age was 29.9 years. The prevalence of CH was 6 % in patients aged below 21 years and 7.9 % in those above 21 years.

This prevalence of CH may be even higher if some of our patients were growth hormone deficiency (GHD). GHD can mask CH in a significant proportion of hypopituitary patients [1]. In a multicenter study we found a severe GHD in 44% of patients with TM [8].

In the general population, the CH prevalence is estimated to be around 1 in 120,000 individuals, and roughly equal in both genders and can arise from a number of pathogenic mechanisms involving the hypothalamus or pituitary [12,18]. Etiology includes all pathologic processes that affect the hypothalamus or pituitary including tumours, trauma, radiation, vascular diseases, infection, lymphocytic hypophysitis, and idiopathic infiltrative diseases; such us hemochromatosis [12,18,20]. Imaging of the brain and the pituitary can help to diagnose the cause.

The precise underlying mechanism of CH in TM patients at present remains not well-known. The mean serum ferritin level was not significantly different between our patients with and without CH, however we cannot exclude iron overload of the hypothalamic- pituitary (central) as an important etiology of CH in our patients because certain tissues, including the pituitary gland, are particularly susceptible to excess iron incorporation when non-transferrin-bound iron (NTBI) is present. This effect is promoted by L-type Ca2+ channels (LTCCs), the front-runners for mediating NTBI transport in iron overload conditions, which are moderately expressed in thyrotrophs [3,14,28]. In addition ferritin is not a sensitive indicator of iron overload in all the tissues.

Tatò et al. [29] studied 14 euthyroid iron- overloaded TM patients (8 females and 6 males, age 15-24 years) with hypogonadotropic hypogonadism . Thyroid-stimulating hormone (TSH), prolactin and free alpha-subunit (FAS) were measured during thyrotropin-releasing hormone (TRH) stimulation test. They observed poor response of FAS to TRH test which they attributed to an involvement of thyrotroph cells.

Chronic liver inflammation is a frequent complication in patients with TM, since over 40% of them have positive anti-hepatitis C virus (HCV) antibodies and more than 50% have chronic (persistent or active) hepatitis [5,27]. Hepatitis C infection may be an additional factor in causing hypothalamic-pituitary thyroid axis dysfunction. A central (hypothalamic-pituitary) dysfunction with growth hormone deficiency, secondary to hepatitis C infection, has been reported by Plöckinger et al [23] in 81% patients with hepatitis C infection before therapy with pegylated interferon-alpha plus either ribavirin or levovirin [4,7,20].

Other alternative explanations for the central etiology of hypothyroidism in addition to different individual sensitivity to iron damage [9], includes increased collagen deposition secondary to increased activity of the iron-dependent protocollagen proline hydroxylase enzyme [15,30] and the hypoxic effect of chronic anemia [6]. The latter cause was excluded in our TM patients who were regularly transfused with packed red blood cells.

In summary, the combination of transfusion and chelation therapy has dramatically extended the life expectancy of these patients, thus transforming TM from a rapidly fatal childhood disease to a chronic life-time illness compatible with longivity. On the other hand, frequent blood transfusions and poor compliance to chelation therapy leads to chronic iron overload that contributed to a whole new spectrum of complications in adolescents and young adults. Central hypothyroidism is not uncommon in young adult TM patients. The diagnosis of CH in TM patients is not clinically noticeable and a normal basal TSH level does

not exclude the diagnosis of CH. Because this pattern is also seen transiently during recovery from severe illness, it the diagnosis should be confirmed on a repeat test when the patient is well.

Clinicians should be alert for the diagnosis of CH through accurate interpretation of thyroid function tests. Brain imaging studies of the hypothalamic-pituitary region could be of help in the investigations of these patients by detecting pituitary iron infiltration (hypointense pattern or heterogeneous intensity) with decreased pituitary volume (Figs. 1, 2).

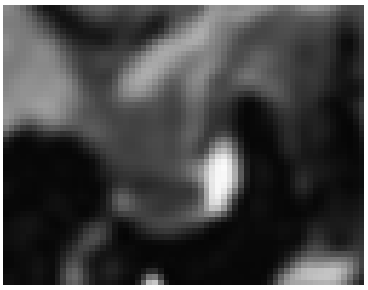

Fig. 1. T1 appearance of he pituitary gland in a thalassemic patient. Diminished pituitary volume with marked hypointense pattern (Soliman A., personal observation)

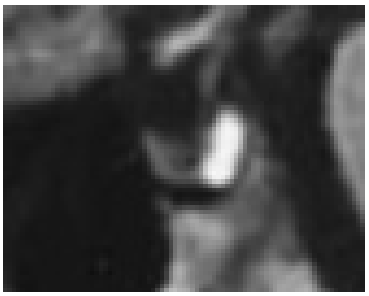

Fig. 2. T1 appearance of the pituitary gland in a thalassemic patient. Diminished volume with heterogeneous intensity (Soliman A., personal observation)

Our recommendation is that if the level of FT4 is consistently low, then these patients should start L-thyroxine treatment. Adrenal function shall be investigated before initiating treatment and adrenal insufficiency shall be treated with glucocorticoid replacement before thyroxine therapy to avoid precipitating an adrenal crisis.

Data from the literature have shown that GH deficiency may mask subclinical forms of CH that become biochemically evident only after institution of GH replacement therapy [17]. Replacement therapy for other pituitary hormone deficiencies may require an adjustment of T4 replacement dose. More specifically, females treated with estrogen and males treated with GH may need a higher T4 dose in order to maintain an euthyroid range [2]. All these factors must be taken in consideration in the management of TM patients with CH.

