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FIVE YEARS RETROSPECTIVE STUDY OF MANDIBULAR FRACTURES IN MECHNIKOV REGIONAL CLINICAL HOSPITAL, DNIPROPETROVSK

Abstract: A retrospective analysis of 1647 case records of patients with mandibular fractures for period as of from 2008 to 2012 is carried out. Among patients were widespread enough alcohol abuses – in 10.1% patients, drag abuse – in 0.8%, smoking – in 62.7%. 64.3% patients are temporally unemployed. In 54,3% with the breaks of lower jaw were found out patients concomitant pathology.

Keywords: fractures of the mandible, complication, delayed consolidation, non-union.

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ПЯТИЛЕТНИЙ РЕТРОСПЕКТИВНЫЙ АНАЛИЗ ПЕРЕЛОМОВ НИЖНЕЙ ЧЕЛЮСТИ У ПАЦИЕНТОВ ДНЕПРОПЕТРОВСКОЙ ОБЛАСТНОЙ БОЛЬНИЦЫ ИМ. И.И. МЕЧНИКОВА

Аннотация: Осуществлен ретроспективный анализ 1647 историй болезней пациентов с переломами нижней челюсти за период с 2008 по 2012 гг. Среди больных было достаточно распространено злоупотребление алкоголем у 10,1% пациентов, наркотических веществ – 0,8%, курение – 62,7%. 64,3% больных – временно не работали. У 54,3% пациентов обнаружена сопутствующая патология.

Ключевые слова: переломы нижней челюсти, статистика, осложнения, замедленная консолидация.

Челюстно-лицевой травматизм был и остается одой из актуальнейших проблем хирургической стоматологии. Частота и тяжесть травматических повреждений костей лицевого черепа увеличиваются год от года. На эти показатели влияет рост дорожного травматизма, техногенных катастроф, локальных вооруженных конфликтов [1, 2361–2366].

Особую остроту проблеме придает распространенность травм челюстно-лицевой области среди наиболее социально активных возрастных групп, что приводит к длительной потере трудоспособности [2, 21–25].

Как отмечает большинство современных авторов, такой вид травм часто возникает среди социально неблагополучных слоев населения, в которых распространены злоупотребление алкоголем, наркотическими препаратами, девиантные формы поведения [3, 69–73].

Также должен обращать на себя внимание факт наличия устойчивых негативных тенденций в состоянии здоровья популяции населения. Согласно данным ВОЗ, только 4,3% населения земного шара можно считать абсолютно здоровым. Отмечается рост хронической патологии, коморбидных заболеваний. Аналитические сводки показывают, что треть живущих людей страдает одновременно от 5 различных заболеваний, а 52% – от десяти [4,1–170].

Вследствие вышеизложеных причин отмечается неуклонный рост различных осложнений переломов нижней челюсти, что требует в дальнейшем затратного и продолжительного лечения [5, 7].

В этой связи изучение этиологии, структуры и эпидемиологии переломов нижней челюсти приобретает особое значение для организации специализированной медицинской помощи, анализа факторов риска развития осложнений и разработки эффективных методов их профилактики и лечения.

Цель исследования: определить структуру повреждений и их осложнений, наличие сопутствующей патологии у пациентов с переломами нижней челюсти на основе ретроспективного анализа историй болезни челюстно-лицевого отделения КУ «Днепропетровская областная больница им. И. И. Мечникова» за 5 лет.

Материалы и методы исследования. Исследование базируется на анализе историй болезней пациентов с переломами нижней челюсти, которые находились на лечении в челюстно-лицевом отделении КУ «Днепропетровская областная больница им. И. И. Мечникова» в 2008–2012 годах. Полученные данные обработаны с помощью методов вариационной статистики с использованием программных пакетов STATISTIKA 5.0 (Лицензионный № 74017–6400000106–57362), Excel 2003 (Лицензионный № 74017–640–0000106–57285) [6, 28–40].

Результаты исследования и их обсуждение: За период с 2008 по 2012 гг. в челюстно-лицевом отделении КУ «Днепропетровская областная больница им. И. И. Мечникова» проходили лечение 1647 больных с переломами нижней челюсти. Среди травмированных было 1528 (92,8%) мужчин и 119 (7,2%) женщин. Распределение по возрастным категориям выглядит следующим образом: от 18 до 25 лет – 583 (35,4%), от 26 до 45 лет – 869 (52,8%), 46–60 лет – 168 (10,2%), 60–75 лет –20 (1,2%), 75–90 лет – 7 (0,4%) больных (табл. 1).

Таблица 1.– Распределение по возрастным категориям больных с переломами нижней челюсти в период с 2008 по2012 гг.

Год	Общее количество больных	Распределение по возрастным категориям				
		18–25 лет	26–45 лет	46–60 лет	61–75 лет	76–90 лет
2008	340	115	194	25	4	2
2009	332	125	165	40	2	-
2010	318	110	163	40	4	1
2011	326	111	171	36	6	2
2012	331	122	176	27	4	2
Всего	1647	583	869	168	20	7

Жители городов области составили 59,9% (987 больных), сельские жители 39,4% (649 больных), 11 больных (0,7%) не имели постоянного места жительства. Постоянным трудом было занято 561 (34,1%) пострадавших, 1059 (64,3%) больных – временно нигде не работали, и этот факт не может не вызывать обеспокоенности, поскольку именно в группе социально неблагополучных пациентов традиционно высоки показатели несвоевременного обращения за медицинской помощью – 76 (4,6%), несоблюдения режима – 39 (2,4%) больных самовольно покинули лечебное учреждение до окончания лечения и 5 (0,3%) пациентов отказались от лечения.

Подавляющее большинство больных было госпитализировано в остром периоде (в сроки до 3-х суток после получения травмы) – 1571 больных (95,4%).

Чаще всего переломы нижней челюсти были следствием бытовой травмы – 1169 случая (71,0%). На втором месте – переломы, возникшие в результате ДТП – 438 случаев (26,6%). Производственные и спортивные травмы составляли одинаковое количество наблюдений – по 20 случаев (1,2%).

В 67 (4,1%) случаях лицевая травма сочеталась с острой черепно-мозговой травмой: у 49 (3,0%) диагностировано сотрясение головного мозга, у 18 (1,1%) – ушиб мозга; 54 (3,3%) больных имели диагноз политравма. Обычно такие пострадавшие поступали в клинику в тяжелом состоянии и в первые недели находились в отделениях интенсивной терапии, нейрохирургии или травматологии. В челюстно-лицевое отделение поступали больные преимущественно в удовлетворительном состоянии –1526 (92,7%) или в состоянии средней степени тяжести –18 (1,1%) пострадавших.

У 146 (8,9%) пострадавших перелом нижней челюсти был получен на фоне острого алкогольного опьянения. Среди больных были достаточно распространены злоупотребление алкоголя – 167 (10,1%) пациентов, наркотических веществ – 13 (0,8%), курение – 1033 (62,7%).

У 894 (54,3%) пациентов выявлена сопутствующая патология: заболевания желудочно-кишечного тракта у 287 (17,4%), сердечно-сосудистые заболевания – у 107 (6,5%), заболевания дыхательной системы – у 65 (3,9%), мочеполовой системы – у 54 (3,2%), эндокринной системы – у 49 (3,0%), хронические заболевания ЛОР-органов – у 66 (6,0%), психические расстройства у 13 (0,8%). В 106 (6,4%) случаях имела место коморбидность, как сочетание двух и более заболеваний у одного больного.

Было диагностировано: 1007 (61,1%) односторонних переломов, 529 (32,1%) – двусторонних, 69 (4,2%) – тройных; множественные и оскольчатые переломы – в 8 (0,5%) и 34 (2,1%) случаев соответственно.

В 14 (0,9%) случаев наблюдали переломы нижней челюсти у пациентов с полной вторичной адентией.

Большинство больных были пролечены консервативно 1157 (70,2%), у 464 (28,2%) больных прибегали к хирургическому лечению (остеосинтезу). У 26 больных (1,6%) использовали модифицированные пращевидные повязки.

Осложнения разного рода диагностировали у 394 (23,9%) больных. Нагноение костной раны диагностировали у 77 (4,7%) пациентов, абсцессы и флегмоны мягких околочелюстных тканей у 69 (4,2%), посттравматический остеомиелит челюсти – у 118 (7,2%) пациентов. Среди невоспалительных осложнений чаще всего сталкивались с замедленной консолидацией отломков 144 (8,7%), сращением отломков в неправильном положении – у 37 (2,3%) и ложным суставом – у 5 (0,3%) больных.

Средние сроки стационарного лечения составили 15,4 ± 0,7 дней, средние сроки временной нетрудоспособности 41,3 ± 2,5 суток.

Выводы: 1. Количество пострадавших с переломами нижней челюсти не имеет тенденции к уменьшению и остается на стабильно высоком уровне.

2. На результаты лечения негативно влияет ряд общих факторов, значительная роль среди

которых отводится образу жизни и вредным привычкам. Среди больных были достаточно распространены злоупотребление алкоголем – 167 (10,1%) пациентов, наркотическими веществами – 13 (0,8%), курением – 1033 (62,7%). 64,3% больных – временно нигде не работали. Больные этой категории чаще нарушали режим лечения – в 120 (7,3%) случаях.

3. У пациентов с переломами нижней челюсти в 54,3% выявляли сопутствующую патологию. В 6,4% случаев имело место сочетание двух и более заболеваний у одного больного.

4. Наиболее распространенным невоспалительным осложнением переломов нижней челюсти была замедленная консолидация отломков 8,7%.

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DEPENDENCE OF THE SEVERE HEMOPHILIA A – RELATED QUALITY OF LIFE IN CHILDREN FROM THE REGIMENS OF PROPHYLAXIS TREATMENT

Abstract: In this article, based on the study of 51 children with severe hemophilia A, we have analyzed the features of the hemophilia A-related quality of life (QoL) at different regimes of prophylactic treatment (PT): a standard regimen of replacement therapy with a frequency of administration of 3 times/week and a mode with a lower multiplicity (1–2 times/week). The conducted study allows us to conclude that in patients who did not have joint hemorrhages a month before the examination, the QoL indices are better with decreasing the frequency of PT. At the same time, in the standard PL mode, the number of joint hemorrhages was smaller.

Keywords: hemophilia A, children, health-related quality of life, prophylaxis treatment.

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ЗАВИСИМОСТЬ КАЧЕСТВА ЖИЗНИ, СВЯЗАННОГО С ТЯЖЕЛОЙ ФОРМОЙ ГЕМОФИЛИИ А, У ДЕТЕЙ ОТ РЕЖИМА ПРОФИЛАКТИЧЕСКОГО ЛЕЧЕНИЯ

Аннотация: В статье, на основе исследования 51 пациента с тяжелой формой гемофилии А, проанализированы особенности качества жизни, связанного с гемофилией А (КЖ), при

разной кратности проведения профилактического лечения (ПЛ): стандартном режиме заместительной терапии с кратностью введения 3 р/неделю и режиме с меньшей кратностью (1–2 р/неделю). Проведенное исследование позволяет нам сделать вывод, что у пациентов, не имевших кровоизлияний в суставы месяц накануне обследования показатели КЖ являются достоверно лучше при уменьшении кратности профилактического лечения. В то же время, при стандартном режиме ПЛ количество кровоизлияний в суставы меньше.

Ключевые слова: гемофилия А, дети, качество жизни связанное со здоровьем, профилактическое лечение.

Введение. Тяжелая форма гемофилии А (тГА) у детей является самым частым заболеванием среди наследственных нарушений системы свертывания крови с тяжелым клиническим течением. По рекомендациям таких авторитетных организаций, как World Federation of Hemophilia (WFH) и National Hemophilia Foundation (NHF), основанных на современных протоколах менеджмента (протокол Malmo, протокол Utrecht, рекомендации United Kingdom Haemophilia Centre Doctors 'Organization и ряд других) лечение тГА у детей предусматривает регулярное заместительное профилактическое лечение (ПЛ) три раза в неделю концентратами дефицитного фактора свертывания крови (ФСК), и, как удачно отметили A. Nijdam и соавт., «при наличии соответствующих ресурсов» [1; 2; 3, с. 9; 4]. Такой режим у большинства пациентов позволяет обеспечить решение двух основных причинно-следственных задач лечения тГА: обеспечить уровень FVIII свертывания крови на постоянном уровне ≥ 1 МО/дл и профилактику формирования артропатии (A. Gringeri и соавт., 2011) [1; 3; 5]. Наряду с этим, ПЛ гемофилии является чрезвычайно дорогостоящим и, соответственно, часто недоступным во многих странах с низким уровнем экономического развития [6]. По данным M. L. Simpson и L. A. Valentino только 25% пациентов во всем мире получают адекватное ПЛ [7]. Именно поэтому, в странах с недостаточным ресурсным обеспечением для проведения полноценной профилактики, дети с тГА лечатся в так называемом режиме «по-требованию» (в англоязычной литературе «on-demand therapy») или в профилактическом режиме «по наличию ресурсов» с уменьшенной частотой введения препарата / дозировкой («гибридная профилактика» (ГП)). Однако, такой режим лечения (ГП) может иметь определенное обоснование с позиции целесообразности даже без учета финансово-экономического эквивалента. В частности, в последние годы значительно возрос интерес ученых к субъективной составляющей гемофлии А у детей, в том числе к качеству жизни, связанной с тГА (КЖ). Многочисленные исследования показали значительное влияние заболевания не только на физические аспекты функционирования детей, но и на психосоциальные [8; 9]. Одним из весомых детерминант влияния на психосоциальное функционирование (ПФ) у детей с тГА является как раз фактор лечения. Так, например, Furlan R. и соавт. (2015) в исследовании 89 пациентов с гемофилией А, проведенном в Канаде, США и Австралии, показали психосоциальные преимущества для пациентов при снижении частоты введения препаратов при ПЛ [10]. Современные рекомендации по менеджменту тГА, разработанные в ходе многочисленных многолетних исследований, основанные в основном на объективных маркерах заболевания, без учета психосоциальных детерминант и индивидуальных особенностей пациентов. Herbert R. D. и соавт. (2018) удачно отметили, что «режим профилактики, который минимизирует риск кровотечений, зависит от особенностей физической активности человека и может сильно отличаться от режимов профилактики, которые оптимизируют фарма-

кокинетические параметры» [11]. Это еще раз подчеркивает необходимость персонифицированного подхода в выборе режима ПЛ пациентов с тГА. Индивидуализация лечебных подходов у детей с тГА обоснована также такими элементами персонификации как возраст, венозный доступ, ресурсное обеспечение и КЖ [3; 6].

Сегодня, количество исследований которые изучают влияние частоты заместительной терапии на КЖ детей с тГА остаются недостаточными, и, по утверждению M. L. Simpson и соавт. (2012) «современные подходы к менеджменту тГА, такие как, скажем, ПЛ один раз в неделю, актуальны для дальнейших многоцентровых, международных, проспективных исследований» [7]. Наряду с этим, следует отметить, что тяжесть гемофилии А в соответствии с классификацией по уровню дефицитного ФСК далеко не в абсолютном большинстве случаев совпадает с клинической тяжестью заболевания. Это обосновано таким понятием, как «геморрагический фенотип». По нашим многолетним наблюдениям, часть пациентов с тГА могут не иметь геморрагических проявлений длительное время даже без заместительного лечения. Такие наблюдения демонстрируют и ряд других ученых, отмечая, что количество «мягких фенотипов» с низким риском кровотечений у пациентов с тГА колеблется в пределах 10–15% (Pavlova A. и соавт., 2013) [12; 13; 14]. Именно поэтому, в последние годы появляются исследования, посвященные изучению возможности индивидуального подхода в выборе профилактического режима с коррекцией частоты заместительного лечения [6; 11].

Бесспорно, основным критерием в выборе лечебного режима тГА у детей остается и должен быть подход по уменьшению риска возникновения кровотечения. Однако, уже в некоторых странах (Таиланд, Китай, Индия), как отметили M. C. Poon и A. Lee (2016) «основная цель ПЛ направлена не на «нулевые кровотечения» и идеальные суставы, а на улучшение КЖ» [6]. Мы также считаем, что на сегодняшний день индивидуализированную профилактику тГА можно считать наиболее оптимальной стратегией как с позиции улучшения КЖ, так и использования доступных ресурсов для лечения.

Таким образом, из проведенного детального осмотра современной литературы по данной проблематике, мы считаем актуальным исследование особенностей КЖ при различных режимах лечения с определением наиболее оптимальных с позиции как раз КЖ.

Цель исследования. Исследовать зависимость КЖ у детей с тГА от режима профилактического лечения и определить возможность персонификации ПЛ с позиции КЖ.

Материалы и методы. Прежде всего хочется отметить, что данная публикация является частью цикла работ, посвященных характеристике КЖ детей с тГА в Украине, часть из которых нами уже была опубликована [15–20]. Для достижения цели данной работы мы обследовали КЖ у 51 ребенка с тГА. Во всех обследованных пациентов проведена оценка КЖ методом социологического опроса в форме индивидуального и электронного анкетирования с использованием валидированной украинской версии опросника Haemo-QoL. Все дети были разделены на три возрастные группы, в соответствии с возрастными версиями опросника: группа I – дети 4–7р .; группа II – 8–12р .; группа III – 13–16р. Все дети, включенные в данное исследование, получали в течение не менее 6 месяцев накануне обследования, заместительное ПЛ концентратами FVIII свертывания крови в дозе 15–50 МЕ /кг. В зависимости от кратности введения препарата пациенты были разделены на две группы: группа «СП» (стандартная профилактика) – пациенты, получавшие стандартную протокольную профилактику 3 р/неделю; группа «ГП» (гибридная профилактика) – пациенты, получавшие ПЛ в меньшей кратности, но не реже 1 р/неделю. Структура обследованных была следующей: I возрастная группа – 16 детей («СП» –

11 «ГП» – 5); II – 22 («СП» – 10, «ГП» – 12); III – 13 («СП» – 4, «ГП» – 9).

