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БЕЛОДРОБНА ТУБЕРКОЛОЗА НА ПОДРАЧЈЕТО НА ОПШТИНА БИТОЛА ЗА ПЕРИОД 2007-2011 година

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Medicus 2012, Vol. 17 (2): 155-159

РЕЗИМЕ: И покрај напредокот во дијагностиката ефикасната терапија и спроведување на вакцинацијата, туберкулозата во денешно време сеуште претставува здравствен, социјален и економски проблем. **Целта на овој труд** е да се прикаже распространетоста на активната белодробна туберкулоза во амбулантно поликлиничката и диспанзерската дејност на подрачјето на општина Битола. **Материјал и метод на работа.** За материјал се користени податоци добиени од Диспанзерот за белодробни заболувања при Клиничка болница - за периодот од 2007-2011година. Применет е дескриптивен метод на работа. **Резултати.** Бројот на болни од активна белодробна туберкулоза е намален од 31 во 2007 на 12 во 2011 година. Учеството на белодробната туберкулоза во вкуниот број на туберкулозни заболувања е 95,9%. Од резултатите видливо е постепено опаѓање на трендот на морбидитетот, со колебање на амлитудата на инцидецата и преваленцата, наизменично од година во година и се движи од 28,5 на 100 000 жители во 2007 година на 11,3 во 2011 година. Слично е и опаѓањет на бројот на новорегистрираните болни. Морталитетот од белодробна туберкулоза е исто така во опаѓање од 4,9 на 100.000 жители во 2007 на 2,9 во 2011 година.**Заклучок.** Добиените параметри укажуваат на успешно спроведување на мерките за спречување и сузбивање на белодробната туберкулоза во општина Битола. Натамошната борба против ова заболување подразбира уште поголемо ангажирање на здравствената служба и заедница во целост.

Клучни зборови: белодробна туберкулоза, здравствен, социјален, економски, проблем.

ВОВЕД

Туберкулозата е заразна болест која е предизвикана од бактеријата Mycobacterium tuberculosis (1). Се пренесува преку воздух, најчесто се инфицирани белите дробови, но можат да бидат опфатени и други органи. Здравиот организам обично успешно ја совладува инфекцијата и не дозволува појава на болест. Ако заради различни причини, имуниот систем не успее да ја совлада инфекцијата, тогаш организмот заболува од белодробна ТБ. Лицето инфицирано од ТБ - бацили може да нема симптоми на заболување, а подоцна (по повеќе години) овие бацили да предизвикаат болест. Кај 5-10% од инфицираните во текот на животот настанува туберкулозно заболување, а тоа се случува кога отпорноста на организмот ќе опадне од разни причини: нередовна и слаба исхрана, преголемо физичко и психичко оптоварување, стрес, консумирање големо количество алкохол и цигари, употреба на дроги, некои други заболувања (шеќерна болест, карцином и др.) (2,3).

Повеќето луѓе кои имаат ТБ немаат никакви симптоми, или симптомите се благи. Најчести симптоми за ТБ се: кашлица која трае подолго од три недели, сува или продуктивна, понекогаш придружена со крвав искашлок, болки во градите, срцебиење, по-

тешко дишење, голем и долготраен замор и при најмал физички напор, малаксаност, губење на апетитот, слабеење, покачена температура, потење (карактеристично попладне или рано наутро, околу вратот и горниот дел на градите). Симптоми од страна на другите заболени органи се: болки и отеченост на лимфни јазли (најчесто на вратот и пазувите); болки, црвенило, оток на зглобовите; присуство на крв во мочката кај бубрежна ТБ; течни столици кај ТБ на цревата, болки во предел на срцето кај туберкулозен перикардитис (воспаление на обвивката на срцето) и т.н. ХИВ/сидата и туберкулозата се блиску поврзани, така што често се зборува за ко-епидемија или за двојна епидемија. ХИВ вирусот го напаѓа одбранбениот систем на домаќинот и ја зголемува можноста за здобивање на нова инфекција, често туберкулоза. Исто така, ХИВ вирусот овозможува и прогресија на латентната во активна туберкулоза или рецидив кај пациенти кои претходно биле лекувани. Туберкулозата е еден од водечките убијци кај ХИВ инфицираните лица, особено во неразвиените земји (4,5). Годишно се инфицираат околу 1,7 билиони луѓе, од кои кај 8 милиони болеста е активна. Годишно умираат 3-5 милиони луѓе. Најчесто се јавува во сиромашни неразвиени земји, каде нема ефикасен здравствен систем. Појавата на болест се должи на економски фактори, медицински фактори и други хронични заболувања (diabetes mellitus, silikosis, малигни тумори, срцеви мани). Во Република Македонија се регистрираат околу 700 нови болни, а во Битола во анализираниот период се регистрирани 52 нови болни (6,7).