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SUMMARY

HIGH PREVALENCE OF CENTRAL HYPOTHYROIDISM IN ADULT PATIENTS WITH B-THALASSEMIA MAJOR

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The commonest form of thyroid dysfunction seen in subjects with TM is primary hypothyroidism due to abnormalities of the thyroid gland. Central hypothyroidism (CH) has been reported as an uncommon clinical entity in TM patients although the anterior pituitary gland is particularly sensitive to free radical oxidative stresses. Diagnosis is usually made on a biochemical basis showing low circulating concentrations of thyroid hormone associated with an inappropriately low TSH levels. The diagnosis is not clinically obvious and a basal normal TSH level does not exclude the diagnosis of CH. Therefore, it is important that clinicians accurately interpret thyroid function tests. In TM patients, CH prevalence differs at different ages is unknown and it is not easy to diagnose because most of the symptoms of symptoms of CH are non specific and are frequently attributed to anaemia or other associated complications . We performed a cross-sectional analysis on a large database using the clinical records of our TM patients to explore the prevalence of CH in prepubertal (<11 years: 25 patients; 13 males) peripubertal (between 11 and 16 years: 9 patients; 3 males), and pubertal TM subjects (>16 years: 305 patients; 164 males). Central hypothyroidism was present in 26 (7,6%) TM patients. Their mean age was 29.9±8.4 years, 14 (53.8%) were males and 12 (46.1%) were females. The prevalence of CH was 6% in patients with a chronological age below 21 years and 7.9% in those above 21 years. Clinicians should be alert for the diagnosis of CH through accurate interpretation of thyroid function tests. We recommend L-thyroxine therapy if the level of FT4 is consistently low provided that the patient has normal cortisol levels.

Keywords: β-thalassemia major, central hypothyroidism, prevalence.

РЕЗЮМЕ

ВЫСОКАЯ ЧАСТОТА ЦЕНТРАЛЬНОГО ГИПОТИРЕОИДИЗМА У ВЗРОСЛЫХ ПАЦИЕНТОВ С БОЛЬШОЙ β-ТАЛАССЕМИЕЙ

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Наиболее частая форма тиреоидной дисфункции у лиц с большой талассемией (БТ) – первичный гипотиреоидизм развивается вследствие изменений в щитовидной железе. Центральный гипотиреоидизм (ЦГ) встречается более редко, хотя передний гипофиз особенно чувствителен к свободнорадикальному оксидативному стрессу. Диагноз ЦГ обычно ставится на основании результатов биохимических исследований (низкое содержание тиреоидного гормона в крови при несоответственно низком уровне TSH – тиреотропного гормона). Диагноз не совсем ясен клинически, базальный нормальный уровень TSH не исключает диагноза ЦГ. Следовательно, весьма важно аккуратно интерпретировать результаты функциональных тестов щитовидной железы. У больных БТ частота ЦГ различна в различных возрастных группах. Постановка диагноза затруднительна, так как симптомы ЦГ в большинстве своем неспецифичны и часто обусловлены анемией и другими ассоциированными осложнениями. Проведен кросс-секционный анализ большой базы данных (клинические записи о наших больных БТ) с целью определения частоты ЦГ в препубертатном (<11 лет, 25 пациентов, 13 мальчиков), перипубертатном (с 11 до 16 лет: 9 пациентов, 3 юношей) и пубертатном (> 16 лет, 305 пациентов, 164 мужчин) возрастных периодах. ЦГ был установлен у 26 (7,6%) больных БТ, их средний возраст составил 29,9±8,4 лет, 14 (53,8%) были мужского пола, 12 (46,2%) – женского. Частота ЦГ составила 6% у больных до 21 года и 7,9% - у больных старше 21 года. Клиницисты должны быть наготове в отношении ЦГ и тщательно интерпретировать данные функциональных исследований щитовидной железы. Если уровень свободного тироксина 4 постоянно низок, авторы рекомендуют лечение L-тироксином при условии нормального уровня кортизола.

რეზიუმე

ცენტრალური ჰიპოთირეოიდიზმის მაღალი სიხშირე დიდი β-თალასემიის მქონე მოზრდილ პაციენტებში

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დიდი თალასემიის (დთ) მქონე ავადმყოფებში თირეოიდული დისფუნქციის უხშირესი ფორმა პირველადი ჰიპოთირეოიდიზმიაა, გამოწვეული ფარისებრი ჯირკვლის დაზიანებით. ცენტრალური ჰიპოთირეოიდიზმი (ცჰ) დთ-ის არახშირი გამოვლინებაა, თუმცა ადენოჰიპოფიზი თავისუფალ-რადიკალური ოქსიდაციური სტრესისადმი განსაკუთრებით მგრძნობიარეა. დიაგნოზი ემყარება ბიოქიმიურ გადახრებს, სისხლში მოცირკულირე თირეოიდული ჰორმონის კონცენტრაცია დაბალია, ხოლო თირეოტროპული ჰორმონის (TSH) კონცენტრაცია - შეუსაბამოდ დაბალი. TSH-ის ბაზალური ნორმული დონე ცჰ-ის დიაგნოზს არ გამორიცხავს. ფრიად მნიშვნელოვანია, კლინიცისტებმა გულდასმით შეაფასონ ფარისებრი ჯირკვლის ფუნქციური სინჯების შედეგები. დთ მქონე პაციენტებში ცჰ-ის სიხშირე სხვადასხვა ასაკობრივ ჯგუფში განსხვავებულია, მისი დიაგნოსტიკა გაძნელებულია, ვინაიდან ცჰ-ის სიმპტომების უმრავლესობა არასპეციფიკურია და, ხშირად, ანემიითა და სხვა ასოცირებული გართულებებითაა განპირობებული. ჩატარებულია მონაცემთა დიდი ბაზის (ჩვენი დთ დაავადებული პაციენტების კლინიკური ჩანაწერები) კროს-სექციური ანალიზი, ცჰ-ს სიხშირის პრეპუბერტულ (<11 წლამდე; 25 პაციენტი, 13 ვაჟი), პერიპუბერტულ (11 და 16 წელს შორის; 9 პაციენტი, 3 ვაჟი) და პუბერტულ (>16 წელზე, 305 პაციენტი, 164 ვაჟი) პერიოდში. ცჰ დაუდგინდა 26 (7,0%) ავადმყოფს. მათი საშუალო ასაკი იყო 29,9±8,4 წელი, 14 (53,8%) იყო ვაჟი, 12 (46,1%) – ქალი. 21 წლამდე ასაკის ავადმყოფებში ცჰ-ს სიხშირე 6% იყო, 21 წელზე უფროსებში – 7,9%. კლინიცისტები