Критериями включения в исследование были следующие: уровень FVIII свертывания крови <1 МЕ/дл, возраст от 4 до 16 лет, анамнестический уровень ингибиторных антител к FVIII свертывания крови < 0,6 БО/мл, ПЛ не менее 6 месяцев накануне обследования КЖ и не реже 1р/неделю за этот период, отсутствие тяжелых психических или неврологических заболеваний. Подтверждение диагноза и формы тяжести заболевания, уровня ингибиторных антител проводилось во время мониторингового обследования методом определения уровня FVIII свертывания крови, теста на уровень ингибиторных антител в модификации Nijmegen, а также методом ретроспективного анализа стационарных карт пациента. Показатели КЖ представлены в виде «transmuted scale score» (TSS) в соответствии с известной формулой [21]; причем, большие показатели TSS свидетельствуют о худшем КЖ, а меньшие – о лучшем. Общее КЖ (оКЖ) определено как среднее TSS всех шкал опросника для конкретного пациента.

Для проверки нормальности розпределения полученных данных мы провели анализ эксцесса и асимметрии, использовали тест Шапиро-Вилка, а также анализ гистограмм и нормограмм розпределения. Учитывая, что розпределение большинства показателей было нормальным, результаты представляли в виде M ± SD (M – среднее, SD – стандартное отклонение). Для оценки достоверности разницы показателей использовался t-критерий для независимых групп. Значение P < 0.05 характеризовалось, как статистически достоверная разница.

Поиск актуальных исследований по теме «Лечение гемофилии у детей» проведен в поисковых наукометрических базах Medline, Google Scoolar, Embase, Cinahl и PsycInfo.

Результаты исследования и их обсуждение. По результатам исследования установлено, что у детей 4–7 р. не выявлено статистически достоверной разницы между показателями КЖ в режиме СП и ГП с показателями 48,94 ± 11,44 и 46,36 ± 7,03 соответственно (p = 0,652 при t = =–0,461). У детей 8–12р. также не выявлено статистической разницы (p = 0,652 при t = –0,461) между КЖ в режиме СП и ГП (45,55 ± 7,79 и 47,25 ± 14,78 соответственно). У старших детей 13–16р. при ГП КЖ было несколько лучше с показателем 45,16 ± 13,33 по сравнению с СП (54,02 ± ± 18,22), однако, такая разница в показателях тоже не была статистически достоверной (p = 0,341 при t = –0,995). Вместе с тем, сравнивая детей II и III групп очевидна тенденция к ухудшению КЖ при применении СП, однако, достоверность таких выводов следует подтвердить проспективным длительным исследованиям, что, безусловно, является перспективным для дальнейших исследований в этом направлении. При сравнении показателей КЖ без разделения на возрастные группы у всех пациентов также не выявлено достоверной разницы при СП и ГЛ (p = 0,548 при t = –0,605) при разнице средних всего 2,03.

Перед формированием выводов данного исследования, мы хотим учесть данные одного из наших предыдущих исследований, где мы продемонстрировали достоверное ухудшение КЖ при увеличении количества кровоизлияний суставы (p = 0,0009 при W = 0) и прямую сильную связь между данными показателями (p < 0,00001 при показателе R Спирмена 0,778) [20]. Одновременно, в данном исследовании мы также определили, что при СП дети имеют меньшее количество кровоизлияний в суставы по сравнению с ГП (разница М достоверная при p < 0.05): по меньшей мере одно кровоизлияние в суставы в течение месяца имели 28% пациентов, получавших СП, и 46% пациентов группы ГП. Без применения методов статистического анализа казалось бы, что с учетом таких исследовательских данных, используя метод компаративного прогнозирования, у пациентов в режиме СП КЖ должно быть лучше, по сравнению с ГП. Однако, полученные данные исследования демонстрируют

отсутствие такой зависимости. Прежде всего, такой результат можно обосновать многокомпонентностью понятия КЖ у детей с тГА, в частности, в данном случае, увеличение частоты лечения при СП компенсаторно ухудшает КЖ в сегментах лечения, улучшая в сегменте физической активности. Именно поэтому, вероятно, такой достоверности нами и не обнаружено. Учитывая это, и пытаясь максимально стандартизировать по критериям сравнения пациентов в исследуемых группах, мы провели сравнение КЖ между группами СП и ГП детей, не имевших кровоизлияний в суставы в течение месяца перед обследованием. Группу сравнения в данном случае составили 32 ребенка из 51 обследованных. Показатели оКЖ у пациентов СП (n = 18) составили 48,11 ± 11.85, тогда как в группе ГП (n = 14) оКЖ было достоверно лучше, с показателем TSS = 40,04 ± 20.19 (при $p < 0.05$). Вместе с тем, у пациентов группы ГП, которые имели 1 и более кровоизлияний в суставы (n = 12) оКЖ было хуже, как у пациентов, получавших СП (n = 7) с показателями 53,72 ± 8.16 и 49,12 ± 10.57 соответственно, однако такая разница не была статистически достоверной (p = 0,15). Полученные данные показывают, что при отсутствии объективных маркеров течения заболевания (кровоизлияния в суставы) КЖ является достоверно лучше при применении ГП. Однако, в таком случае не выясненным остается риск возникновения кровотечений, который, вероятно, будет выше в группе ГП.

Выводы. Количество суставных кровоизлияний (СК) у детей с тГА, получавших лечение в режиме СП достоверно менше, чем при использовании терапии ГП. В то же время, при отсутствии геморрагического синдрома в виде СК дети с тГА имеют лучшие показатели КЖ при уменьшении частоты введения препарата. Таким образом, ПЛ с меньшей кратностью введения препарата целесообразно применять у детей с отсутствием кровоизлияний в суставы и низким риском их возникновения. Соответственно, крайне перспективным для дальнейших исследований остается определение прогностических маркеров риска возникновения кровотечений у пациентов с тГА.

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ANALYSIS OF MORTALITY CASES FROM CONCOMITANT INJURIES: EXTRACRANIAL INJURIES COMBINED WITH CRANIOCEREBRAL INJURIES

Abstract: Concomitant injury is the mechanical damage of two or more organs of different cavities or simultaneous damage of the internal organ (or organs) and the musculoskeletal system (single or multiple). Concomitant injury is defied by two or more injuries, which require the help of different medical specialists (neurosurgeon, traumatologist, abdominal surgeon, oculist, maxillofacial surgeon, etc.). CCCI, which occurs in 43% of patients with all kinds of injuries, deserve special attention. Mortality among these patients ranges from 28.6% to 30.7% [4; 5; 6;].

Analysis of the treatment's result used in this study shows that out of 251 patients, 46 died after hospitalization. The overall mortality rate was 18.3%. Depending on the types of injuries in combination with extracranial injury, the mortality rates were as follows: in combination with damage to the maxillofacial region, 3 cases (6.5%), chest and thoracic injuries – 14 (30.4%), abdominal cavity organs damage – 11 (23.9%) and multiple extracranial injuries – 18 (39.1%).

Researchers all over the world continue to improve the diagnostic techniques and treatment of combined extracranial injuries, aiming to find better means of treatment and to improve its quality and efficient use of available resources [7; 8].

Keywords: severe concomitant craniocerebral injury, injury of thoracic organs and organs of abdominal cavity, analysis of mortality cases from extracranial injuries.

Rationale. Concomitant craniocerebral injury (CCCI) is the most common type among all concomitant injuries. Those injuries make up from 43 to 68% cases and most predominate are craniocerebral injuries. Concomitant injuries are more than combination of various organs' injuries, they can be considered as a special category of injuries, where pathological process has its own peculiarities and laws [1; 2; 3].

General characteristics of patients and research techniques. This research has a detailed

analysis of the complex assessments and the results of treatment of 251 patients with severe craniocerebral injuries, combined with extracranial injuries of different localization in the acute stage, as the basis. The patients treated were from Osh Interregional Clinical Hospital, Osh City Clinical Hospital, Nookat Subdistrict Hospital, Jalal-Abad Regional Hospital and Kyzyl-Kiya Sub-district Hospital in 2013–2016.

Out of 251 patients, 46 died after hospitalization, the overall mortality in the present research was 18.3%. A number of factors were found significant in relation to the probability of mortality. High mortality rates were determined in cases of patients older than 61 years, whereas the sex of patients was found not significant to the mortality probability. In our research, there were 201 (80.1%) males and 50 (19.9%) females ($p < 0.05$). The average age of patients ranged from 13 to 86 years and was 45.2 ± 7.6 years.

Out of 65 people who were hospitalized in the state of shock or terminal state, 32(49.2%) died.

The main causes of death in case of extracranial injuries were:

1) severe craniocerebral injury combined with severe extracranial injuries –16 patients (34.8%);

2) diagnostic errors – 5 cases (10.9%), including intracranial injuries (hematomas) in 3 patients, 1 – liver rupture and 1 – spleen rupture;

3) secondary complications – 14 patients (30.4%) including inflammatory – 12 (pneumonia-9, peritonitis-2, meningitis-1), thromboembolism of the pulmonary artery-2. Total mortality rate with compression of the brain in patients with combined craniocerebral injury and shock was 70.8%, and postoperative mortality – 63.0%.

Given the long-term anchorage of the patients in the position on the back during reanimation and anti-shock treatment, preoccupation of the surgical team with the implementation of urgent anti-shock measures, such as surgical interventions, the implementation of some techniques of treating severe craniocerebral injury becomes difficult or impossible [8; 9; 10]. We have developed some treatment techniques, the use of which is possible in these conditions. It includes techniques of intensive sanitation of subarachnoid spaces and the removal of intracranial hematomas through mini burr holes.

Treatment of chest injuries was also dependent on the predictors of severity and outcome of injury. All modern types of conservative and surgical treatment were used, except for thoracobrachial dressings, which, because of the complexity of application, bulkiness, difficulties associated with examining and caring for the patients, were not used. Treatment of patients with injuries of the chest, which have had a favorable prognosis and duration of shock up to 8 hours, was performed as early as possible, using all available techniques of surgery and conservative treatment.

We propose a technique for treating injuries of the thorax and ribs in a concomitant craniocerebral injury. To perform this technique, 1 ml of a 2% solution of lidocaine should be slowly injected intradermally to the patient. The administration should be performed below the xiphoid process once a day for 3–5 days. This technique allows the provision of a steady decrease in the pain syndrome of the patients with chest injuries and fracture of the ribs in a concomitant craniocerebral injury with a minimal expenditure of analgesics due to a decrease in the activity of the sympathetic nervous system.

This technique increases the effectiveness of treatment, shortens the recovery period and reduces disability rate among patients with concomitant craniocerebral injuries.

The proposed technique allowed us to achieve a qualitative improvement in the results of treatment of patients with concomitant craniocerebral injuries, reduce the incidence of disability, and prevent possible complications, both in the acute period of severe craniocerebral injury and in the postoperative period. This technique was used in the diagnostic and treatment of 15 patients. After the treatment, using this treatment technique, all patients were discharged in a satisfactory condition.

Practical use of the presented technique with the introduction to the therapeutic and diagnostic tactics, based on the objective indicators of the severity of the condition of the patients, allowed to improve the quality and effectiveness of treatment of one of the most severe categories of patients – the patients with a concomitant craniocerebral injury, which suffered from shock. So, the mortality rate of patients with severe cerebral contusion decreased by 5.4% and with compression of the brain by 2.2%. The mortality in the complicated injuries has decreased by 6.8% (from 48.5 to 41.7%). The overall mortality rate decreased by 18.3%, and the mortality in the shock period was 10% compared lower to the mortality in a similar group of patients treated according to usual schemes.

The highest mortality was observed in the presence of compression of the brain and in the combination of severe craniocerebral injury with multiple thoracoabdominal injuries. It was noted that the lethal outcome was more often observed in the I group of patients with concomitant craniocerebral injury in the first and second periods of wound dystrophy (respectively 81.6% and 63.3%) and was mainly due to shock, acute blood loss, and the development of early pneumonia and thromboembolic complications.

Clinical and morphological comparisons of 46 deceased patients showed a lack of parallelism between clinical manifestations of injury and brain injuries of the patients. Contusions of the brain differed in multiplicity, depth and expressed hemorrhagic component, perifocal edema was expressed slightly.

Outcomes of a concomitant craniocerebral injuries depended on the severity and concomitant factor of this type of injury, the presence of shock, blood loss, alcohol intoxication, the dynamics of impaired consciousness, the types and the mechanism of injuries, the speed, volume, and adequacy of the provided treatment up to the hospital stage, the terms of hospitalization, the time of the beginning and completeness of the treatment, complications and accompanying diseases. Prediction of immediate outcomes and early complications of concomitant injury required a comprehensive development of approaches taking into account all the necessary clinical and paraclinical data and information on the age aspect.

To determine our strategy and surgical treatment of intracranial hematomas, we analyzed the causes of death of operated patients to retrospectively evaluate in what cases with modern neurosurgery and resuscitation facilities, the intervention will be ineffective, and in what cases, with more correct and timely treatment, one could hope for benign outcome. Patients of young and middle age group and elderly (people of 60 years and older) were studied by us in terms of the causes that led to death.

The mortality rate prevailed among the patients with a bilateral injury of the midbrain (3 cases) and bilateral brain bridge (8 cases), and duration of coma was noted in 4(1–16) ± 2.4 cases with a supratentorial lesions, in 8(3–17) ± 2.8 – with damage to the brainstem and in 14(2–24) ± 6.1 – bilateral injury of the midbrain.

In the group of patients of 21–40 years old, out of 73 patients, 3 patients died. When analyzing the causes of mortality, it was revealed that 1 of them died in the first hours or days, from a bruise-hemorrhage to the brainstem, 2 patients – in the postoperative period from cerebral edema with an incision that developed suddenly on day 3–6 after the removal of intracranial hematoma. At first, the condition of these patients improved, they became available to the contact, but then, within a few hours, a cerebral symptomatology developed followed by addition of the stem symptomatology and termination of the vital functions. On pathoanatomical dissection, these patients showed a sharp edema and swelling of the brain, which led to compression of the brainstem, patients died due to hemodynamic disorders in the brain.

Results. Therefore, 3 patients from this group died, because of the reasons that made treatment ineffective. Removal of foci of crushing, intensive anti-edematous therapy could rescue the patients.

In the middle age group (41–60 years), 13 patients died. In this group, on the background of the hypertensive disease, atherosclerosis and chronic diseases present in patients, the severity extends beyond cerebral complications in the form of hypostatic pneumonia, fibro-purulent tracheobronchitis, and hemorrhages to the parenchymal organs. In this group, the frequency of damage to the bones of the skull and internal organs was high, 1 patient of this group died in the postoperative period during the first day of primary stem hemorrhage. This patient, except for the main focus and hemorrhages in the hemisphere and the brain stem, had small hemorrhages in the stomach, pericardium, lungs, intestines and adrenal glands. This combination of hemorrhages should be considered as a vasomotor or diapedesis. Hemorrhages in the adrenal glands, as well as hemorrhages in the stem are considered incompatible with life. The dependence of the frequency of combined hemorrhages in the parenchymal organs on age was noted. Their frequency increases with age. In all cases of hemorrhage, edema-swelling of the brain developed with infringement in the occipital foramen of the brain stem sections. Such compression of the stem leads to its ischemia with violation of cardiac and respiratory functions.

Thus, it was not possible to save one patient with a hemorrhage in the brain stem, despite the use of existing techniques and means.

Among the elderly group (61 years and older), 15 patients died, 2 died on the first day due to the increasing stem decompensation.

Two patients of this group died of hemorrhagic stroke on the background of the developed hypertensive crisis. Their condition after the operation improved, but then on day 2–3 they died as a result of rapidly developing vital disturbances. The biopsy has shown that fresh hemorrhages were found in the brain tissue. The remaining 2 patients died on the 5–14th day with gradual deterioration of the condition. A few hours before death, the condition deteriorated sharply, followed by a loss of consciousness and a syndrome of "floating" eyeballs, tonic convulsions and termination of vital functions, which led to death. Autopsy has shown areas of traumatic softening of the cortex and white matter of the brain, secondary hemorrhage in the brainstem, more often found in the bridge. Secondary hemorrhages were associated, evidently, with the growing pathological vascular reactions that led to hemorrhage into the brain stem. All had hypostatic pneumonia of varying severity, atherosclerotic changes in blood vessels, in particular cerebral vessels.

Out of 46 (18.3%) of the deceased patients, 6 developed primary hemorrhages of the stem, which were the main cause of death of the patients. The severity of the injury in these patients did not give any real reason to believe that any of them could be saved. Other 5 patients had different causes of death. Here, death was due to secondary reactions to injury. The causes of such violent reactions that led to death were the centers of extensive traumatic brain lesions.

Conclusion. Therefore, we can note that as the age of the patients we observe increases, the frequency of secondary stem hemorrhages, pathological vascular reactions of the brain, hypostatic pneumonia increases on the background of already existing cardiovascular and pulmonary pathologies.