ЦЕЛ НА ТРУДОТ

Целта на овој труд е да се прикаже распространетоста на активната белодробна туберкулоза во амбулантно поликлиничката и диспанзерската дејност на подрачјето на општина Битола, според разни обележја (пол, возраст, фреквенција на јавување, морбидитет, морталитет, стапка на инциденца, преваленца и друго). Da se utvrdat merkite i aktivnostite koi treba da se sprovedat vo uspe{noto sovladuvawe na tuberkulozata koi se del i se prepora~uvaat vo nacionalnite programi koi gi podgotvuvaat Министерството за здравство и Институтот за белодробни заболувања и туберкулоза.

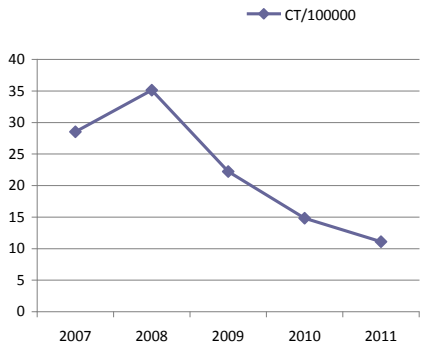
МАТЕРИЈАЛ И МЕТОД НА РАБОТА

За материјал се користени податоци за состојбите со морбидитетот на белодробната туберкулоза од Извештаи на службата за општа медицина и специјалистичките служби, Обр. бр. 3-01-60 и Извештај на службата за белодробни (пулмонални) болести и туберкулоза, Обр. бр. 3-06-60 за лекуваните лица на подрачјето на општина Битола за период 2007-2011 година. Koristeni se i podatocite od lekarskiot izve{taj za pri~inata za smrt. Применет е дескриптивен метод на работа.

РЕЗУЛТАТИ

Во службата за белодробни заболувања и туберколоза и дејноста за општа медицина на подрачјето на Битола, во 2011 година регистрирани се вкупно 12 случаи на заболени од туберкулоза со стапка на инциденца од 11,1 на 100000 жители. Овој број е намален за 61,3 индексни поени во однос на 2007 година, кога биле регистрирани 31 случаи на заболени од белодробна туберкулоза со стапка на инциденца од 28,5 на 100000 жители (Графикон 1).

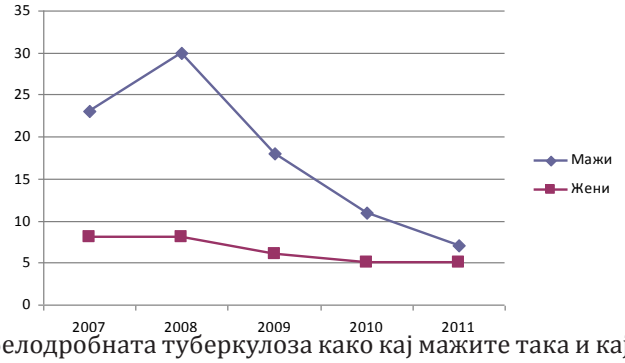
Графикон 1. Стапка на белодробна туберкулоза во дејноста за општа медицина и службата за белодробни болести и туберкулоза во Битола, 2007 – 2011 година.



Анализата според пол покажува поголема застапеност на мажите во вкупниот број на регистрирани случаи и тоа 23 во 2007 година и 7 во 2011 година, значи 73,6% наспроти жените чии процент изнесува 26,4% (Графикон 2).

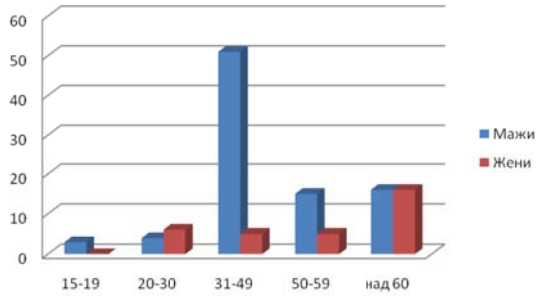
Графикон 2. Структура на заболени од белодробна туберкулоза во дејноста за општа медицина и службата за белодробни болести и туберкулоза според пол во Битола, 2007 – 2011 година.