მზად უნდა იყვნენ ცჰ-ის დიაგნოზის დასასმელად და სწორედ მოახდინონ ფარისებრი ჯირკვლის ფუნქციური კვლევის შედეგების ინტერპრეტაცია. თუ თავისუფალი თიროქსინ 4-ის დონე სტაბილურად დაბალია, კორტიზოლის ნორმალური დონის შემთხვევაში მიზანშეწონილად მიგვაჩნია ამ მდგომარეობის მკურნალობა L-თიროქსინით.

ATHENS UNIVERSITY THALASSEMIA EXPERTISE UNIT: EVOLUTION, STRUCTURE, PERSPECTIVES AND PATIENTS' EXPECTATIONS

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Thalassemia Expertise Centres (TECs), for the management of thalassemia started operating in developed countries with a high prevalence of thalassemia trait in the 70's, soon after the implementation of frequent transfusions treatment. Previously the medical and nursing needs of management were manageable, because of lack of any effective therapy and the short survival of patients. Frequent transfusions improved survival, increased considerably the cohort of patients with thalassemia and aggravated the medical and nursing burden of management. To meet the unbearable burden of management, thalassemia expertise centers were established, initially in Children's Hospitals or in Pediatric Departments of General hospitals in developed countries. Latter, when the cohort of adult patients increased, Adult TEC were organized jointly to Departments of Hematology, Internal Medicine or Transfusion Medicine (Blood Banks).

Evolution and Structure of Thalassemia Expertise Centres

Basic prerequisites for the organization of a TEC are: 1) the precise epidemiological studies to define the magnitude and the burden of thalassemia on health services and 2) the evaluation of the scientific and social activities of the TEC which the existing health system can support and handle.

The implementation of frequent transfusions in the treatment of thalassemia was most effective in ameliorating the clinical features of the disease and in improving survival and quality of life [10]. In parallel medical and nursing burden increased considerably because of frequent admissions and time-consuming transfusion procedure. An example of the extreme differentiation of medical and nursing burden related to implementation of frequent transfusion treatment is illustrated in Fig. 1. It represents the changing pattern of the ratio of annual admissions of patients to the total admissions in the University Department of Pediatrics in the period 1964-1980. In 1964, (the year prior transfusion) there were 115 admissions of thalassemic patients corresponding to 2% of total. Since then a rapid and steady increase of patients' admissions was observed; in 1980 the total annual admissions of patients rose to 4,760, representing 32% [3].

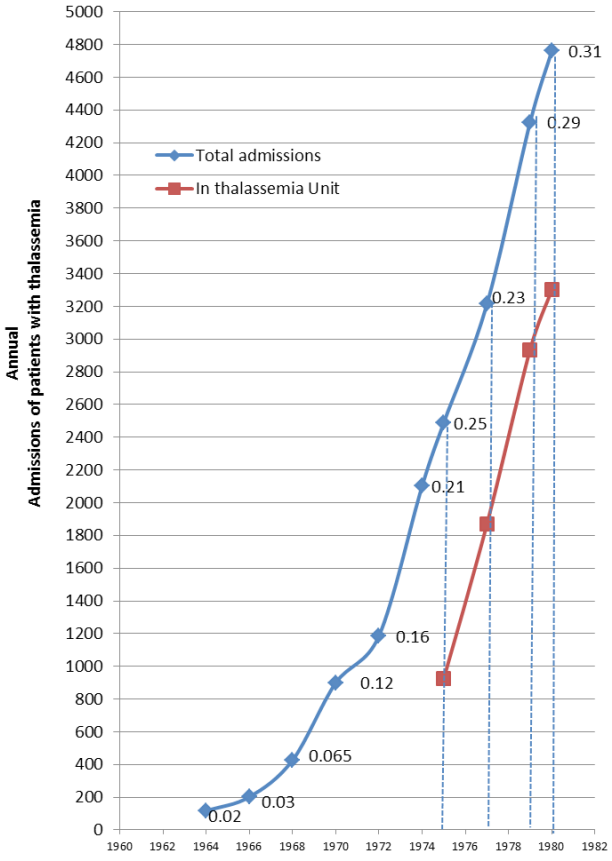

Fig. 1. Annual admission of patients with thalassemia, in relation to total admissions in the University Department of Pediatrics during the period 1964-1980

To confront the progressive increase of medical and nursing demands, an independent unit, solely devoted to the treatment of thalassemia, operating on a daily outpatient basis was organized in 1975. For this period, the organization, function , and multidiscipline nature with interconnection to other specialties units of the University Department of Pediatrics and the Children's hospital, could be considered as prototype of TEC, along with few others operating, in the same period in Italy and later in Cyprus. The Unit fulfilled most of the criteria recently proposed by EUCERD for the designation of Centres of Expertise for rare diseases [1].