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CORRECTIVE EFFECT OF LASER THERAPY ON THE STATE OF LOCAL ORAL CAVITY RESISTANCE IN PATIENTS WITH SJOGREN'S DISEASE

Abstract: the effect of laser therapy on the state of oral cavity resistance in the complex treatment of Sjogren's disease was investigated. It has been established that laser irradiation of parotid salivary gland reduces manifestations of xerostomia, increases salivation, normalizes the cell composition of the liquid of oral cavity, reduces the Greene-Vermillion index.

Keywords: Sjogren's disease, oral cavity resistance, laser therapy.

Background. Saliva is an important biological substance which is part of oral fluid. About 1,5 liters of saliva are produced in one day in healthy body, the saliva moisturizes the mucous membranes of the mouth and esophagus, digested food; saliva with the cellular population of the oral cavity contributes to controlling the microbial population of the mouth due to presence of antibacterial factors, promotes mineralization and strengthening of teeth [1; 2].

Reduction of saliva production occurs in healthy people with emotional overload, in diseases – diabetes mellitus, hyperthyroidism, taking some medications (antidepressants, diuretics, etc.). The prevalence of xerostomia correlates with age – among people under the age of 50 years old it is observed in 6% of population, and in people of 65 years old – in 15% [2; 3]. However, the most expressed xerostomia is observed rheumatic pathology – rheumatoid arthritis [4–7], systemic scleroderma, and especially – in Sjogren's disease (SjD) [9–12].

Sjogen's disease is a systemic disease of glands of external secretion with predominant affection of salivary, lacrimal glands and also glands of gastrointestinal tract. Subjective signs of this disease are damages of eyes (dry keratoconjunctivitis) and oral cavity (from decrease of saliva production to it's complete absence) [9–12]. There may be signs of decreased functions of other exocrine glands (sweat glands, glands of gastrointestinal tract), which lead to development of dry skin, atrophy of gastric and intestinal mucous membranes [12–15].

Systemic manifestations of this disease include lymphadenopathies, arthralgia or arthritis, Raynaud's syndrome, affection of gastrointestinal tract, lungs, heart, kidneys, cytopenia, phenomena associated with hyperproduction of antibodies (hyperviscosity syndrome, the appearance of cryoglobulins, rheumatoid factor, antinuclear antibodies, antibodies Ro/SS-A and La/SS-B) [9; 15; 16].

The activity in the pathological process of structures related to immune response system changes the cellular composition of the oral cavity fluid, which reduces the protective capabilities of oral mucosa and leads to rearrangement of the microbial landscape of the oral cavity and promotes the development of autoimmune inflammation in the salivary glands and oral cavity [9; 10].

Therefore, the most important task of treatment of such patients is to reduce the intensity of autoimmune inflammatory process in the salivary glands. Systemic glucocorticoids are used for the treatment of SjD, and in the presence of vasculitis the cytostatics should be used. Also locally artificial tears, mouthwash and drinking water while eating can be applied

[9; 13; 17; 18]. However, the desired effectiveness of therapeutic complexes is not always achieved. It should also be noted that the inclusion of physical factors in the treatment of SjD is not fully worked out, particularly the ability to influence by these factors on the resistance of mucous membrane of oral cavity for correction of autoimmune inflammation. The purpose of the study is to determine the effect of laser irradiation on the cellular composition of oral cavity as important component of local resistance.

Materials and methods. 42 patients with Sjogren's disease were examined, all of them are women, the average age of patients was 53.5 ± 1.1 years old. The duration of disease was from 2 to 6 years (average duration 3.7 ± 0.2 years).

A clinical, laboratory and instrumental investigation was conducted, which included analysis of complaints, anamnesis. The research was carried out by rheumatologist, stomatologist and ophthalmologist. In the objective examination, particular attention was paid to the condition of mucous membrane of eyes and oral cavity. We conducted the Schirmer's test.

For studying of cellular composition of the oral liquid in patients, the fluid was collected from sublingual fossa during 10 minutes with the use of special capsule. The resulting liquid was centrifuged at 10,000 rpm for 4 minutes. From the precipitate which formed in the test tube, smears were prepared, which stained by hematoxylin-eosin after fixation in vapors of alcohol-ether during 2 hours. The obtained specimens were studied using a light microscope. In 10 fields the number of lymphocytes, neutrophils, epithelial cells, "bare nuclei" (a sign of activity of the autoimmune reaction) were counted, in total at least 150 cells. Then we counted their relative number in the cell population of oral cavity.

Laboratory investigation included general blood and urine tests, determination of total protein and fractions, glucose, bilirubin, creatinine, rheumatoid factor and interleukin-1 in blood. The antinuclear antibodies, anti-Ro/SS-A and anti-La/SS-B antibodies were detected. The contents of lysozyme and secretory IgA (SIgA) in saliva before and after treatment were studied. Electrocardiogram and X-ray of chest organs were performed.

The diagnosis was verified according to the criteria of the Institute of Rheumatology (2001) [9].

All patients were treated with methylprednisolone in adequate doses, in the main group with complex therapy we added the infrared laser on the area of parotid glands. We applied the apparatus of laser therapy "Uzor", on the projection of parotid glands by local contact, radiation power 7 W, frequency 300 Hz, exposure 3–4 minutes, 10 procedures per course, every day.

Results and discussion. As a result of examination, it was found that complaints of dry mouth, difficulty on swallowing of dry food and need to take some water after it were in all patients, recurrent parotitis were noted in 34 patients (81%). Complaints of dry eyes and sense of "sand" were presented in 40 patients (95%). Periodic arthralgia was observed in 36 patients (85,7%), periodic dysphagia – 6 patients (14%).

The enlargement of submandibular, infraauricular and cervical lymph nodes was observed in 27 patients (64%). Raynaud's syndrome was observed in 15 patients (35.7%).

All patients had precervical caries of various degree of severity and partial adentia. The mucous membrane of the oral cavity in all patients was dry, hyperemic, there were cracks in the corners of the mouth, and in 3 patients there was a severe damage of the mucous membrane of oral cavity – expressed hyperemia of the mucous membrane, smooth, red mucous of tongue – "burning mouth". The Green-Vermillion index (index of hygiene of the oral cavity) was 5.4 ± 0.1, which corresponds to unsatisfactory level of hygiene of the oral cavity.

The decrease in salivation by Schirmer's test less than 10 mm/5 minutes (on average – 5.5 ± 0.05 mm) was observed in 40 patients, all patients had decrease in spontaneous salivation to 1.5 ml/15 minutes. The decrease in the content of lysozyme in saliva to 74 mcg/ml and SIgA to 0.34 g/l was detected.

A decrease in the amount of hemoglobin less than 110 g/l was detected in 28 patients (66.7%), an increase in ESR more than 15 mm/h in 10 patients (23.8%). Rheumatoid factor was detected in 19 patients (45%), antinuclear antibodies in low titre – in 32 patients (76%), anti-Ro/SS-A and anti-La/SS-B antibodies – in 17 patients (40%).

The results of investigation of oral cellular population are given in (Table 1). Before analyzing the changes in the composition of cell population, it should be emphasized that a large amount of dense protein was detected in the oral liquid. As we can see from Table 1, before treatment macrophages (neutrophils) in the oral cavity were absent in all patients, which indicates the lack of nonspecific phagocytosis, that is, there is practically no antimicrobial protection in the oral cavity of these patients.

Perhaps it causes the changes in the microbial population. At the same time, there is a significant increase in the content of lymphocytes and "bare nuclei", which indicates the high activity of autoimmune inflammation.

Table 1.– Effect of laser irradiation on the cellular composition of oral cavity in patients with Sjogren's disease (%)

Group/Indicator	Control group	Before treatment	After treatment
Lymphocytes	7.4%	32.12%	26.34%
Neutrophils	18.45%	0	17.76%
Epithelium	71.0%	58.78%	56.9%
"Bare nuclei"	1.47%	9.0%	0

The inclusion of laser therapy in complex treatment led to decrease in the hygienic Green Vermillion index, the content of IL-1 in serum, increase in the spontaneous salivation rate and the content of lysozyme in saliva (Table 2) more significantly than in the control group (Table 2), that is, there was a significant increase in the amount of saliva and its bactericidal capacity. The obtained results indicate an additional inhibitory effect of laser therapy on autoimmune and inflammatory processes in the salivary glands, which are the pathogenetic basis of Sj D. Decrease in the content of IL-1, which is a systemic inflammatory mediator, may indicate a decrease in the intensity of the autoimmune inflammatory process in the salivary glands.

Table 2.– Effect of complex treatment on the level of interleukin-1, oral hygiene, the quantity and quality of saliva in patients with Sjogren's disease

№	Indicator	Basic group (n = 21)	Control group (n = 21)
1.	Hygienic Greene-Vermillion index, before/after treatment, units	5.4 ± 0.1 4.0 ± 0.1*	5.4 ± 0.09 4.6 ± 0.07*»
2.	Salivation rate, before/after treatment, ml/min	0.15 ± 0.01 0.26 ± 0.02*	0.15 ± 0.02 0.21 ± 0.01*»
3.	Lysozyme content in saliva, before/after treatment, mcg/ml	75.0 ± 0.7 91.5 ± 0.9*	74.7 ± 0.6 86.1 ± 0.6*»
4.	SIgA concentration in saliva, before/after treatment, g/l	0.34 ± 0.03 0.46 ± 0.04*	0.33 ± 0.02 0.43 ± 0.03*
5.	IL-1 concentration in blood, before/after treatment, pg/ml	19.7 ± 0.1 14.2 ± 0.2*	19.6 ± 0.2 16.6 ±0.3*»

*Notes: * – the significance of difference in the group before and after treatment, $p < 0.05$;*
– the significance of difference after treatment in basic and control groups, $p < 0.05$.

Reduction of tension of autoimmune processes positively affects the state of protective capabilities of the oral cavity. This is evidenced by the appearance of neutrophil cavity in the cell population of the oral cavity (Table 1). In addition, the inhibition of autoimmune reactions by laser irradiation of the region of parotid gland contributes to decrease in the intensity of inflammatory processes (decrease in the content of lymphocytes), and evidence of inhibition of autoimmune reactions is the disappearance of "bare nuclei" from the cellular population of the oral cavity.

Conclusions. Thus, Sjogren's disease is characterized by obligate damage of mucous membrane of the oral cavity and teeth due to the autoimmune process in the glands of the external secretion (salivary and lacrimal). The application of laser therapy on parotid glands in the complex treatment of Sjogren's disease potentiates the positive effects of systemic therapy, greatly reduces the subjective and objective manifestations of xerostomia, achieves more significant increase in salivation, improvement of the protective properties of saliva and oral hygiene (reduction of Green-Vermillion index), and also increasing of oral mucous membrane resistance.

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STUDYING QUALITATIVE CHARACTERISTICS OF BONE TISSUE OF THE HUMAN MAXILLA ON THE QUANTITATIVE CONTENT OF TRACE ELEMENTS (K, Fe, Co, Sr, Zn) IN THE DYNAMICS OF PRENATAL ONTOGENESIS

Abstract: *Purposes.* The updated and substantiated understanding of the patterns of the upper jaw growth in prenatal ontogenesis contributes both to the diagnosis of congenital malformations and the prevention of prenatal injury to the maxillofacial area. In this regard, determining the density of bone tissue, that is, its mineralization, whose impairment forms the basis for the development of various defects, is as important as studying inter-tissue relations in the process of histo- and organogenesis, including epithelio-mesenchymal ones. This research was conducted to study the quantitative content of trace elements (K, Fe, Co, Sr, Zn) in the dynamics of prenatal ontogenesis as a fundamental material of bone tissue. The research was carried out as part of the implementation of a fragment of the planned comprehensive research work "Features of morphogenesis and structural and functional peculiarities of tissues and organs in human ontogenesis", No. of state registration 0116U002938.

Methods. By means of atomic emission (AES) and atomic absorption (AAS) spectrometry and statistical processing, as well as variational and dynamic analysis programs, the relative values for each

trace element were obtained while studying the bone tissue from abortion and sectional material of the upper jaw in 131 human fetuses (Ukrainians).

Results. These results as the mean value of the studied parameter (M), standard deviation (m), paired student's t-test or reliability value (t), and the probability level, which are shown in Tables 2–4, where the comparison values of the first and fourth groups are: for potassium (K) – 0.188 ± 0.006 in the first and 0.144 ± 0.019 in the fourth group ($t = 2.21$, $p < 0.05$); for iron (Fe) – 0.348 ± 0.027 and 0.435 ± ± 0.057($t = 1.38$, $p > 0.05$); for cobalt (Co) – 0.086 ± 0.006 and 0.059 ± 0.008 ($t = 2.70$, $p < 0.01$); for zinc (Zn) –0.905 ± 0.035 and 0.303 ± 0.032 ($t = 12.81$, $p < 0.001$), which substantiates the high reliability of the findings, the quantitative determination of the content of trace elements simultaneously reflects the quality of the bone tissue of the upper jaw of human fetuses in prenatal ontogenesis.

The investigated growth rate (%) for potassium (K) in the three groups is negative: between the first and second groups (–4.39%), between the second and third (–68.94%). However, a sharp increase in potassium in the fourth group, compared to the third one, is + 318.63%. The overall growth for potassium between the first and fourth groups is + 24.33%.The dynamics of iron trace content (Fe) has a positive growth pattern in almost all age periods of prenatal ontogenesis; accordingly, there is a positive growth rate (%): between the second and third groups it increases by 34.62%; between the third and fourth – by 52.15%; between the first and the fourth – by 102.67%, except for a moderate decline in the second group (17–24 weeks), therefore, the growth rate between the first and second groups has a significant but negative value (–1.05%). The maximum growth rate (%) is set for cobalt (Co) (Figure 5) in the middle (22–27 weeks) of the intrauterine development (IUD) of the fetus, which is more than one hundred and fifty percent (+150.51%), which confirms the intensity of the vascular system development and that of metabolic transformations, is observed in this age period. A slight increase (%) of zinc (Zn) with the sign "+" is observed in the second experimental group (+3.99%), and rapidly decreases in the third (–9.34%) and the fourth (–42.43%) groups, which is a positive reflection of the qualitative parameters of fetal bone tissue.

Conclusion. Our study have found that the age dynamics of all values of the trace elements content in the prenatal ontogenesis of the upper jaw of human fetuses significantly correlates with both the decrease and growth – in the first (11–16 weeks of IUD), the second (17–24 IUD), the third (25–29 weeks of IUD) and the fourth (30–40 weeks of IUD) experimental groups, which is directly proportional to the re-distribution of trace elements for the construction of organs and systems in these age periods. The regularity of the dependence and ratio of the content of cobalt (Co) and iron (Fe) in the first, second and third experimental groups was studied. There was a slight correlation between dependence and direct correlation (Fig. 2) on the reduction of zinc (Zn) and iron (Fe) in all groups. Studying the patterns of dynamics of the density of bone tissue of the upper jaw in human fetuses, depending on the mineral composition and the presence of the revealed synchronism of these processes, suggests that the change in density is indicative of a change in the content of certain mineral elements. In our opinion, this provision may be the basis for the development of new techniques for early diagnosis of congenital anomalies of the maxillofacial area at the preclinical stages of its development and the methods of their prevention, through the correction of the mineral composition.

Keywords: prenatal ontogenesis, maxilla, maxillofacial region, human.

Introduction: Biological behavior of bone in different developmental periods is determined by its main properties: biochemical (content of mineral and organic substances and their correlations), morphological (degree of heterogeneity and features of topographic correlations of major vessels [22], which ensure vascularization and proper construction of the body), biological (content and the ratio of different types of cells per unit of the bone volume [32]). That is, the bone tissue is a dynamic open system that is characterized by a complex multi-level organization with the ability to change its structure and properties in the ontogenetic modeling process in accordance with the state of the regulatory systems and conditions in which it is located. As a result, it has a significant individual and topographic variability of the morphological structure, chemical composition and biological potential [18; 26; 29; 32].

Studying the chemical composition of bone tissue is associated with considerable difficulties, since in order to study the inorganic matrix, it is necessary to isolate the organic saturation and further dissolution of the inorganic one, which may also partially lead to the loss of macro- and micronutrients. In addition, their content is directly proportional to both components, organic and inorganic parts [12; 28].

With the current level of theoretical ideas about the structure and function of the bone tissue, the concept of "bone quality" should be considered as a collective integrated one, which is a certain generalized characteristic of architectonics, density, mineral bone density and its biological potential [23; 30].

A number of authors have described that all these parameters are quite varied in the prenatal ontogenesis, and their impairment leads to a significant pathology of the maxillofacial area [1; 2; 15; 31].

Materials and methods. We have studied the bone rudiments of the upper jaw of the 131-nd human fetuses of Ukrainians, aged 11–40 weeks of the intrauterine development (IUD), obtained from abortion and sectional material of spontaneous miscarriages or stillbirths in preterm labor, who had died of conditions associated with diseases of the maxillofacial area and had developed in the uterus in the absence of the effects of manifestly expressed harmful factors of the human external and internal environment.

The bone tissue sampling was carried out on both sides of the upper jaw of a fetus at different sites, with the most pronounced, macroscopically, density. Methods of macroscopy, morphometry of objects were applied during the study, using the gradation of periods of the intrauterine development on the basis of classical periodization of embryogenesis and post-fetus human ontogenesis according to G. A. Schmidt (1972), which determines: the embryonic period – 45 days, the prefetal period lasting 30 days and the fetal period of 192 days, from 55.0 to 376.0 mm of crown-rump length (CRL). The study involved both gross specimens from the Museum of Chairs of the Medical University and materials received in accordance with the agreement on scientific cooperation with the Chernivtsi Oblast Communal Medical Center "Post-mortem Department" in Chernivtsi (Ukraine).

All studies were conducted in compliance with the main provisions of the GCP (1996), the Council of Europe Convention on Human Rights and Biomedicine (of 04.04.1997), the Helsinki Declaration of the World Medical Association on the Ethical Principles of Scientific Medical Research with Human Participation (1964–2013), orders of MoH of Ukraine No. 690 of September 23, 2009, No. 616 of 03.08.2012, and according to the guidelines [16] and "Procedure for the extraction of biological objects from the dead individuals, whose bodies are subject to forensic examination and pathological anatomical investigation for scientific purposes" [17].

Applying the methods of flame atomic emission and atomic absorption, determination of potassium metal ions (K), strontium (Sr), zinc (Zn) and cobalt (Co) was carried out directly from the initial solutions with the corresponding wavelengths:

– K(potassium) – λ = 766.5 nm, linearity 0.1–0.2 mg/l, C_n = 0.01 mg/l

– Zn(zinc) – λ = 231.9 nm, linearity 0.4–2.0 mg /l, C_n = 0.05 mg/l

– Co (cobalt) – λ = 240.7 nm, linearity 0.1–5.0 mg/l, C_n = 0.05 mg/l

– Sr(strontium) – λ = 460.7 nm, linearity 0.05––0.5 mg/l, C_n = 0.005 mg/l

using atomic absorption spectrophotometer AAS-1N (Carl Zeiss Jena, Germany) by means of the flame of propane-butane-air. We also used a drying chamber 2B-151 (Ukraine) and analytical scales of the second class of accuracy XAS100 / C (RADWAG, Poland).

Determination of Fe (iron) content was carried out using a photometric method, light absorption was measured by means of a photocolorimeter КФК-2-УХЛ 4.2 (Ukraine), as ferrum (III) –yellow three sulfosalicylic complex in an ammonia medium with a wavelength λ = 400.0 nm, linearity 0.1–0.2 mg l, C_n = 0.03 mg/l. Statistical processing of the findings was conducted using the unified program STATISTICA 10, applied in scientific clinical and epidemiological studies in medicine. Standard statistical programs of variation and dynamic analysis by means of computer technology were used as well.

Using the method of statistical groupings in the study of qualitatively homogeneous aggregates, where there are no qualitative transformations yet, but there are quantitative differences, grouping for a large number of observations has been carried out (Table 1).

Table 1.– Grouping the research objects

Ordinal group number	Age, weeks	Number of observations
1	11–16	35
2	17–24	33
3	25–29	32
4	30–40	31
Total number of observations		131

To study the quantitative composition of trace elements of the bone tissue in the human maxilla in the dynamics of the fetal development period, a variational analysis of statistical data with the determination of the average values for each trace element, the error of the average values, was used. An assessment was made of the reliability of the averages and the probability of an error-free prediction.

A comparative analysis of the dynamics of density and content of trace elements in the bone tissue of the maxilla of human fetuses in different age groups of prenatal ontogenesis was carried out with the determination of the reliability of the difference of the indices using the Student's reliability test (t).

As a result of statistical research in the processing of statistical data we received absolute numbers, indicating the size of phenomena. Although absolute numbers have some cognitive value, their use is limited. To determine the level of the phenomenon and to compare the values in dynamics, we took into account the relative values (indices, coefficients), which result from the correlation of statistical quantities among themselves.

All the findings (relative and average values) were evaluated to determine their reliability. The assessment of the reliability of the relative values was carried out by calculating their error ($m_{\%}$) by the formula for a large number of observations.

The result was considered reliable when the fraction from the division of the indicator to its error $\left(P/m\right)$ equaled 2 or more. The criterion of reliability (t) = 2 showed that the result obtained in a sampling population, in 95.5% of cases differed from the result of the general population by 2m. That is, the probability of an error-free prediction (P) was 95.5%. This result is considered acceptable for statistical research in the field of medicine, as shown in (Figure 1).

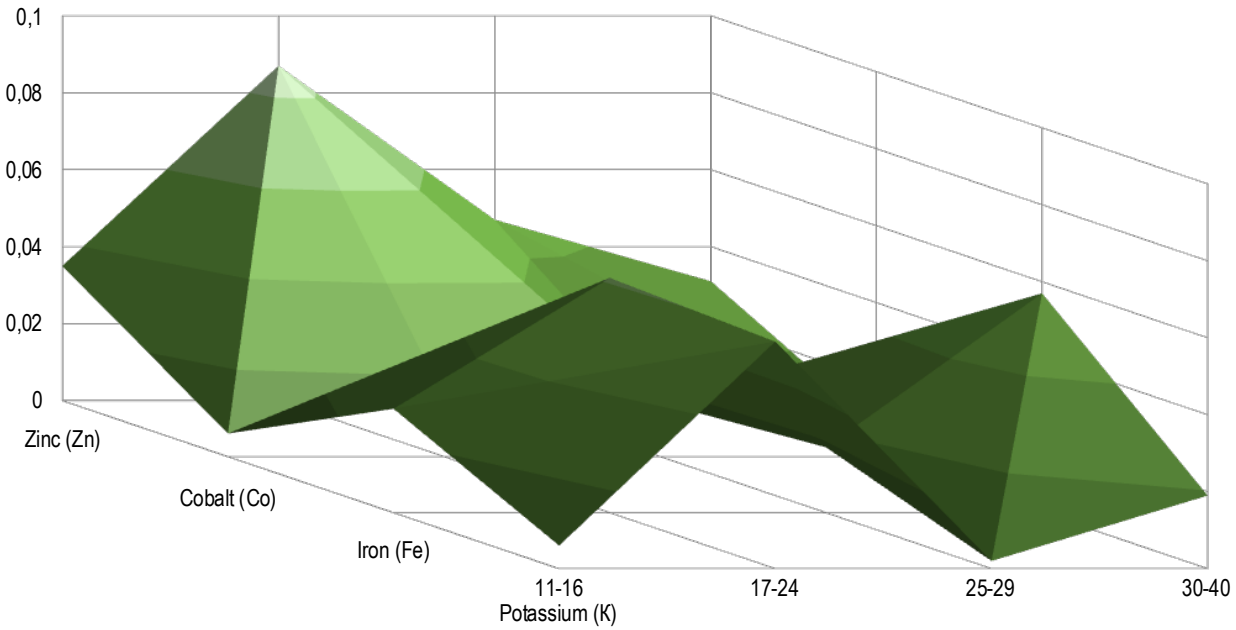

Figure 1. A standard error (11–16, 17–24, 25–29, 30–40) in age groups, weeks

Estimation of reliability of average values (average values of the content of minerals – trace elements of the maxillary tissue of the human fetus) was carried out by calculating their error (m_M) by the formula for a large number of observations.

To determine the significant difference between the values in different groups of prefetal and fetal periods, the reliability of the difference between the values presented in tables 2–4 was done which are given in this section (materials and methods) as the major attention in our study is drawn to analyzing the dynamics – growth rate (%). For relative and average values, Student's test t was calculated using the corresponding formulas. The difference was considered significant at $t \geq 2$.

Table 2.– Reliability of the difference between the values of trace element content in the bone tissue of the upper jaw in human fetuses in the age groups of 11–16 weeks (group 1) and 17–24 weeks (group 2)

Groups / Trace elements	Group 1		Group 2		Student's t-test	P
	M_1	m_1	M_2	m_2		
Potassium (K)	0.188	0.006	0.237	0.059	0.83	> 0.05
Iron (Fe)	0.348	0.027	0.455	0.061	1.57	> 0.05
Cobalt (Co)	0.086	0.006	0.081	0.015	0.31	> 0.05
Zinc (Zn)	0.905	0.035	1.244	0.087	3.64	< 0.001

Table 3.– The reliability of the difference between the indexes of trace element content in the bone tissue of the maxilla of human fetuses in the age groups of 11–16 weeks (group 1) and 25–29 weeks (group 3)

Groups / Trace elements	Group 1		Group 3		Student's t-test	P
	M_1	m_1	M_2	m_2		
1	*2*	*3*	*4*	*5*	*6*	*7*
Potassium (K)	0.188	0.006	0.043	0.002	23.02	< 0.001
Iron (Fe)	0.348	0.027	0.359	0.017	0.37	> 0.05

1	*2*	*3*	*4*	*5*	*6*	*7*
Cobalt (Co)	0.086	0.006	0.119	0.014	2.20	< 0.05
Zink (Zn)	0.905	0.035	0.660	0.047	4.22	< 0.001

Table 4.– The reliability of the difference between the indexes of trace element content in the bone tissue of the maxilla of the human fetuses in the age groups of 11–16 weeks (group 1) and 30–40 weeks (group 4)

Groups / Trace elements	Group 1		Group 4		Student's t-test	P
	M_1	m_1	M_2	m_2		
Potassium (K)	0.188	0.006	0.144	0.019	2.21	< 0.05
Iron (Fe)	0.348	0.027	0.435	0.057	1.38	> 0.05
Cobalt (Co)	0.086	0.006	0.059	0.008	2.70	< 0.01
Zink (Zn)	0.905	0.035	0.303	0.032	12.81	< 0.001

To assess the homogeneity of statistical data (average values of the content of trace elements in the human maxillary bone marrow), the determination of the type and reliability of the mean value was determined by the coefficient of variation (CV) presented in Tables 5–8 and assessed according to the scale (low, medium and high variety of the feature). At the same time, it was found that the coefficient of variation (CV) in the second group (17–24 weeks) for potassium (K) was 143.49 and for cobalt (Co) – 104.31, indicating a high diversity of the feature.

Table 5.– Mineral composition of the bone tissue of the upper jaw of a human fetuses aged 11–16 weeks (group 1), trace elements, mg / g, n = 35

Trace elements	mean M	Confidence interval (M-2m)	Confidence interval (M+2m)	Standard deviation (Δ)	Dispersion (δ^2)	Coefficient of variation (CV)	Standard error (m)	Probability of error-free forecast (P)
Potassium(K)	0.188	0.175	0.201	0.039	0.002	**20.56**	0.006	< 0.001
Iron (Fe)	0.348	0.294	0.403	0.158	0.025	**45.58**	0.027	< 0.001
Cobalt (Co)	0.086	0.073	0.098	0.035	0.001	**41.55**	0.006	< 0.001
Zink (Zn)	0.905	0.833	0.976	0.208	0.043	**22.99**	0.035	< 0.001

Table 6.– Mineral composition of the bone tissue of the upper jaw of the human fetuses aged 17–24 weeks (group 2), trace elements, mg / g, n = 33

Trace elements	mean M	Confidence interval (M-2m)	Confidence interval (M+2m)	Standard deviation (Δ)	Dispersion (δ2)	Coefficient of variation (CV)	Standard error (m)	Probability of error-free forecast (P)
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
Potassium (K)	0.237	0.117	0.358	0.340	0.116	**143.49**	0.059	< 0.001
Iron (Fe)	0.455	0.331	0.579	0.350	0.123	**76.97**	0.061	< 0.001

1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
Cobalt (Co)	0.081	0.051	0.111	0.084	0.007	**104.31**	0.015	< 0.001
Zink (Zn)	1.244	1.068	1.421	0.498	0.248	**40.04**	0.087	< 0.001

Table 7.– Mineral composition of bone tissue of the human fetuses upper fetal jaw aged 25–29 weeks (group 3), trace elements, mg / g, n = 32

Trace elements	**mean M**	**Confidence interval (M-2m)**	**Confidence interval (M+2m)**	**Standard deviation (Δ)**	**Dispersion (δ2)**	**Coefficient of variation (CV)**	**Standard error (m)**	**Probability of error-free forecast (P)**
Potassium (K)	0.043	0.038	0.047	0.012	0.0001	**29.02**	0.002	< 0.001
Iron (Fe)	0.359	0.323	0.394	0.097	0.009	**27.28**	0.017	< 0.001
Cobalt (Co)	0.119	0.089	0.148	0.081	0.006	**68.38**	0.014	< 0.001
Zink (Zn)	0.660	0.563	0.757	0.268	0.072	**40.60**	0.047	< 0.001

Table 8.– Mineral composition of the bone tissue of the upper jaw of the human fetus aged 30–40 weeks (group 4), trace elements, mg / g, n = 31

Trace elements	**mean M**	**Confidence interval (M-2m)**	**Confidence interval (M+2m)**	**Standard deviation (Δ)**	**Dispersion (δ2)**	**Coefficient of variation (CV)**	**Standard error (m)**	**Probability of error-free forecast (P)**
Potassium (K)	0.144	0.104	0.183	0.106	0.011	**74.02**	0.019	< 0.001
Iron (Fe)	0.435	0.318	0.551	0.319	0.101	**73.29**	0.057	< 0.001
Cobalt (Co)	0.059	0.043	0.076	0.045	0.002	**75.49**	0.008	< 0.001
Zink (Zn)	0.303	0.238	0.368	0.177	0.031	**58.43**	0.031	< 0.001

To identify the trends of qualitative characteristics of bone tissue of the maxilla of human fetus in the dynamics of prenatal ontogenesis, a dynamic analysis was used. The patterns of growth or decrease in the content of certain trace elements in the bone tissue of the human upper jaw depending on the age period of prenatal ontogenesis of the fetus have been established.

Using the method of comparative analysis, age dynamics of density values and content of mineral elements of bone tissue of the human upper jaw in prenatal ontogenesis was determined. The processing of the research results was carried out using modern computer technology.

Thus, using the above mentioned methods of photometric research and statistical processing allowed us to obtain qualitatively new and reliable data, which are the basis of the scientific substantiation of the peculiarities of the structure and mineral composition of bone tissue of human fetuses' upper jaw in the early prenatal period of ontogenesis.

Results. Numerical data on the content of trace elements in the structure of human bone tissue at the age of 11–40 weeks of IUD, with dimensions (l = 55.0–376.0 mm of CRL) in grading of periods of fetal development on the basis of classical embryogenesis periodization and after germinal human ontogenesis by G. A. Schmidt are presented in Tables 2–4 (M ± m,

mg/g) and in Table 9 (%). In order to make the analysis convenient and to implement it in practice in future, we conducted a dynamic analysis with the determination of the percentage of trace elements in the investigated fragments of the bone tissue of the samples (weighing from 0.15–0.55 g), which, at the same time, provide a complete basis for mineralization and qualitative characteristics of the bone tissue development in prenatal ontogenesis, which presented in (Table 9) and graphically depicted in (Figure 2).

Table 9.– Structure of the bone tissue of the upper jaw of the human fetus based on the content of trace elements,%

Group	Age, weeks	A number of observations	Trace elements,%			
			Potassium(K)	Iron (Fe)	Cobalt(Co)	Zink (Zn)
1	11–16	35	12.29	22.80	5.61	59.30
2	17–24	33	11.75	22.56	4.02	61.67
3	25–29	32	3.65	30.37	10.07	55.91
4	30–40	31	15.58	46.21	6.32	32.19

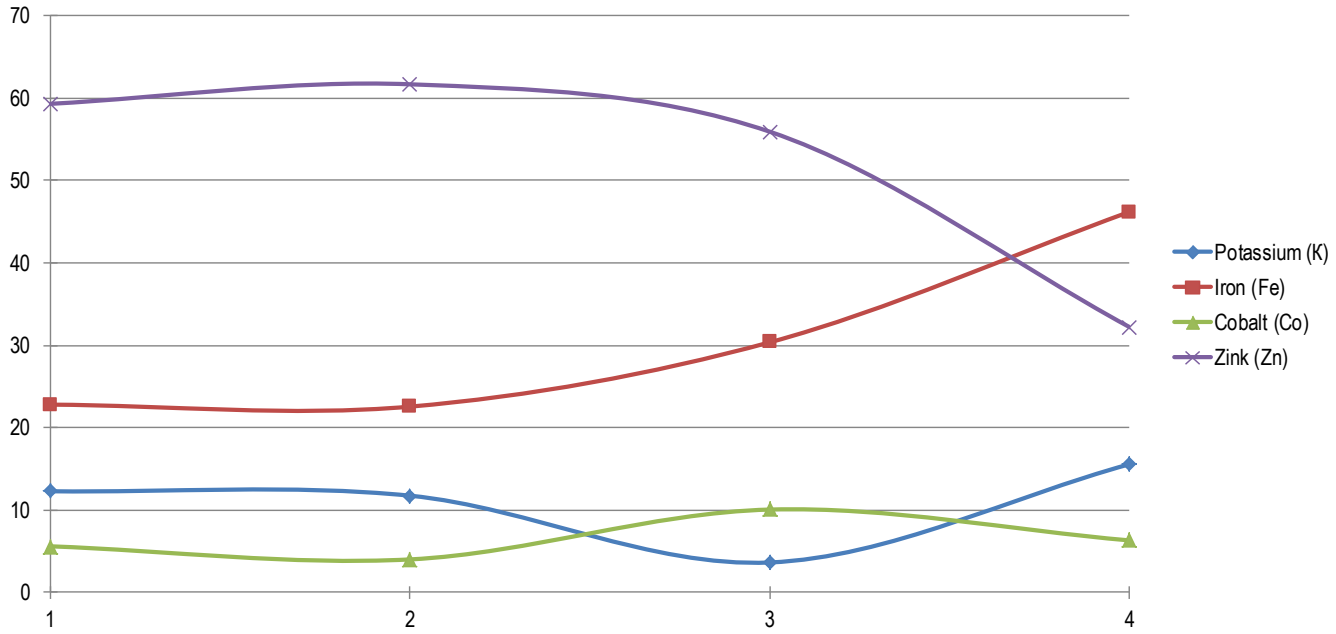

Figure 2. Structure of the bone tissue of the upper jaw of the human fetus based on the content of trace elements,%

It has been established that age dynamics of all indices of trace element content in prenatal ontogenesis significantly correlates both in the direction of decrease and in the direction of growth – in the first (11–16 weeks of IUD), the second (17–24 weeks of IUD), the third (25–29 weeks of IUD) and the fourth (30–40 weeks of IUD) groups, which is directly proportional to the re-distribution of trace elements for the development of fetal organs and systems in these age periods.