The flow-chart of the structure of the Thalassemia Unit of the University Department of Pediatrics in Aghia Sophia Children Hospital (Fig. 2) consists of: 1) the Out-patient Clinic, where patients are followed regularly for all health problems, including psychological and social. The clinic is also involved in genetic counseling and in programming and monitoring treatment. 2) The Day-Treatment Clinic involved in the clinical and laboratory evaluation of patients, the administration and monitoring of transfusions and chelation and the implementation of appropriate treatment for the complications related to the disease or to the treatment.
3) The special Laboratory and Research Unit, which runs specific and advanced procedures used in diagnosis, follow and monitoring of treatment. A major activity of the Unit focuses on basic and clinical research on thalassemia, hemoglobinopathies and other common and rare anemias. The TEC collaborates closely with the hospital Blood Bank, the Hospital General Laboratory and the General and Sub-specialties Units of the University Department of Pediatrics.

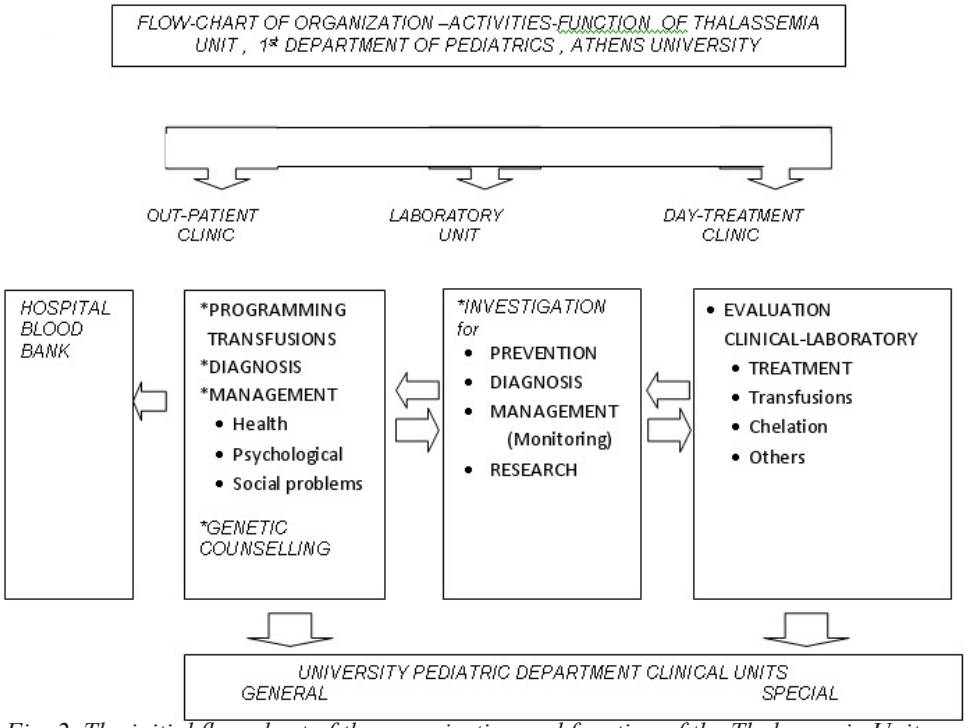

Fig. 2. The initial flow chart of the organization and function of the Thalassemia Unit of the First Department of Pediatrics of the University of Athens in "Aghia Sophia" Children's' Hospital

Another main objective of the TEC joined to research is education and training of heath professionals on thalassemia.

The original structure and activities of the Unit were gradually modified supplemented and upgraded following the international evolution and advances in the management and prevention of thalassemia. More precisely, in the 80's when a good proportion of patients reached adulthood, the permanent medical staff was enriched with adult specialists jointly with collaboration and adult specialties departments of endocrinology, cardiology and others. Thus the Unit was modified to a combined Pediatric and Adult TEC capable to cover children, adolescents and adults.

Other additional activities were and are: 1) the active participation, in the implementation of national prevention program which started in 1980 covering: genetic counseling, screening for detection of couple at risk, prenatal and preimplantation diagnosis using advanced molecular techniques.
2) Implementation of cure treatment by bone marrow transplantation which started in 1995 in collaboration with the Bone Marrow Transplantation Unit of our Hospital.
3) Collaboration with the High Risk Pregnancy Unit of the University Maternity Hospital.
4) Collaboration with MRI centre specialized in evaluation of iron content of liver, heart and endocrine glands.

The structure and activities of Thalassemia Unit cover the proposed criteria of European Commission of Health for reference centres for rare diseases [1]. In Greece, parallel to the combined TEC, a number of purely Pediatric and Adult Units operate, covering basically the treatment of patients following national and international recommendations, while the prevention program is coordinated by the National Prevention Center. In other developed countries with a high prevalence of thalassemia, TEC have similar structure.

In developed countries where thalassemia is considered a very rare anemia for the native population, thalassemia patients are referred for diagnosis and management either to Expertise Centres for rare anemias or to pediatric or adult hematology departments or units. Special units for the management of hemoglobinopathies has been established in developed countries hosting large immigrant communities with high prevalence of hemoglobinopathies.

In European Union, a pilot program of Rare Disease Reference network (RD ERNS) for congenital anemias (ENERCA) is operating since 2002 [1]. Thalassemia and hemoglobinopathies are covered by the network of rare anemias as the prevalence of hemoglobinopathies, for the whole European Union population, is considered low. At this time the European Union of Experts for Rare diseases (EUCERD) is working to define criteria for the designation of Centres of Expertise for rare diseases, that have to operate on a multidiscipline basis. The structure of the TEC described, fulfilled, from its initial form, the prerequisite of the multidiscipline function [1].

Expectations of patients and their families
Older patients with longstanding follow and treatment in Athens University TEC, are in general satisfied from the continued improvement of the efficacy of treatment that results in significant amelioration of clinical symptoms, and improvement of survival, quality of life and social adaptation [4].

They do expect that in the near future, conventional treatment will be more efficient and more easily applicable, (especially chelation) and the risks for severe complications from the endocrine glands and the heart will be further minimized. They also expect that complete cure with bone marrow transplantation will be extended to an increasing proportion of patients, using HLA compatible non relative donors, or a compatible sibling in case of artificial fertilization combined with appropriate preimplantation genetic diagnosis.