The regularity of the dependence and ratio of the content of cobalt (Co) and iron (Fe) in the first, second and third experimental groups has been studied.

There is also a slight correlation between dependence and direct correlation with zinc reduction and iron growth in all experimental groups.

For more detailed analysis, tables (10, 11) provide numerical data on the rate of growth (%) that reveal the essence of the distribution dynamics of

these trace elements of the maxillary bone in the prenatal ontogenesis of human fetuses.

The growth rate for potassium (K) in the three groups is negative: between the first and second groups (–4.39%), between the second and third (–68.94%). However, a sharp increase in potassium (K) in the fourth group, compared with the third, is +318.63%. The overall growth rate for potassium (K) between the first and fourth groups is + 24.33%, which is shown in (Figure 3).

Table 10.– Age dynamics of trace element content in the bone tissue of the human fetuses' upper jaw,%

Trace elements	Groups		Growth rate,% (+) – growth (–) – decrease	Groups		Growth rate,% (+) – growth (–) – decrease
	1	2		2	3	
Potassium (K)	12.29	11.75	– 4.39	11.75	3.65	– 68.94
Iron (Fe)	22.80	22.56	– 1.05	22.56	30.37	+ 34.62
Cobalt (Co)	5.61	4.02	– 28.34	4.02	10.07	+150.51
Zink (Zn)	59.30	61.67	+ 3.99	61.67	55.91	– 9.34

Table 11.– Age dynamics of trace element content in the bone tissue of the maxilla in human fetuses,%

Trace elements	Groups		Growth rate,% (+) – growth (–) – decrease	Groups		Growth rate,% (+) – growth (–) – decrease
	1	2		2	3	
Potassium (K)	3.65	15.58	+ 318.63	12.29	15.58	+ 24.33
Iron (Fe)	30.37	46.21	+ 52.15	22.80	46.21	+ 102.67
Cobalt(Co)	10.07	6.32	– 37.24	5.61	6.32	+ 12.65
Zink (Zn)	55.91	32.19	– 42.43	59.30	32.19	– 45.72

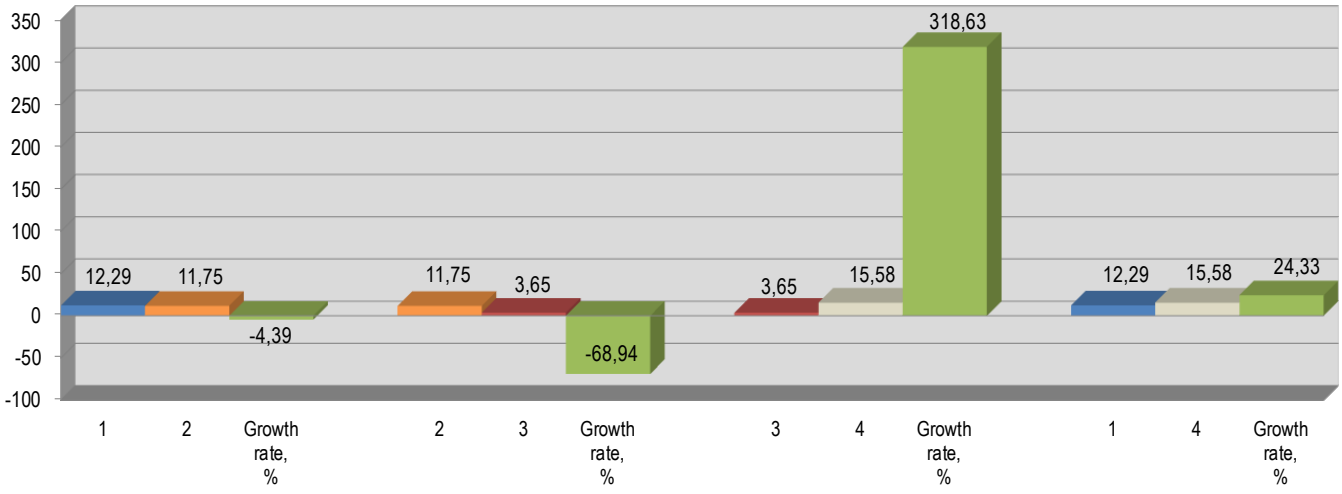

Figure 3. Rate of growth of potassium (K) trace elements,%

The dynamics of the iron (Fe) trace element content (Figure 4) has a positive growth pattern in almost all age periods of prenatal ontogenesis, therefore it increases: between the second and third groups by 34.62%; between the third and fourth by 52.15%; between the first and the fourth by 102.67%, except for a slight decrease in the second group (17–24 weeks), consequently, the growth rate between the first and second groups has an insignificant but negative value (–0.05%).

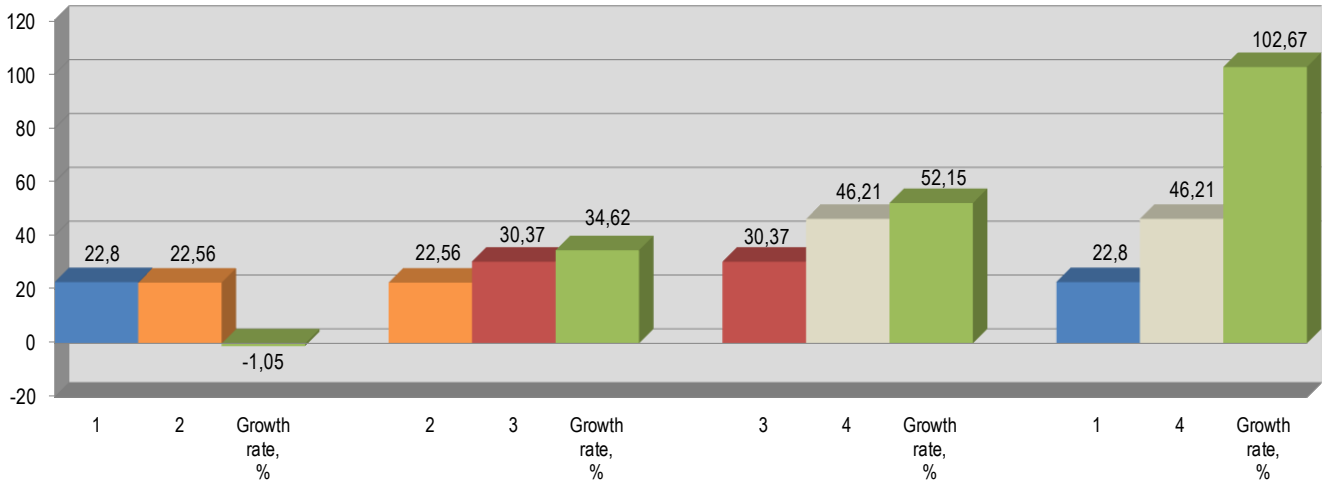

Figure 4. Rate of growth of iron (Fe) trace elements, %

The maximum growth rate was found for cobalt (Co) (Figure 5) in the middle of 22–27 weeks of IUD of human fetuses, which is more than one hundred and fifty percent (+150.51%), which confirms the intensity of development of the vascular system in this age period.

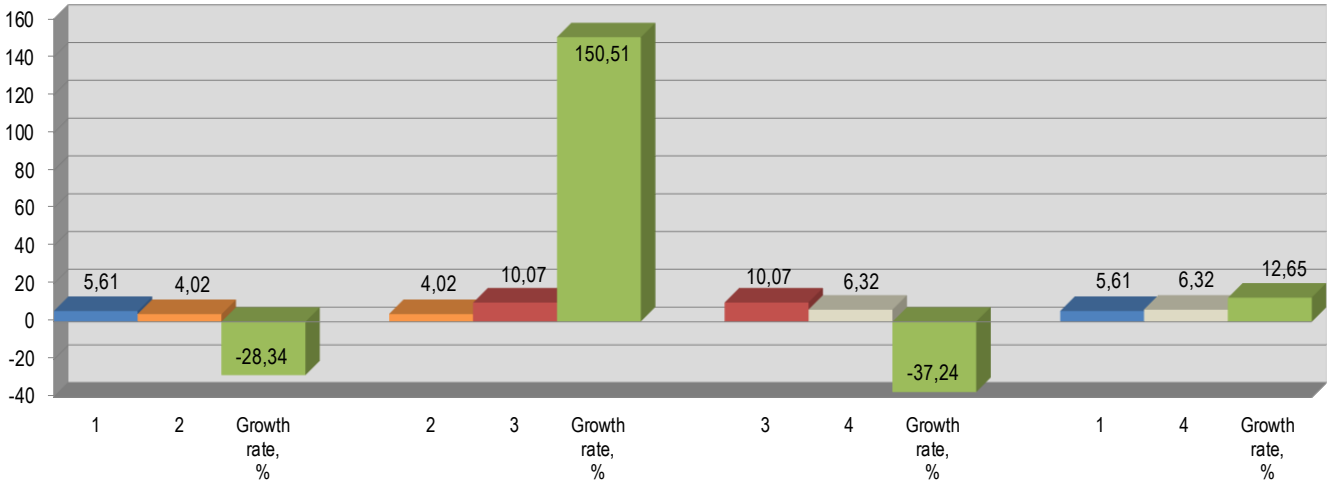

Figure 5. Growth rate of cobalt (Co) trace elements, %

A slight increase in zinc (Zn) (%) (Figure 6) is observed in the second experimental group (+ 3.99%), and rapidly decreases in the third (–9.34%) and fourth (–42.43%) groups, which is a positive reflection of the qualitative indices of the fetal bone tissue.

Analyzing the results of the average value of the studied parameter (M), the standard deviation (m), the paired Student's test, or the reliability indicator (t) and probability level, which are given above (see Table 2–4), stating that the comparisonvalues between the first and fourth groups are: for potassium

(K) – 0.188 ± 0.006 in the first and 0.144 ± 0.019 in the fourth group (t = 2.21, p < 0.05); for iron (Fe) – 0.348 ± 0.027 and 0.435 ± 0.057 (t = 1.38, p > 0.05); for cobalt (Co) – 0.086 ± 0.006 and 0.059 ± ± 0.008 (t = 2.70, p < 0.01); for zinc (Zn) – 0.905 ± ± 0.035 and 0.303 ± 0.032 (t = 12.81, p < 0.001), which indicates the high reliability of the difference between the indicators in the quantitative determination of the content of trace elements in the bone tissue of the upper jaw of human fetuses in the results obtained.

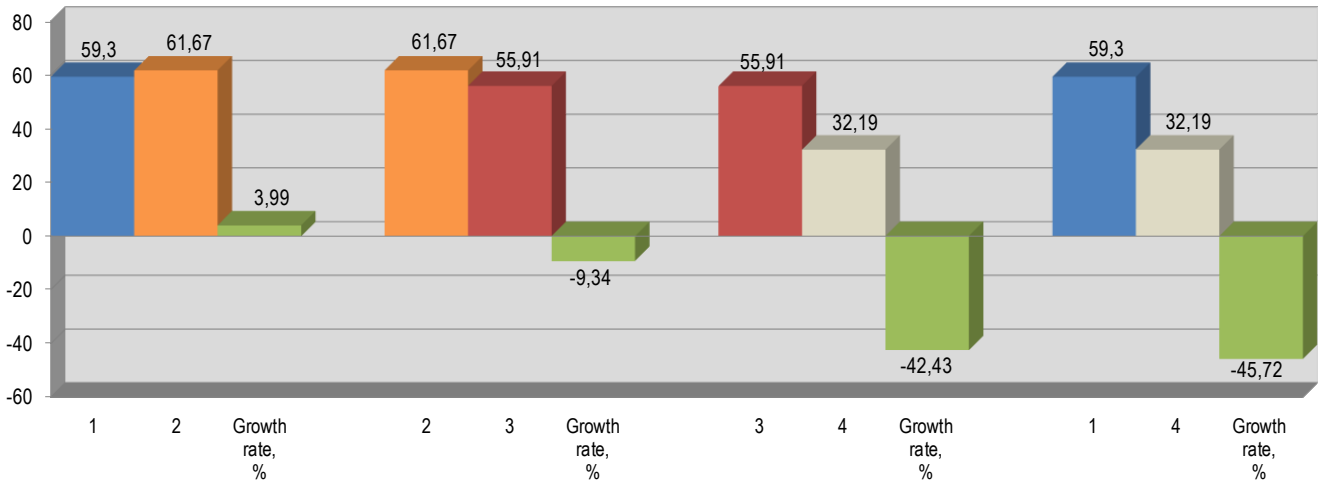

Figure 6. Growth rate of zink (Zn) trace elements, %

Discussion. Nowadays, the advancement of science often requires a determination of the content of trace elements, which cannot be found by means of a certain method of analysis [10; 23; 28].

With scientific progress and the ever-increasing manifestation of birth defects [1; 2; 15; 31], the question arises of the need to choose simple, precise and sensitive techniques that will make it possible to identify a component in complex biological mixtures.

To solve this problem, we have chosen the methods of separation and concentration for this study, which allow us to largely eliminate difficult low predicted situations. In addition, in many cases, concentration has expanded the limits of application of instrumental methods (atomic absorption spectrometry, spectrophotometry) [11; 12].

The amount of scientific research is growing significantly as well as the prospect of using combined and hybrid methods to determine the qualitative and quantitative characteristics of biological objects during relevant and progressive research directions [10–12].

At the same time, the data of scientific literature show that in recent years there has been an increasing interest in the laws of the development of the jaw system components [2; 18; 22; 26; 29; 32]. The processes of the formation and development of jaws and teeth have attracted the attention of many researchers [25].

The results of our literature review and our own histological studies, which are presented in papers [19–22], confirm that epithelio-mesenchymal interactions with the transformation of the latter into the coarse-fiber tissue (7–12 weeks of IUD) and the subsequent gradual formation of jaw bone during the studied periods of prenatal ontogenesis is a precursor to the development of the upper jaw in the human fetus.

The scientific studies [14; 26; 32] say that the bone tissue of the jaws in the 17–24-week old fetuses is represented by areas of a spongy bone of varying degrees of formation with heterogeneous forms and sizes of bone beams and interstitial spaces surrounded by peripheral zones of cartilage tissue.

In 25–32-week old fetuses the bone tissue of the jaw is represented by the formed bony beams, some

of which lie separately, and the other – are connected by bridges, forming a spongy bone, surrounded by periphery islets of cartilage tissue. The bone tissue in the jaws of fetuses aged 33–40 weeks of IUD and newborns is represented by heterogeneous shaped and sized bone beams with hemopoiesis islets in the interstitial spaces. The bone beams, among which the large ones predominate, form a spongy bone of a usual structure.

A considerable number of scientific works study the mineral composition of hard tissues of the jaw system [9–12; 23; 24; 28]. Meanwhile, such important aspects of the problem as the study of the mineral composition of human bone tissue in the dynamics of prenatal ontogenesis, patterns of their correlation and distribution of mineral elements in the bone tissue remain beyond the attention of researchers.

That is why the purpose of our work was to study the features of the mineral composition of the bone tissue of the upper jaw of the human fetus during the stages of prenatal ontogenesis and the patterns of distribution of the content of mineral elements in the investigated objects, which ensure the quality and proper ossification of the bone itself.

We know that the collarbone [27] and the mandible are the bones, in which the islets of osteogenesis appear first, but the upper jaw is significantly lagging behind in time with its development and proper mineralization, which ensures the density and quality of bone tissue. Therefore, malformations are most common in the upper jaw [1; 2; 15; 31; 30] due to the influence of conditions of the internal environment in which the fetus stays.

Scientific papers [3–6; 8; 13] cover the responses of the bone tissue on the effects of dehydration, which is associated with the value of bones as depots of mineral substances in the body, which in turn determines their role in the water-salt metabolism.

The bone tissue is characterized by a constant renewal of its components, accompanied by processes of formation and destruction, compensatory-adaptive reactions in response to changing conditions of existence. These facts indicate bone tissue as a dynamic rather than static structure as evidenced by the authors of the works (Prylyska O. I., 2004; Pushyna S. A., 2004; Tekuchenko E. V., 2006; Pashkova I. G., 2016;). The plasticity and dynamism of the bone tissue make it one of the important factors in maintaining the homeostasis of an organism: it is an active participant in the general metabolism, it actively participates in salt metabolism. Nevertheless, a number of aspects in the study of this problem remain unclear, in particular, the change in the bone tissue mineral composition depending on the influence of the environmental conditions of the fetal bone marrow, which is a topical issue of the present [5; 7].

Numerical data on the content of trace elements in the structure of human bone tissue at the age of 11–40 weeks of IUD, with dimensions (l = 55.0– – 376.0 mm of CRL) in grading of periods of fetal development on the basis of classical embryogenesis periodization and after germinal human ontogenesis by G. A. Schmidt are presented in Tables 2–4 (M ± ± m, mg/g) and in Table 9 (%).

In order to make the analysis convenient and to implement it in practice in future, we conducted a dynamic analysis with the determination of the percentage of trace elements in the investigated fragments of the bone tissue of the samples (weighing from 0.15–0.55 g), which, at the same time, provide a complete basis for mineralization and qualitative characteristics of the bone tissue development in prenatal ontogenesis. Due to the fact that we are not aware of similar studies, we were not able to conduct a comparative analysis of the findings, which obviously limits the discussion on this issue.

The quantitative data we obtained can help to monitor the normal development of bone tissue of the human fetal maxilla, as well as to screen early for birth defects and anomalies, as well as significantly improve the study of quantitative morphology with relative ossification and be selected for a theoretical basis in the development of new preventive and therapeutic measures.

Conclusions. In the course of our research, we found that age dynamics of trace element content in prenatal ontogenesis in all values significantly correlated both with the decrease and the growth – in the first (11–16 weeks of IUD), the second (17–24 weeks of IUD), the third (25–29 weeks of IUD) and the fourth (30–40 weeks of IUD) experimental groups, which is directly proportional to the redistribution of trace elements for the construction of organs and systems in these age periods.

The regularity of the dependence and the ratio of content of cobalt (Co) and iron (Fe) in the first, second and third experimental groups have been studied.

There is also a slight correlation between dependence and direct correlation (see Fig. 2) with respect to the reduction of zinc (Zn) and iron (Fe) in all experimental groups.

Studying the patterns of density dynamics depending on the mineral composition of the bone tissue and the presence of the revealed synchrony of these processes, suggests that the change in density is indicative of a change in the content of certain mineral elements. In our opinion, this provision may be the basis for developing new methods of early diagnosis of congenital anomalies of the maxillofacial areas at pre-clinical stages of its development and methods of their prevention, by correcting the mineral composition.

Compliance with ethical standards. All studies were conducted in compliance with the main provisions of the GCI (1996), the Council of Europe Convention on Human Rights and Biomedicine (of 04.04.1997), the Helsinki Declaration of the World Medical Association on the Ethical Principles of Scientific Medical Research with Human Participation (1964–2013), Orders of MoH of Ukraine No. 690 of September 23, 2009, No. 616 of 03.08.2012, and according to the guidelines [16] and "Procedure for the extraction of biological objects from the dead, whose bodies are subject to forensic examination and pathological anatomical investigation, for scientific purposes" [17].

Conflict of interest. The authors declare that they have no conflict of interest.

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MODELING OF THE CYTOKINE ENVIRONMENT PROVIDING THE OPTIMAL IMMUNE RESPONSE OF ANTITUMOR VACCINES BASED ON DENDRITIC CELLS

Abstract: The inclusion of interferons and TLR ligands in the maturation cocktail provides a mature phenotype of DC with the predominance of costimulatory molecules over co-inhibitory, active directed migration in response to chemokines (CCR7 ligands), and also a high level of secretion of IL-12p70 as a response to CD40L stimulation. In a mixed medium of lymphocytes, these signals

are functionally interpreted by polarizing the immune response toward IFNγ-secreting Th1 cells and suppressing the concomitant activation of regulatory T-cells. As a contrast to standard DC vaccines, the proposed maturation protocol creates a αDC1 culture with the cells that simultaneously combine the necessary, often contradictory, properties of antitumor vaccines.

Keywords: DENDRITIC CELLS, ANTITUMOR VACCINES, TH1– CELL, PGE2, IL – 12, CCL19, CCL21, CCR7, TLR LIGANDS.

Dendritic cells (DC) are used in cancer immunotherapy for more than 20 years because of their ability to induce antitumor immunity [1]. The clinical effectiveness of DC remains limited, although the antigen-specific immune response has been detected in most cases [2; 3]. Autologous monocytes of peripheral blood are used to create DC vaccines in the majority of studies, which undergo a two-stage process of differentiation and maturation [4]. The composition and concentration of cocktail components for maturation have crucial significance for the future DC. The "gold standard" is the combination of TNFα, IL-1β, IL-6 and PGE_2, however, different sources suggest a wide variety of cytokine composition variants for maturation of DC [5].

The ability of the DC to secrete IL-12p70 has crucial importance for the polarization of the immune response in the direction of Th1, which is most needed for an effective reaction against tumor cells. However, bioactive IL-12p70 cannot be produced by DC, which has matured under the conditions of a standard combination of cytokines [6]. This negative effect is mostly created by PGE_2, which prevents the production of IL-12p70 and in parallel induces the production of IL-12R antagonist (IL-12p40 homodimer) [7]. Moreover, PGE2 selectively disrupts production IL-2 and IFNγ by T-cells, inhibits the activation of T-cells in response to Th1-cytokines such as IL-2 and IL-12p70, affects the appearance of the ability to attract regulatory T-cells (Treg), and also stimulates naive CD4+ T-cells to differentiate towards Treg. At the same time, in the presence of obvious suppressor activity, PGE_2 shows a synergy with TNFα and promotes maturation of DC expressing CCR7. The latter provides the reaction of DC to chemokines CCL19 and CCL21 (both are CCR7 ligands) and active migration of DC to the lymph nodes [10].

The inconsistency of these effects lead to an active search for alternative ways of generating mature DC, which lead to a discovery that Toll-like receptor agonists (TLR) induce maturation of DC capable of inducing Th1 polarization [11]. These agents have been used in cocktails for maturation of DC, and TLR4-ligand-lipopolysaccharide (LPS), TLR3-ligand-polyI:C [13] and TLR7/8-ligand-R848 (resiquimod) are the most known among them [12; 14]. It has been shown that the combination of pro-inflammatory cytokines, interferons and TLR ligands can lead to generation of DC, which actively secret IL-12p70 [15].

Mature DCs express costimulatory (CD80 = = B7–1 and CD86 = B7–2) and co-inhibitory (CD274 = PD-L1) molecules, and the interaction of corresponding T-cell ligands with them influences the direction of differentiation of the latter [16]. Various influences of these molecules on the phenotype and function of T-cells have been found, but their manifestation pattern depends on the conditions of maturation of the DC, needs further research and clarification. Not sufficiently studied are the questions about the effect of different maturation protocols on the migration properties of DC, their ability to attract T-cells and induce Treg. In this relation, test of DC in a mixed leukocytes culture (MLC) with autologous T-cells without the influence of exogenous cytokines and allogeneic stimulation will reveal more subtle differences in the nature of the immune response induced by DC, matured under different conditions.

Therefore, the purpose of this study was to research the various protocols of DC generation which,

through the expression of a pattern of costimulatory molecules and secreted cytokines, could provide the optimal functional effect of these signals on the activation of T-cells. In particular, the effect of various TLR ligands and pro-inflammatory cytokines on the properties of DC was compared to determine the type of polarization of the immune response of T-cells.

Materials and techniques

Cultivation of DC and acquisition of naive $CD4^+$ T-cells.

Reagents: interleukin-4 (IL-4, Thermo Fisher Scientific) granulocyte-macrophage colony-stimulating factor (GM–CSF, Neostim, Russia), interleukin-1β (IL-1β, Betaleikin, Russia), interleukin-6 (IL-6, Thermo Fisher Scientific), interferon-α2 (IFNα, Roferon-A, Roche, Switzerland), interferon-γ (IFNγ, Inharon, Russia), lipopolysaccharide (LPS, *E.coli* 0111: B4, Sigma-Aldrich), prostaglandin-E2 (PGE_2, Sigma-Aldrich), tumor necrosis factor-α (TNFα, Life Technologies), polyinosinic-polycytidylic acid (poly-I: C, Sigma-Aldrich), resiquimod (R848, Sigma-Aldrich).

To cultivate DC of 14 conditionally healthy donors (age group from 25 to 37 years), samples of peripheral heparinized venous blood were obtained in a volume of up to 20 ml, which was processed no later than 6 hours after sampling. Peripheral mononuclear cells (PMC) were isolated in accordance with standard protocol in Histopaque-1077 density gradient (density 1.077 g/ml, Sigma-Aldrich). Monocytes were obtained from the BMD fraction by adhesion to plastic, followed by culturing for 6 days in a serum-free medium Panserin 413 (PAN Biotech GmbH, Aidenbach, Germany) containing 15% Panexin basic serum replacement (PAN Biotech GmbH, Aidenbach, Germany) and recombinant human cytokines (GM–CSF-50 ng/ml and IL-4–25 ng/ml), in a humidified atmosphere with 5% CO_2 at 37 °C. On the 6th day for 48 hours, a different combination of pro-inflammatory interleukins, interferons and TLR ligands was used to induce maturation of two DC populations: standard DCs-sDC: TNFα (50 ng/ml), IL-1β (12 ng/ml), IL-6 (25 ng/ml) and PGE_2 (1 μg/ml); Polarized DCs inducing Type I immune responses of T-helpers (αDC1): TNFα (50 ng/ml), IL-1β (12 ng/ml), IL-6 (25 ng/ml), IFNα (3000 U/ml), IFNγ (1000 U/ml), LPS (2.5 μg/ml), poly-I: C (20 μg/ml) and R848 (3 μg/ml). Mature cultures of the DC were washed twice and used for further research.

To study the stimulating and polarizing effects of different DC populations (sDC and αDC1) on autologous naive $CD4^+$ T-cells under MLC, the latter were isolated from the suspension of the non-adhesive PMC depleted monocyte fraction by negative immuno-magnetic separation using the EasySep $CD4^+$ kit (StemCell Technologies) in according to the manufacturer's protocol.

Flow cytometry using marked monoclonal antibodies (BD Biosciences) and the corresponding isotypic controls was carried out for the phenotyping of DC–CD14 (FITC, M5E2), CD83 (APC, HB15e), HLA-DR (PerCP, L243), CD80 (FITC, L307), CD86 (PE, 233), CD274 (APC, MIH1), CCR7 (FITC, 3D12), CCL19 (PE, T50–867) and regulatory T-cells — CD4 (FITC, RPA-T4), CD25 (PE-Cy7, M-A251), CD127 (AlexaFluor 647, HIL-7R-M21), FoxP3 (PE, 259D/C7). Expression of intracellular cytokines in different T-cell populations was determined after 4 days of incubation of MLC according to protocols of BD FastImmune™ CD69/CD8/CD3 (#340365) reagents, BD FastImmune™ Anti-Human IL-4 (#340451) and FastImmune™ IFNγCD69/CD8/CD3 (#346048). Cell population analysis was performed on a BD FACSCanto flow cytometer (Becton Dickinson, USA), with 3 to 6 color marks and data analysis in the BD FACSDiva v.6 program (Becton Dickinson, USA). Expression was assessed for the mean fluorescence intensity (MFI), the ratio of the MFI value for coloring with a specific marked antibody to MFI for the corresponding isotype.

Cytokine secretion and T-cell proliferative response were evaluated in autologous MLC, where

naive $CD4^+$ T-cells (1×10^6/cavity) were used as the responding cells and cultured in 24-well plates in serum-free Panserin 413 medium supplemented with 10% serum replacer Panexin basic in humid atmosphere at 37 °C in 5% CO_2. DC served as stimulants, which were added to autologous T-cells in the ratio 1:10. Secretory and proliferative response of T-cells was evaluated at the end of 4 days of incubation. The concentration of soluble cytokines IFNγ and IL-4 was evaluated using the Multi-Analyte Profiling (xMAP) technology on a Luminex 200 2-beam laser analyzer (Luminex Corporation, USA), according to the manufacturer's methodology for a commercial test system (Procarta® Mix & Match Assays, eBioscience) and using ProcartaPlex Analyst 1.0 software (concentration range from 1 to 32000 pg/ml).

The proliferative response of T-cells in MCL was evaluated by the incorporation of bromodeoxyuridine (BrdU) in DNA synthesis according to the protocol for the BD Pharmingen™ BrdU Flow Kits reagent kit.

The secretion of IL-12p70 and IL-10 by dendritic cells was stimulated during 24-hour incubation in a neutral medium (without cytokines) with CD40L. MEGACD40L® Protein (Enzo Life Sciences Inc.) was used as the latter, which is an oligomeric construct that efficiently mimics the membrane-associated aggregation of CD40L observed *in vivo*. Stimulation with CD40L recreates the interaction of DC with naive $CD4^+$ T-cells expressing CD40 receptor. The concentration of IL-12p70 and IL-10 in the supernatant was measured using xMAP technology (see above).

Chemotaxis of DC and naive T-cells *in vitro* was performed on the basis of the Migratest (Glycotope Biotechnology) kit using 24-cavity plates equipped with membranes with a pore diameter of 3 μm. The lower chamber was filled with a medium of chemokine CCL21 (Thermo Fisher Scientific) in a volume of 350 μl, and 100 μl of a DC suspension (5×10^4) was added to the top, after which the plate was incubated for 2 hours at 37 °C. Chemotaxis of naive $CD4^+$ T-cells was examined in similar 24-cavity membrane plates. A suspension of naive $CD4^+$ T-cells (2×10^5) in a volume of 100 μl was introduced into the upper chamber and the lower chamber was filled with supernatant from DC culture (5×10^5 DC in 1 ml of neutral medium stimulated with CD40L for 24 hours), after which the plate were incubated for 3 hours at 37 °C. To study the CCR7-dependent migration component of naive $CD4^+$ T-cells for 30 minutes prior to chemotaxis, they were incubated with blocking unlabeled anti-CCR7 antibodies (Purified, 3D12, BD Biosciences).

The statistical processing of data was carried out using the "Statistica 6.0 for Windows" application software package. To detect significant differences in the compared indicators, the non-parametric Wilcoxon-Mann-Whitney U-test was used. Differences were considered significant at a significance level of $p < 0.05$.

Results

Migration activity of the DC

DC of monocytic origin maturing with introduction (sDC) or in the absence of PGE_2 (αDC1), represent two types of mature DC that are usually cultured in serum-free media for clinical use.

Although the DC maturation protocols using PGE_2 are associated with the suppression of IL-12p70 production [17], the ability of PGE_2 to enhance CCR7 expression and, accordingly, the migration activity of DC as a response to chemokines CCL19 and CCL21 (CCR7 receptor ligands that attract DC to lymph nodes) justifies the use of PGE_2 in standard DC maturation protocols (sDC) when creating cellular vaccines [18].

In contrast to sDC, the use of another maturation protocol involving interferons and TLR ligands leads to the formation of mature polarized DCs (αDC1) capable of secreting high levels of IL-12 and inducing tumor-specific cytotoxic T-lymphocytes (CTL) *in vitro*. However, αDC1 is characterized by low migration activity *in vitro* in response to chemokines that bind to CCR7 (CCL19 and CCL21).

In accordance with the data obtained (Fig. 1), DC, which matured in the presence of PGE_2 (sDC) showed not only a statistically significant higher level of CCR7 expression than αDC1, but also high migration activity in response to CCL21. However, even significant differences in the expression of CCR7 in sDC and αDC1 cells rapidly disappeared after being transferred from the maturation medium to the neutral medium containing only GM–CSF. This equalization of the differences in the expression of CCR7 after transfer to a neutral medium was naturally reflected in the disappearance of the differences in migration activity in sDC and αDC1.

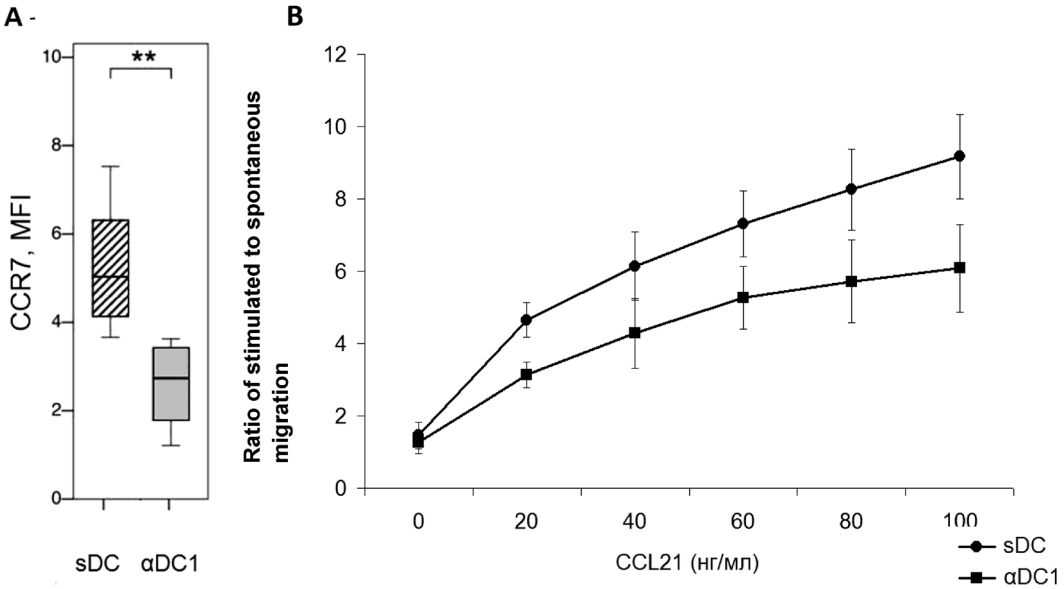

Figure 1. CCR7 expression (A) and *in vitro* migration capacity as a response to chemokine CCL21 (B) in two types of DC populations (sDC and αDC1)

To analyze the causes of differences in the expression of CCR7 in two types of DC (sDC and αDC1), the levels of its surface and intracellular expression were compared. Surprisingly, despite the marked predominance of surface expression of CCR7 in sDC, the determined total content of this receptor (surface and intracellular) did not show significant differences between the two types of DC. Taking into account the existence of the mechanism of ligand-induced internalization of CCR7, the possibility of leveling differences in the levels of surface expression of this receptor in sDC and αDC1 was studied by adding exogenous CCL19 (it is known that CCL21 does not affect internalization).