Last but not least, they wish the studies on gene replacement therapy to move more rapidly and successful clinical trials start soon.

Perspectives for Thalassemia Expertise Centres
The evaluation of the efficacy of longitudinal implementation of national programs of prevention and treatment in developed countries with high prevalence of thalassemia trait, demonstrated the valuable contribution of TECs in the management of thalassemia , the change in epidemiology and the age distribution of the cohort of patients.

Published data from Italy, Greece and Cyprus showed impressive and similar results on improvement of survival and quality of life [2,6,8].

Most interesting observations of the longitudinal evaluation of prevention and treatment programs is the change of the prevalence of patients with thalassemia major as well as the age distribution of thalassemia population.

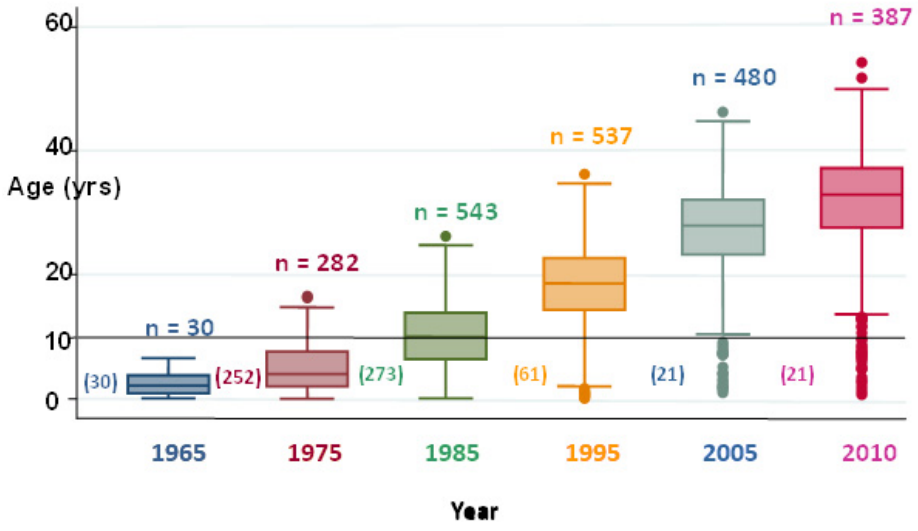

Fig. 3. Changes in annual input, total number and age distribution in patients with thalassemia treated in the Thalassemia Unit of the University Department of Pediatrics at "Aghia Sophia" Childrens Hospital during the period 1965-2010 n=total number of patients (n): number of new patients during intervals

Publication on a recent registry of hemoglobinopathies in Greece [9] and another on the efficacy of the prevention program [7] demonstrated:
1) A very low prevalence of thalassemia (3.2 patients per 10,000 population) compared to the expected (7.7 patients per 10,000), if only conventional treatment was implemented.
2) A dramatic change of the age distribution of patients with thalassemia to older ages. In 2010 of the whole cohort of 3,241 patients only 3,8% were below the age of 10 years while the mean age was 36 years. Characteristic are the longitudinal changes of age distribution of patients followed in our unit from 1965-2010 (Fig. 3). The figure illustrates how a short survival fatal disease of childhood turned to a chronic disease of adolescence and adulthood, after conventional treatment and how a predominant monogenic disease of childhood after successful implementation of prevention turned to a very rare disease of childhood [5].

These data combined with amelioration of the impact on health services indicate that countries with successful implementation thalassemia programs through TECs have to reconsider the activities of these centres.

At least for Greece and following the continuous trend of reduction of the number of patients, a subsequent reduction of the number of TEC seems logical. Based on the changes of the age distribution, a modification of the activities of Pediatric TECs which follow children and adolescents is indicated. For these ages the present prevalence of thalassemia major is extremely low and comparable to other transfusion dependent rare congenital anemias. Exploiting the wide experience of the medical and nursing staff in transfusion and chelation treatment these centres could accommodate all transfusion dependent rare anemias, following the prototype of Centres of Expertise for rare disease and particularly of rare anemias. It is also proposed that Pediatric TEC upgrade their participation to the prevention program to nullify the birth of neonates with thalassemia.

It is hoped that the experience gained from the longitudinal implementation of prevention and management programs for thalassemia in developed countries combined with continued advances in biotechnology ,will facilitate developed and under development countries to organize the most efficient Thalassemia and hemoglobinopathies Expertise Centres to control these diseases.

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SUMMARY

ATHENS UNIVERSITY THALASSEMIA EXPERTISE UNIT: EVOLUTION, STRUCTURE, PERSPECTIVES AND PATIENTS' EXPECTATIONS

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Thalassemia Expertise Centres (TECs), were first organized in developed countries with high thalassemia prevalence in the 70's to meet the increasing demands of the implemen-

tation of frequent transfusions in the treatment of thalassemia, and to consequently adopt, the rapid advances in the management of the disease.

Recent evaluation of longitudinal implementation of the national programs for prevention and treatment, demonstrated their efficacy for patients and public health. The beneficial effects focused on clinical symptoms amelioration, reduction of incidence and severity of complications and considerable improvement in survival, quality of life and social adaptation.National programs leaded to the modification of the most common genetic, fatal pediatric disease with short survival, to a chronic long-lived disease for adults and a very rare disease for children. In the few developed countries new perspectives for pediatric TECs need to be considered.

Keywords: thalassemia, Expertise centres, evolution, structure and perspectives.