According to the data obtained, the addition of exogenous CCL19 (100 ng / ml) significantly reduced the level of surface expression of CCR7 only in the αDC1 culture, which initially actively expressed CCR7 due to its maturation conditions without the addition of PGE_2. At the same time, in both cultures of DC (sDC and αDC1), the overall expression level of CCR7 (surface and intracellular) did not change under the influence of exogenous CCL19.

PGE_2 is a potent inhibitor of CCL19 DC products (sDC)

Given the selective increase in the surface (but not general) expression of CCR7 in DC culture maturing in the presence of PGE_2 (sDC), and the ability of exogenous CCL19 to eliminate it, the effect of PGE_2 on the endogenous production of CCL19 in DC cultures was compared (Fig. 2).

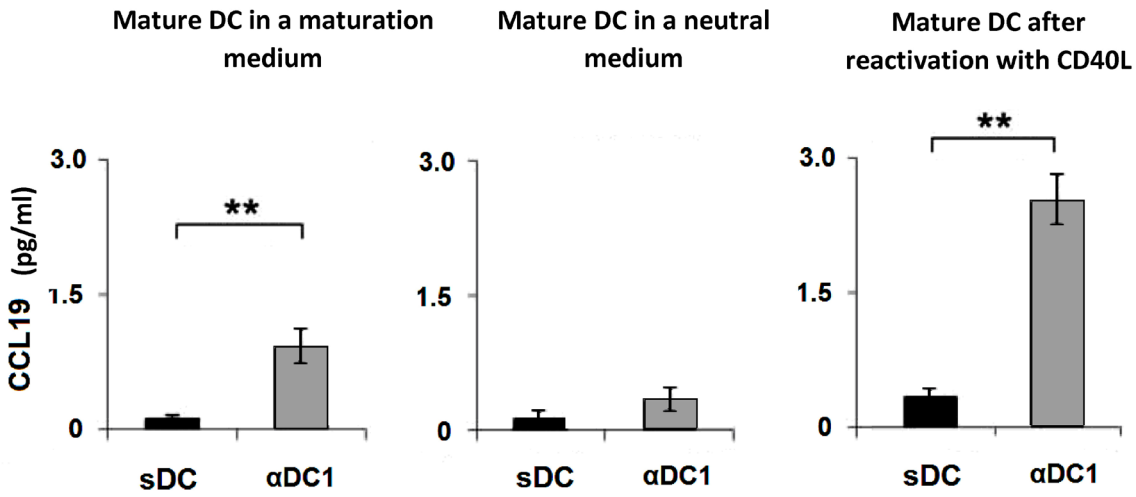

Figure 2. Secretion of CCL19 by DC populations (sDC and αDC1) under different incubation conditions: in a maturation medium; in a neutral medium; in a neutral medium after reactivation with CD40L; (*, $p < 0.05$; **, $p < 0.01$)

By taking in consideration, that CCL19 can affect the intensity of internalization of CCR7, we detected a significantly higher level of endogenous secretion of CCL19 in mature αDC1 culture compared to sDC. Considering that *in vivo* the source of the chemokine CCL21 is the cells of the lymphatic endothelium, we did not find its significant secretion in both types of DC cultures. Analysis of factors inducing maturation of DC and their effect on the regulation of CCL19 production in cultures of sDC and αDC1 showed that the main inducers of CCL19 are TNFα, IFNα, poly-I: C (TLR3 ligand), LPS (TLR4 ligand) and R848 (TLR7/8-ligand), while PGE_2 exerts a potent inhibitory effect on the secretion of this chemokine. In addition, PGE_2 also suppresses the production of CCL19 originally induced by LPS (ligand TLR4).

The stability of CCL19 secretion by both populations of mature DC was evaluated after transferring them to a neutral medium (without stimulating factors), as well as in the presence of a CD40L stimulator. Despite the fact that after the transfer to a neutral medium, the secretion of CCL19 at the end of the 24 hour incubation sharply decreased in both populations that had differently matured DC, it was restored to a much greater extent after their reactivation with the help of CD40L. The population sDC, in contrast to αDC1, despite the absence of PGE_2 in the medium, weakly secreted CCL19 both in a neutral medium and after CD40L stimulation. The data obtained indicate that the maturation conditions of DC play a decisive role in their ability to secrete CCL19 in a neutral medium. By means of this chemokine, the DC are able to attract recirculating T-cells (naive and central memory) that drain into the drainage lymph nodes, which express the corresponding receptor (CCR7).

To determine the functionality of the chemokine CCL19 secreted by αDC1, the ability of sDC and αDC1 to target naive $CD4^+$ T-cells expressing CCR7 was evaluated. For a more revealing assessment of the contribution of the CCR7 pathway to the migration of naive T-cells, blocking monoclonal antibodies against CCR7 were used. While a large number of naive $CD4^+$ T-cells migrated towards the supernatant αDC1, the ability of the supernatant sDC to cause migration of naive $CD4^+$ T-cells did not exceed the control level corresponding to a pure medium. Expectedly, the migration activity of naive $CD4^+$ T-cells completely disappeared in the presence of blocking antibodies against CCR7.

Profile of costimulatory molecules

At the end of the maturation stage, both DC populations (sDC and αDC1) exhibited a mature phenotype, which manifested itself in significant expression of such markers as CD83 and HLA-DR against the background of the disappearance of CD14. However, analysis of the expression of costimulatory and inhibitory markers (CD40, CD80 = B7–1, CD86 = = B7–2, CD274 = PD-L1) revealed significant differences between them (Fig. 3).

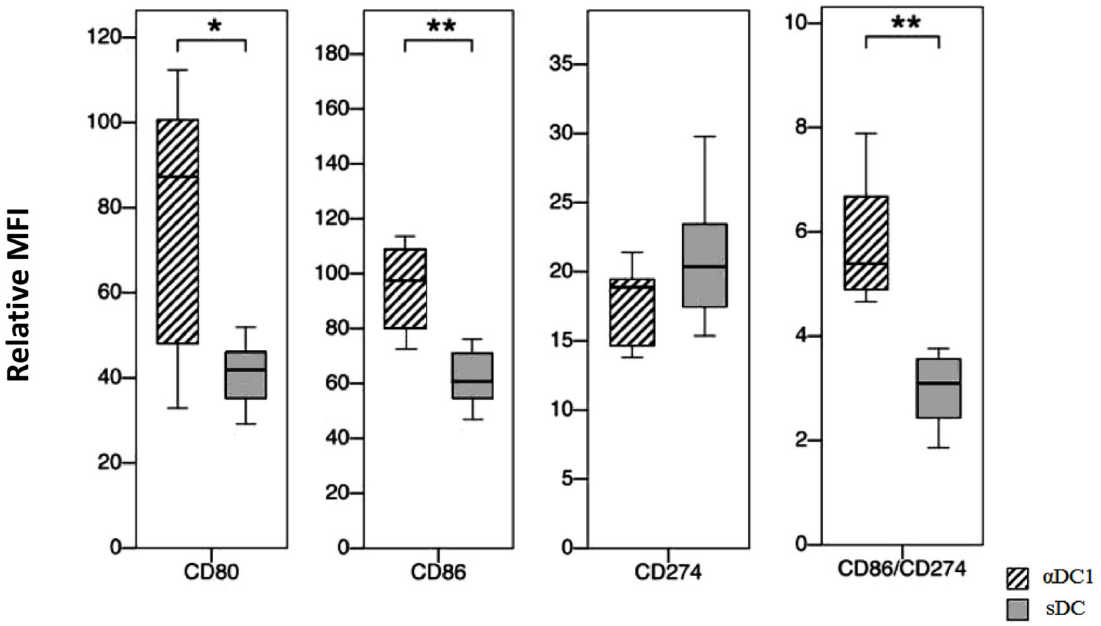

Figure 3. Profile of the expression of costimulatory molecules by populations of dendritic cells sDC and αDC1 (*, p < 0.05; **, p < 0.01)

As we can see, in the αDC1 population, the expression level of CD40 was more than 2 times the corresponding value (MFI) for sDC. The expression of the CD80 and CD86 costimulation molecules showed the highest MFI values in both DC populations, but a significant prevalence was observed in αDC1 87.2 and 97.6, respectively (versus 41.9 and 60.8 in the sDC population). The expression of the inhibitory marker CD274 was higher in the sDC population (17.1 vs. 23.4), with the MFI CD86/CD274 ratio, which characterizes the degree of positive costimulation potential, significantly prevailed in the αDC1 population (5.4 vs. 3.1, $p = 0.005$).

Secretion of IL-12p70

Analysis of the supernatant obtained after a 24 hour stimulation of DC with CD40L under neutral conditions showed a high IL-12p70 content (2.5×10^3 pg/ml) in the αDC1 population, while in the sDC supernatant its concentration was extremely low (38 pg/ml, $p = 0.005$) and slightly exceeded the detectable threshold (Fig. 4).

The result of measuring the concentration of IL-10 was at the boundary of the detected level and did not exceed 8 pg/ml in the supernatants of both DC populations. The difference in the secretion of these interleukins in two DC populations demonstrates the ratio of concentrations of IL-12p70 / IL-10 ($p = 0.007$).

αDC1 induce the activation of Th1 cells producing IFN-γ

The tolerogenic activity of various DC populations was assessed by their ability to induce proliferation of regulatory and non-regulatory activated T-cells in MLC for 4 days. The surface marker CD127 and intracellular FoxP3 were used to isolate subpopulations: $CD4^+CD25^{high}FoxP3^+CD127^-$ is a phenotype of regulatory T-cells (T-reg); $CD4^+CD25^{high}FoxP3^-CD127^+$ is a phenotype of non-regulatory activated T-cells (Fig. 5).

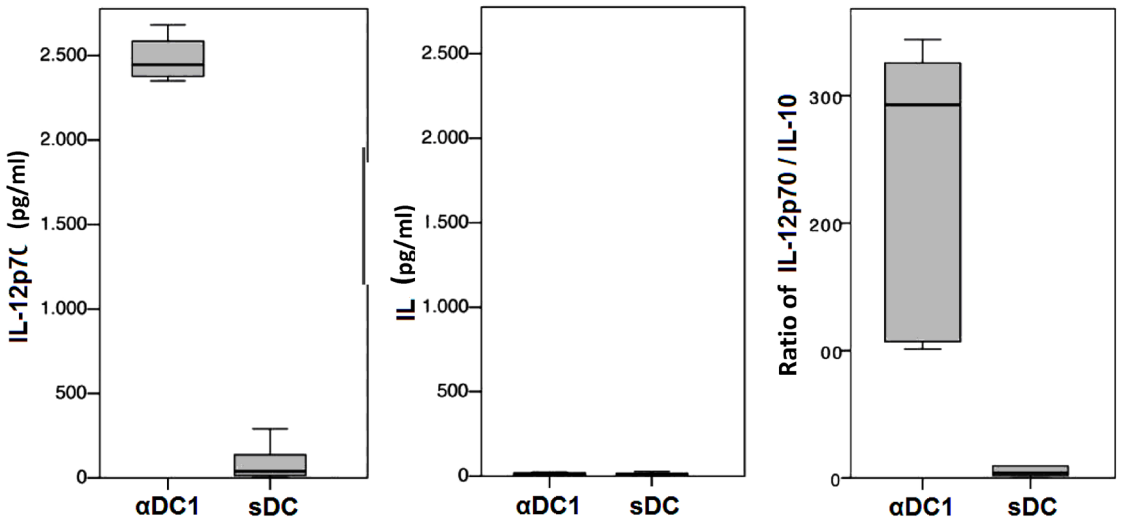

Figure 4. Concentrations of IL-12p70 and IL-10 in the supernatant after 24-hour stimulation of sDC and αDC1 with CD40L in neutral medium

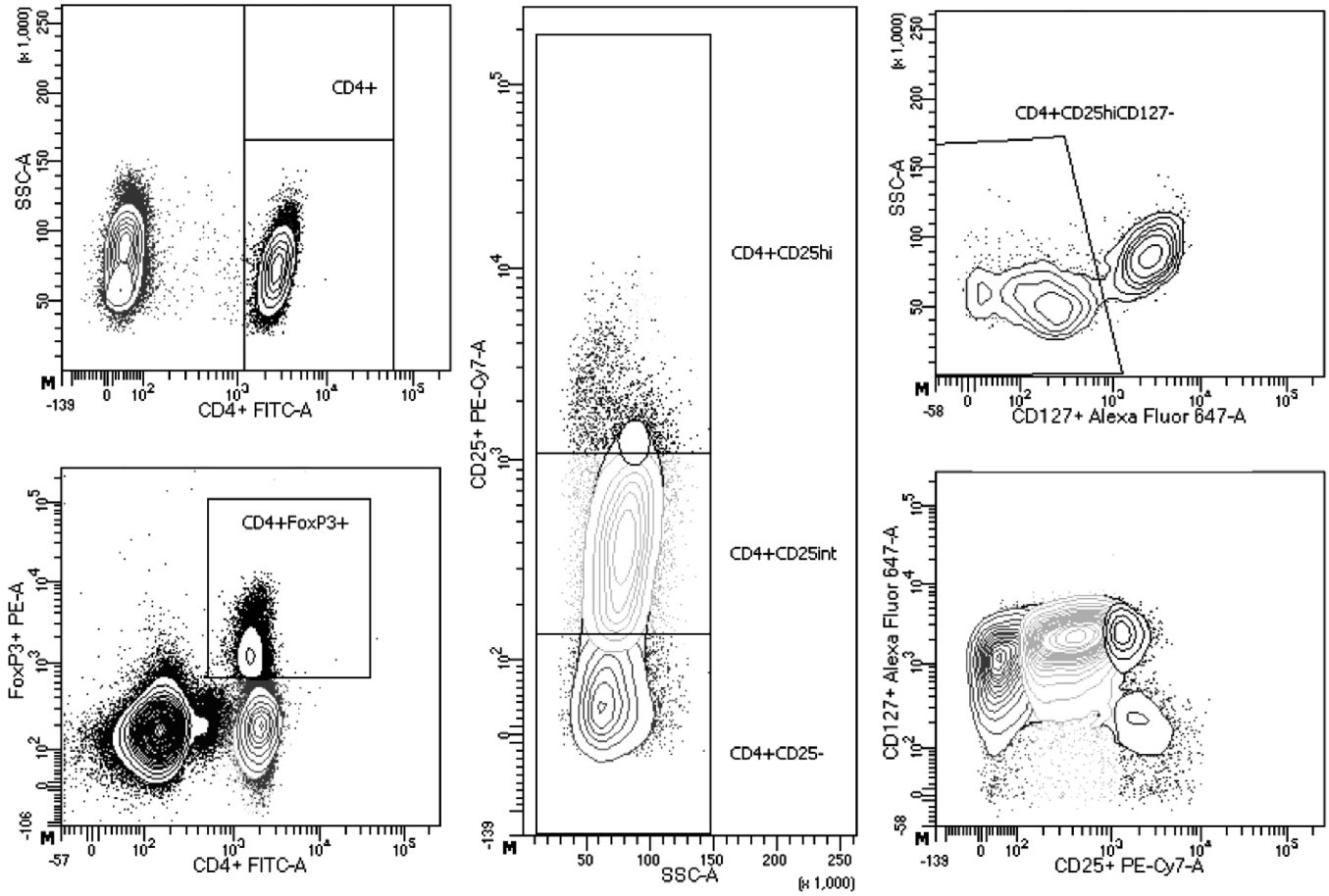

Figure 5. Gating strategy for a subpopulation of regulatory T-cells induced in MLC, by a representative example of one of the donors

Compared to the control, both types of DC induced a significant increase in the relative content of FoxP3$^+$ cells, as well as activated cells with CD4$^+$CD25^{high}CD127$^-$ phenotype. Cultivation in the presence of αDC1 provided a higher level of activated CD4$^+$CD25^{high} cells than in the presence of sDC (5.3% vs. 4.1%; $p = 0.09$). At the same time, sDC significantly induced T-reg compared to αDC1 (2.5% vs. 2.0%, $p = 0.037$). On the contrary, the percentage of activated non-regulatory T-cells predominated when cultured in the presence of αDC1 (0.8%) and significantly exceeded the percentage of these cells in culture with sDC (0.2%; $p = 0.007$).

The ratio of the percentage of activated T-cells to regulatory (CD4$^+$CD25^{high} / T-reg) further emphasized this difference (the median ratio for αDC1 is 0.35, for sDC = 0.09, $p = 0.005$).

At the end of 4 days of cultivation in mixed culture supernatants, the concentration of soluble cytokines IFN-γ and IL-4 was determined (Fig. 6). After co-cultivation with the presence of αDC1, compared with sDC, a high concentration of IFN-γ(3318 pg /ml vs. 638 pg/ml, $p = 0.007$) was observed, while IL-4 concentration in both cases was slightly higher than the detection threshold.