РЕЗЮМЕ

ЦЕНТР ЭКСПЕРТИЗЫ ТАЛАССЕМИИ АФИНСКОГО УНИВЕРСИТЕТА: ЭВОЛЮЦИЯ, СТРУКТУРА И ПЕРСПЕКТИВЫ

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Центры экспертизы талассемии (ЦЭТ) впервые были организованы в развитых странах с высокой частотой талассемии в 70-ые годы XX века. С целью ответа на возросшие потребности внедрения частых переливаний крови в лечение талассемии и, следовательно, адаптации новейших успехов менеджмента этого заболевания. Недавняя оценка лонгитудинальной имплементации национальных программ для превенции и лечения, показало их эффективность для пациентов и общественного здравоохранения. Благотворные эффекты заключались в уменьшении интенсивности клинических проявлений, частоты и тяжести осложнений, в значительном улучшении выживаемости, качества жизни и социальной адаптации. Национальные программы привели к модификации наиболее частых генетических фатальных педиатрических заболеваний с короткой продолжительностью жизни в хронические долготекущие заболевания взрослых. В будущем следует обсудить новые перспективы ЦЭТ в некоторых развитых странах.

რეზიუმე

ათენის უნივერსიტეტის თალასემიის ექსპერტიზის ცენტრი: ევოლუცია, სტრუქტურა და პერსპექტივები

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თალასემიის მკურნალობაში სისხლის ხშირი გადასხმისა და ამ დაავადების მენეჯმენტში უახლესი წარმატებების ადაპტაციის მზარდი მოთხოვნების საპასუხოდ, განვითარებულ ქვეყნებში XX საუკუნის 70-იან წლებში მაღალი სიხშირით დაიწყო თალასემიის ექსპერტიზის ცენტრების (თეც) ორგანიზება. პრევენციისა და მკურნალობის ნაციონალური პროგრამების ლონგიტუდინური იმპლემენტაციის შეფასებამ აჩვენა მათი მაღალი ეფექტურობა პაციენტებისა და საზოგადოებრივი ჯანდაცვისთვის. სასარგებლო შედეგი გამოვლინდა კლინიკური სიმპტომატიკის გაუმჯობესებაში, გართულებების სიხშირისა და სიმძიმის შემცირებაში, სიცოცხლის ხანგრძლივობის და ხარისხის,სოციალური ადაპტაციის მნიშვნელოვან გაუმჯობესებაში. ნაციონალურმა პროგრამებმა განსაზღვრა ყველაზე ხშირი გენეტიკური ფატალური პედიატრიული დაავადებების გარდაქმნა მოზრდილების ქრონიკულად მიმდინარე დაავადებებად. მიზანშეწონილად მიგვაჩნია ზოგიერთ განვითარებულ ქვეყანაში თეც-ის ახალი პერსპექტივების განხილვა.

HEALTH ISSUES IN ADOLESCENTS' INTERNET USE - BENEFITS AND RISKS

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The media represent one of the most powerful influences on child and adolescent development and health [38]. The Internet has turned during the past decade into a major information resource in various domains of life and a communication venue among adolescents who seek health information via the net. Both "old" media (television, movies, and magazines) and "new" media (the Internet and social networking sites, video/computer games, cell phones) can have an impact on virtually every health concern that practitioners and parents have about young people [37]. Adolescents are avid Internet users; data have suggested that more than 90% of teens have access to the Internet and most teens report daily use of the Internet [27]. Adolescents far outnumber adults in their use of e-communication technologies, such as instant messaging and social network sites [42]. The increasing availability of computers in homes, as well as wireless Internet access, means that adolescents today can go online anywhere, at any time [36]. It has been estimated that 55% of Internet-using adolescents use online social networking Web sites [24] and 28% get health information via online sources [21]. Digital media have become an important source of information, and sometimes misinformation, about health problems [14]. The media are not the leading cause of any major health problem, but they do contribute significantly to a variety of adolescent health problems: aggressive behavior, sexual activity, drug use, obesity, sleep disorders, eating disorders, depression, suicide and self harm [7, 30, 36]. Unmonitored Internet use may place adolescents at a significant risk, such as cyberbullying, unwanted exposure to pornography, and potential revealing personal information to sexual predators [32].

This paper focuses on 3 major health issues in adolescents' Internet use: Body image and eating behaviors; sexuality and reproductive health behaviors; and self harm and suicidal behavior. Finally, this paper demonstrates Internet venues where reliable health information is provided to young people by health professionals highlighting also some difficulties in the provision of such information.

Body image and eating disorders

The media are saturated with images promoting thinness, and links between disordered eating behaviors and exposure to thin-ideal images have been identified in both cross-sectional and longitudinal research [25,41]. Indeed, studies have concluded that thin-ideal internalization can trigger eating disorder symptoms [40], thus highlighting the vulnerability individuals high in thin-ideal internalization might have to media exposure [2]. Although there are insufficient data to state that the media cause eating disorders, media exposure can certainly be considered as a significant risk factor [16,20,25], and may even contribute to the development of eating disorders [36]. Google search of pro-eating disorder Web sites revealed about 7,280,000 results within 0.30 seconds. On the Internet there are now over 100 pro-eating disorders websites that not only encourage disordered eating but offer specific advice on purging, restricting caloric intake, and exercising excessively (e.g. pro-Ana and pro-Mia) [9]. These websites are popular among youth who wish to be thinner, because they offer spaces, where one can find support as well as express one's feelings and thoughts around the disturbing eating lifestyle. Participants describe blogging as a cathartic experience and perceive the social support they receive from other members of the pro-Ana community as a benefit. The main motive for joining the online community is to be provided with both advice regarding weight loss and support and many pro-anorexic websites members equate thinness with happiness and are satisfied with their membership [33]. Pro-Ana and pro-Mia are social and harmful movement on the Web. They may have undesired and negative effects in adolescents because they contribute to the encouragement of disruptive eating behaviors or to the maintenance and aggravation of already existing eating disordered behavior. They also present graphic materials and photographs to encourage, support, and motivate site users to continue their efforts with anorexia and bulimia [9,13,46].

Sexuality and reproductive health

The Internet has become a widely used resource for sexual health information, especially among adolescents. The appeal lies in the ease and anonymity with which online seekers can obtain advice and reassurance, particularly regarding sensitive sexuality topics [22,23]. Media play an important role in providing sexual information to adolescents [8,12] and in shaping their beliefs about how males and females behave in romantic relationships [11]. Indeed, the increased use of the Internet by teens has dramatically increased exposure to x-rated materials, with more than 50% of teens indicating incidental exposure to sexual materials [44].

Longitudinal correlation studies that allow cause-and-effect conclusions to be drawn show an impact of sexual content in the media on adolescents' sexual behavior [36].

A Google search for "teen-pornography" revealed about 38,200,000 results within 0.28 seconds. Nationwide US studies found that by age 18,93% of males and 62% of

females reported seeing pornography [34], and that nearly half of the Internet users had been exposed to on-line pornography in the previous year [43]. A longitudinal study of more than 1500 10 to 15 year olds found a nearly six fold increase in the odds of self-reported sexually aggressive behavior with exposure to violent x-rated material over time [31].

Although all of this sounds alarming and concerning, both "old" and "new" media can be a powerful source of positive sexual information as well. New technology is also exploding with possibilities, such as text-messaging safe sex information and information of testing for sexually transmitted infections as well as using computer video games to increase knowledge and attitudes favoring avoiding teen pregnancy. Online media education about social networking sites has been shown to reduce displays of risky sexual behaviors [36].

Suicide and self-harm

One of the major concerns in the current debates around suicide as applied to the media in general and the Internet in particular, is related to the role that the computer-generated systems, services and devices play in suicide stimulation. The potential influence of the Internet on its suicide-sensitive users was especially relevant as regards the influence exerted upon young people. Studies have linked media coverage and portrayals of suicide with an increase in actual suicides, a type of "suicide contagion" that affects teens far more than adults [36]. Cybersuicide is a term used in reference to suicide on the Internet, and it is associated with websites that lure vulnerable members of society and empower them with various methods and approaches to deliberate self-harm [7]. The Internet as a means of communication may encourage suicidal behavior by depicting ways by which suicide may be committed. Moreover, some internet websites may discourage people from seeking help, condone suicide, and forbid entry to anyone offering to discourage users from committing suicide. However, the internet may be a resource to help a potentially suicidal person get help, and can be used to identify those at risk for suicide, communicate with them, and potentially prevent suicide. If used appropriately, the internet is a powerful communication tool that can be used to benefit suicidal patients [1]. Interviews with suicide and self-harm websites users revealed that participants perceived these sites as sources of empathy and understanding, and as a way of coping with social and psychological distress, thus giving users access to important, socially valued identities, such as being understood, belonging to a community and coping with their problems [3].

The behaviors of non-suicidal self-injury (NSSI) and deliberate self-harm (DSH) are prevalent among adolescents, and an increase of NSSI and DSH rates in recent years has been postulated [28]. Many youth who self-injure go online to connect with others who self-injure and view others' NSSI experiences and share their own through text and videos platforms. These exposures can introduce young people to risks, such as NSSI reinforcement, through the sharing of stories and strategies, as well as risks for triggering of NSSI urges and hiding them from parents and friends. Some pro self harm sites also offer information that promotes eating disorders. Sites with pro self injury and pro self harm agendas target vulnerable people, but their reach extends far beyond the self injurers themselves. When a person suffers irreparable physical or mental damage as a result of following the advice on these sites, the harm extends outward in a concentric fashion affecting parents, siblings, extended family, friends, and even strangers. Therefore, intervention in this area should encourage substitution of healthier online activities for the activities that may currently foster harm [26].

Major publicity now surrounds suicides precipitated by Internet bullying and harassment [4,6,15,45]. Cyberbullying has been shown to possess different ramifications from traditional school-yard bullying [39]. This modern mode of bullying performed using electronic forms of contact (e.g., SMS, MMS, Facebook, YouTube), has been considered as being worse than traditional bullying in its consequences for the victims. This difference was mainly attributed to the increased potential for a large audience, the anonymity of the bullying perpetrators, the lower levels of direct feedback, and the lower levels of supervision [35].

Internet venues for reliable health information provided to young people

Despite the evidence of potential harm, there is also evidence that media can be beneficial for youth [19]. New media technologies give youth the opportunity to create their own expressions of individuality, whether through social networks like Facebook or file-sharing sites like YouTube [10]. New media also allow adolescents to experience community communication in a time of life when they often feel unmoored, by increasing empathy and acceptance of diversity through modeling of pro-social behaviors. For example the site "It Gets Better Project", which was created after a series of suicides by youth who had been bullied over their sexuality, gives lesbian, gay, bisexual, and transgender (LGBT) youth the space to tell their stories and to hear the encouragement of LGBT adults who have successfully navigated the turbulent teen years [36]. The site "Recover Your Life" helps people who suffer from self harm, as well as people with other issues such as eating disorders, mental health problems, and abuse. However, the benefits of socializing on-line are not equal for every child or adolescent, and the positive Internet effect holds only when adolescents also talk directly with their friends and family [5].

In order to address the vast amount of uncontrolled and sometimes dangerous health information messages on the Internet, specific health websites have been developed,

where reliable health professionals respond on line to health related questions raised anonymously by adolescents [18,29]. The advantage of these websites is that the on-line communication is between the young person and the health professional, and is free of any non-professional intervention. Other health websites that function as "forums" allow non-professionals to respond to the discussed issues, and thus enabling larger online audiences the chance to receive reliable health information.