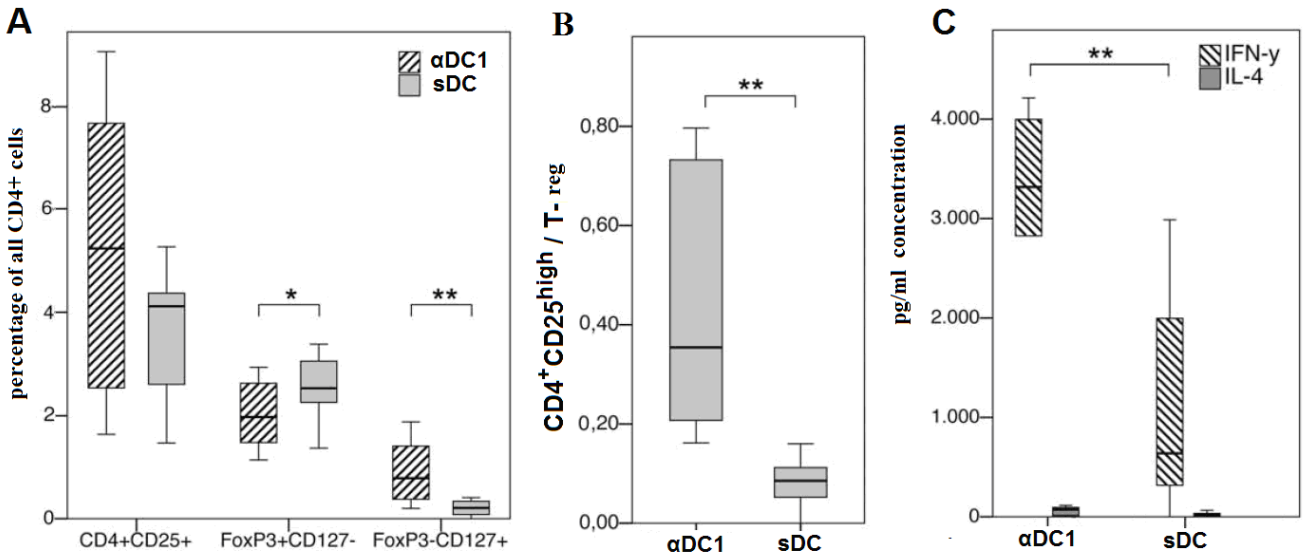

Figure 6. The content of various subpopulations of CD4$^+$ cells (A, B) induced in MLC upon co-cultivation with sDC and αDC1 after subtracting the corresponding values in the control MLC (not stimulated by DC), as well as the concentration of soluble cytokines IFN-γ and IL-4 (C) in the supernatant (*, $p < 0.05$, **, $p < 0.01$)

To assess the ability of sDC and αDC1 to activate the Th1 / Th2 response in MLC, the relative number of CD3$^+$ cells expressing intracellular cytokines IFNγ and IL-4 was examined (Fig. 7). For this purpose, the last 6 hours of MLC incubation was performed in the presence of a protein transport inhibitor (brefeldin). Compared to the control in culture with αDC1, the CD3$^+$IFNγ$^+$T-cells (Th1) content increased by an average of 6.2-fold, and CD3$^+$IL-4$^+$T-cells (Th2) increased 4.1-fold, and in culture with sDC – by 1.5-fold and 1.1-fold, respectively.

Proliferative response of T-cells in MLC

One of the integral indicators of functional activity of DC is their ability to stimulate the proliferative response of autologous T-cells in MLC, which is associated with the degree of maturity of the DC, as well as the spectrum and level of cytokines produced by them. Two different populations of DC (sDC and αDC1) and T-cells from the same donor (autologous) were used to formulate MLC to exclude possible differences associated with the expression of HLA antigens.

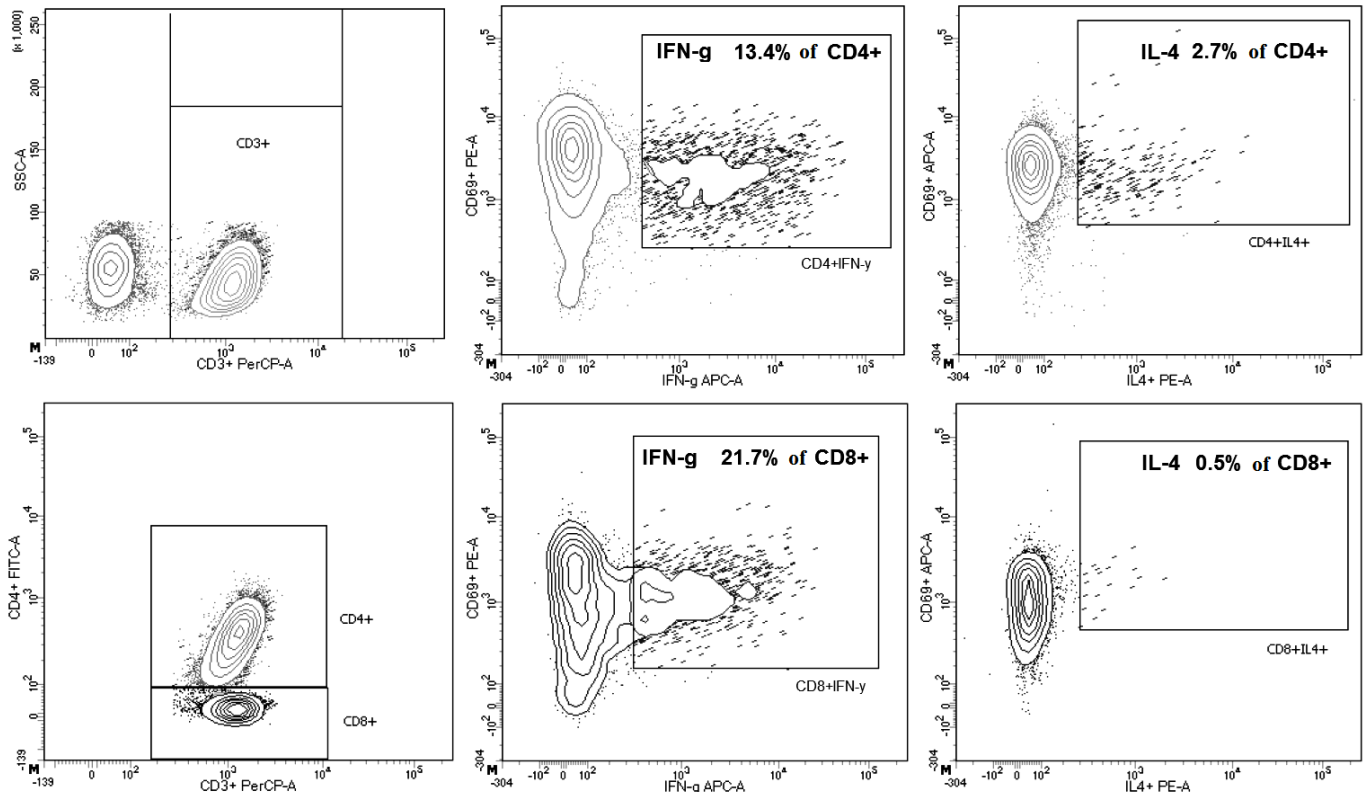

Figure 7. Gating strategy for T-cell subpopulations expressing intracellular cytokines IFNγ and IL-4 in response to PMC incubation in mixed culture with DC (sDC and αDC1) by a representative example of one donor

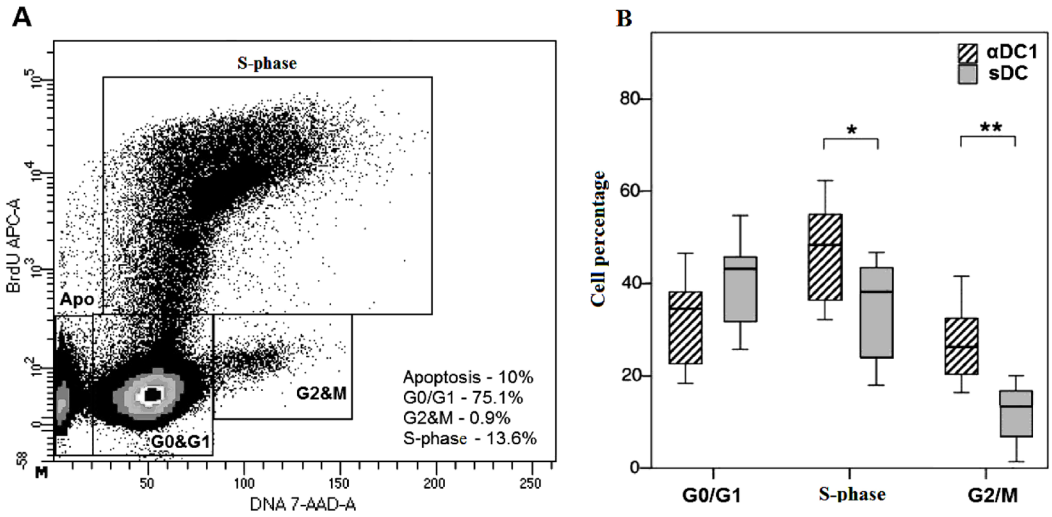

Figure 8. Gating strategy for proliferating T-cells (A) in different phases of mitosis and the corresponding percentage (B); (*, $p < 0.05$; **, $p < 0.01$)

The results of the study indicate that there is a pronounced effect of DC on the proliferation of autologous T-cells. In the absence of DC (control), the proportion of proliferating lymphocytes (S-phase + +G2/M) was less than 20%. In response to the presence of DC in MLC, after 72 hours of culture, the formation of clones of daughter cells was observed, the proportion of which in the S-phase and G2/M

for αDC1 was 50.30% and 26.4%, respectively, and for sDC38.4% and 18.2% (Fig. 8).

The data obtained indicate that αDC1 in MLC possessed a more pronounced ability to stimulate the proliferative response of T-cells.

Discussion

Our attempt to improve the ability of DC to induce an antitumor response motivated us to develop a protocol for DC serum-free cultivation in an optimized cytokine environment that allows achieving the key properties of DC necessary for high vaccine efficiency: 1) fully mature DC status; 2) high expression of IL-12p70; 3) responsiveness to chemokines of secondary lymphoid organs.

To attract and interact DC with T-cell subpopulations (naive and central memory), the stability of CCL19 products matured at the periphery of the DC after their migration to regional lymph nodes is of great importance. The data obtained demonstrated that PGE_2 acts as a potent inhibitor of the production of chemokine CCL19 in the culture of sDC. At the same time, the ability for increased production of CCL19 in the αDC1 population quickly disappeared as soon as it was transferred from the maturation medium to the neutral medium. However, in response to CD40L stimulation of αDC1, in contrast to sDC, repeated chemokine-inducing signals were obtained that led to a second wave of increased production of CCL19.

It is shown that PGE_2 inhibits the proliferation of T-cells, directly and indirectly affects the secretion of proinflammatory and antitumor cytokines, and also facilitates the interaction of DC with regulatory T-cells, enhancing their proliferation. However, it was paradoxical that PGE_2 promotes the enhancement of CCR7 expression and thereby the chemotaxis of DC in response to chemokines CCL19 and CCL21. The latter provide directional migration of antigen-primed DCs to the draining lymph nodes, where they interact with T-cells (naive and central memory cells). This attractive property of PGE_2 justifies its inclusion in the composition of standard cytokine cocktails for the maturation of dendritic cell vaccines.

This study attempts to explain this paradoxical property of PGE_2 to cause increased expression of CCR7 on the sDC surface and simultaneously to reduce the production of CCL19. It is known that in high concentrations CCL19 induces ligand-dependent internalization of CCR7 and its disappearance from the cell surface. For this reason, the addition of exogenous CCL19 to sDC culture resulted in a dramatic decrease in the surface expression of CCR7. However, the effect of exogenous CCL19 did not alter the level of total (surface and intracellular) expression of CCR7 in the sDC population. It appears that PGE_2 inhibits the secretion of CCL19 and, as a consequence, ligand-dependent internalization of the CCR7 receptor, which explains its increased surface expression on sDC. It is important to note that the differences in the surface expression of CCR7 on sDC and αDC1 disappeared rapidly after their transfer from the maturing medium to the neutral one.

The weak migration capacity of αDC1 *in vitro* was due to the low expression of CCR7, since in the conditions of a maturing medium they actively secrete CCL19, which leads to internalization of this receptor. However, the migration activity of αDC1 was rapidly restored, to a level no lower than that of sDC, after their transfer to a neutral medium that did not contain CCL19.

The results obtained do not abolish the possible benefits of using PGE_2, which have been shown in a large number of other studies [18]. In either case, the potential benefits of using PGE_2 adversely affect the ability of the DC to secrete CCL19 necessary to attract T-cells. In addition, PGE_2 is able to locally program the DC for preferential interaction with Treg, which together can explain the generalized immunosuppression associated with chronic inflammation and the tumor process.

Specific binding of the antigen peptide in the composition of the molecule of the major histocompatibility complex presented by APC with the T-cell

receptor is the main signal for activation and differentiation of T-cells (signal 1). However, the type and degree of expression of the resulting response of T-cells depends both on the nature of the interaction of costimulatory molecules on APC with their ligands on T-cells (signal 2) and on the secretion of the corresponding cytokines (signal 3) [19].

It is known that the expression of costimulatory molecules from the B7 family (CD80 and CD86) is a decisive event in the maturation of DC [16]. The signal from the interaction of these molecules with ligands (CD28) on the surface of T-cells is important for the initiation of the cell cycle, IL-2 secretion and clonal expansion. At the same time, molecules from the B7 family partially or completely initiate co-inhibitory signals to the T-cells, thereby regulating the degree of expression of the immune response. Comparison of the maturation conditions of DC in a large number of donors made it possible to demonstrate that a positive costimulatory profile predominated in αDC1, while sDC demonstrated a stronger expression of the co-inhibitory molecule (CD274 = =PD-L1 = B7-H1) from the B7 family. This need to be taken into account in future when receiving DC for adoptive immunotherapy, especially in connection with the fact that most clinical trials used sDC-based vaccines. The unfavorable balance of costimulatory molecules expressed on DC may be one of the reasons for the unsuccessful results of clinical trials.

In addition to costimulation, cytokines secreted by DC after contact with T-cells (signal 3) are also of paramount importance in the implementation of an adequate antitumor immune response. The ability of DC for the secretion of cytokines was modeled with the help of CD40L, which allows simulating the natural interaction of DC with naive $CD4^+$ T-cells. Secreted by mature DC, IL-12p70 is an essential component of the cytokine environment for inducing polarization of naive $CD4^+$ T-cells in the direction of the Th1 response, while IL-10 is responsible for the development of tolerance. In most recent studies, preference has been given to cultivation techniques that provide mature DC with the highest potential for secretion of IL-12p70 [6; 7; 11; 17]. These publications report the possibility of reaching the concentration of IL-12p70 in the culture supernatant for maturation of the DC at a level of several tens of thousands of pg/ml [20]. However, when using DC in *in vivo* conditions, it is more reasonable to analyze the secretion of IL-12p70 not in an artificial maturation medium, but in a neutral medium in response to the interaction of DC with naive T-cells (signal 3). The concentration of IL-12p70 induced under such conditions is significantly lower. Dohnal et al. [21] consider a concentration of 100 pg/ml as the lower limit for the use of DC as an antitumoral vaccine, but the real value needed to achieve Th1 of the T-cell response *in vivo* and clinical efficacy remains unknown. According to our results, sDC in response to stimulation of CD40L *in vitro* produces a very small amount of IL-12p70, while the secretion of this interleukin αDC1 is many times higher than the above threshold of 100 pg/ml. The level of IL-10 in supernatants of both DC populations was extremely low. The ratio of concentrations of IL-12p70/IL-10, characterizing the "strength" of the Th1-polarizing ability of the DC, was maximal in the αDC1 population.

Stimulating ability of DC for autologous $CD4^+$-T-cells was also examined in terms of their ability to induce regulatory and activated T-cells under autologous MLC. Despite the absence of a significant difference in the total number of $CD4^+$ $C25^+$ cells, the subdivision of this heterogeneous population by the degree of expression of CD127 and FoxP3 showed that sDC mainly induces proliferation of regulatory T-cells, while αDC1 – of non-regulable activated T-cells. The study of such opposite effects of DC on the activation of autologous T-cells is of paramount importance for achieving adequate immunotherapy.

When studying the potential of DC to direct the development of T-cells towards Th1, Th2 or Th17 polarization in the supernatant of MLC with αDC1, we detected a high concentration of IFNγ. This effect with respect to αDC1 was expected, since

IL-12p70 is a powerful inducer of Th1 polarization. At the same time, the concentration of IFNg in MLC with sDC was also sighificantly increased, although the latter weakly secreted IL-12p70. An explanation for this may be a highly positive, although different in severity, profile of the expression of costimulatory molecules CD80 and CD86 in both DC populations.

The use of the TLR (LPS, poly-I: C, R848) in the cocktail for maturation of interferons and ligands provided not only the mature phenotype αDC1, but also the predominance of costimulatory molecules over co-inhibitory, high secretion of IL-12p70, but not IL-10, in response to CD40L stimulation, active directed migration, and proliferation of a significant number of CTL *in vitro*. In contrast to standard DC-based vaccines, which are based on the use of either insufficiently mature DC (highly productive for IL-12 but with low migration and stimulatory activity) or mature DC (with high migration and stimulatory activity but with reduced production of IL-12), the protocol developed in this study makes it possible to obtain a culture of αDC1 cells of which are simultaneously combine all the necessary, often contradictory, properties for antitumor vaccines.

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