An Israeli survey of a representative sample of contacts to a teen-health-forum ("The body during adolescence") run by 6 adolescent medicine pediatricians was performed in 2009. Among 412 adolescents' contacts (51% females), 44% of the questions were related to sexuality issues and 17% were related to self image and body composition. [17]. This Internet health forum enables adolescents and parents to ask questions and raise doubts and anxieties regarding various health issues without the fear of being exposed and of expressing their concerns face-to-face with a healthcare provider. Sensitive issues regarding sexuality and self-image that frequently are not raised during clinical encounters are expressed and receive professional responses in the forum. Notwithstanding the significance of a rapid professional contribution, physicians responding to contacts in Internet venues need to recognize the barriers related to their communication with persons whom they have not met and for whom follow up is impossible. When a youngster expresses acute distress, the responding health professional may feel helpless in suggesting medical or psychosocial advice, not knowing the patient and without the possibility to directly examine the patient. Delivering bad news in an Internet health forum is also problematic and may be detrimental to the young person on line. Telemedicine requires adherence to ethical issues including refraining from criticism of other physicians, who may have been involved in the health care of the individual on line. These barriers may be the reason for the high referral rate to clinical medical consultation in the teen-health-forum survey that reached 40% [17]. Health professionals who see adolescents at their clinics need to understand that spending some time with their adolescent patients discussing media use, may be as important as discussing school difficulty, aggressiveness, disordered eating, or poor sleep patterns [37].

In summary, the Internet of the 21st century is affordable, convenient and anonymous and may provide unique potential health education. However, health professionals need to recognize the hazards of adolescents Internet use, and to address potential Internet abuse when encountering adolescents in clinical settings.

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SUMMARY

HEALTH ISSUES IN ADOLESCENTS' INTERNET USE - BENEFITS AND RISKS

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The Internet has turned during the past decade into a major information resource in various domains of life

and a communication venue among adolescents who seek health information via the net. The increasing availability of computers in homes, as well as wireless Internet access, means that adolescents today can go online anywhere, at any time. The media are not the leading cause of any major health problem, but they do contribute significantly to a variety of adolescent health problems, including aggressive behavior, sexual activity, drug use, obesity, sleep disorders, eating disorders, depression, suicide and self harm. This paper focuses on 3 major health issues in adolescents' Internet use: Body image and eating behaviors; sexuality and reproductive health behaviors; and self harm and suicidal behavior. This paper also demonstrates Internet venues where reliable health information is provided to young people by health professionals. Health professionals need to recognize the hazards of adolescents Internet use, and to address potential Internet abuse when encountering adolescents in clinical settings.

Keywords: adolescents, health, internet, benefits, risks.

РЕЗЮМЕ

ВОПРОСЫ ЗДРАВООХРАНЕНИЯ ПРИ ИСПОЛЬЗОВАНИИ ИНТЕРНЕТА ПОДРОСТКАМИ – ВЫГОДА И РИСК

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В последнюю декаду интернет превратился в главный информационный ресурс в различных областях жизни и коммуникационное средство для подростков, ищущих информацию о здоровье в сети. Растущая доступность компьютеров дома, а также доступа к беспроводному интернету означают, что сегодня подростки могут войти в сеть всюду и в любое время. Средства массовой информации не являются ведущей причиной основных медицинских проблем, тем не менее, они вносят значительный вклад в развитие различных проблем, касающихся здоровья подростков, включая агрессивное поведение, сексуальную активность, применение наркотиков, ожирение, нарушение сна и питания, депрессию, суицид, самоповреждение. В статье фокусируется внимание на 3 основных медицинских проблемах, возникающих при использовании подростками интернета: образ тела и пищевое поведение, сексуальное и действующее на репродуктивное здоровье поведение; самоповреждающее и суицидальное поведение. В статье также указаны интернет ресурсы, в которых соответствующая информация о здоровье предоставляется молодым людям профессионалами здравоохранения. Профессионалы здравоохранения должны распознавать опасности использования интернета подростками и учитывать их в клинической деятельности.

რეზიუმე

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უკანასკნელ წლებში ინტერნეტი ცხოვრების სხვადასხვა სფეროში ინფორმაციის მთავარ წყაროდ გადაიქცა, ასევე, კომუნიკაციის საშუალებად მოზარდებისათვის, რომლებიც ქსელში მოიპოვებენ ინფორმაციას ჯანმრთელობის შესახებ. კომპიუტერების მზარდი ხელმისაწვდომობა, უკაბელო ინტერნეტის დანერგვა განაპირობებს იმას, რომ მოზარდებს შეუძლია ქსელში შესვლა ყველგან და ყოველთვის. მედია არ არის ჯანმრთელობასთან დაკავშირებული პრობლემების მთავარი მიზეზი, მაგრამ იგი მნიშვნელოვან ზეგავლენას ახდენს მოზარდების ჯანმრთელობასთან დაკავშირებულ ქცევაზე, ასეთს მიეკუთვნება აგრესიული ქცევა, სექსუალური აქტივობა, ნარკოტიკების ხმარება, კვების დარღვევები, სიმსუქნე, დეპრესია, ძილის დარღვევები, სუიციდი. სტატიაში ყურადღება ფოკუსირებულია 3 სამედიცინო პრობლემაზე, რომელიც დაკავშირებულია მოზარდების მიერ ინტერნეტის გამოყენებასთან: სხეულის ხატი და კვების ქცევა, სექსუალურ და რეპროდუქციულ ჯანმრთელობაზე მოქმედი ქცევა, თვითდაზიანებაზე და თვითმკვლელობაზე მიმართული ქცევა. სტატიაში მითითებულია, ასევე, ინტერნეტ-რესურსები, რომლებშიც სათანადო სამედიცინო ინფორმაცია წარმოდგენილია ჯანდაცვის პროფესიონალების მიერ. ჯანდაცვის პროფესიონალებმა აუცილებელია გაითვალისწინონ მოზარდების მიერ ინტერნეტის გამოყენების შესაძლო საფრთხე და მხედველობაში მიიღონ იგი კლინიკური საქმიანობისას.