Анализата според возраст покажува дека



белодробната туберкулоза како кај мажите така и кај жените со најголема фреквенција се јавува кај возрасната група од 20 - 49 години со 56 или 54,5% заболени и над 60 години 26,4% (Графикон 3).

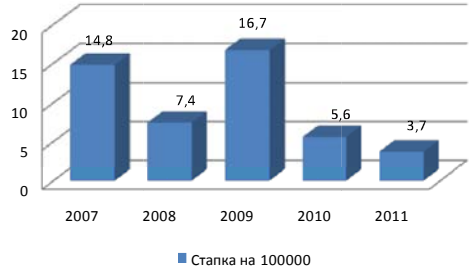
Графикон 3. Структура на заболени од белодробна туберкулоза во дејноста за општа медицина и службата за белодробни болести и туберкулоза според пол и возрасни групи во Битола, 2007 – 2011 година.



Сите органи заболуваат од туберкулоза, но најчесто страдаат белите дробови. Белодробната локализација на болеста е забележана кај 95,9% од вкупниот број на туберкулозни заболувања.

Во анализираниот период 2007 - 2011 година стапката на инциденца покажува тренд на пад 14,8 на 100000 жители во 2007 година на 3,7 на 100000 жители во 2011 година (Графикон 4).

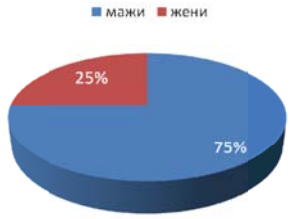
Графикон 4. Стапка на инциденца на белодробна туберкулоза во службата за белодробни болести и туберкулоза во Битола, 2007 – 2011 година.



I mortalitetot od belodrobna tuberkuloza vo Bitola vo period 2007 - 2011 godina registriran vo doma{n uslovi od strana na ovlasteni lica - lekari mrtvoproveriteli vo slu`bata za itna medicinska pomo{ ( I M P ) poka`uva trend na pad. Od belodrobna tuberkuloza vo 2011 godina umrele 3 lica so stapka na mortalitet od 2,9 na 100.000 `iteli, dodeka stapkata na mortalitet vo 2007 godina e 4,9 na 100.000 `iteli ili 6 umreni lica. Vo 2011 godina mortalitetod od belodrobna tuberkuloza e za 50,0 indeksni poeni pomal vo odnos na 2007 godina.

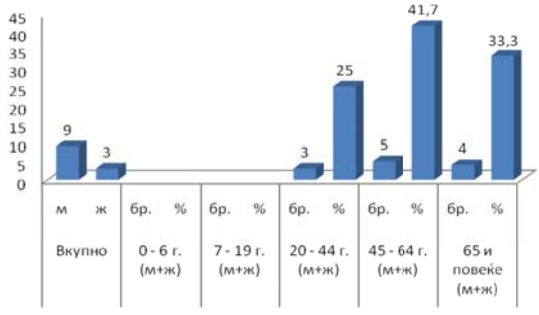
Analizata spored pol poka`uva pogolema zastapenost na ma`ite vo vkupniot broj na registrirani slu~ai 75,0% nasproti `enite ~ii procent iznesuva 25,0%(Графикон 5).

Grafikon 5. Procentualno u~estvo na umreni od belodrobna tuberkuloza registrirani vo dejnosta za I M P , spored pol vo Bitola, 2007 - 2011 godina



Analizata spored vozrast poka`uva deka mortalitetot od belodrobna tuberkuloza so najgolema frekvencija se javuva kaj vozrasnata grupa od 45 - 64 godini so 5 umreni lica ili 41,7% i nad 65 godi{na vozrast so 4 umreni ili 33,3%. (Grafikon 6).

Grafikon 6. Broj i struktura na umreni od belodrobna tuberkuloza registrirani vo dejnosta za IMP, spored vozrasni grupi vo Bitola, 2007 - 2011 godina



ДИСКУСИЈА

Во согласност со извештајот на Светската Здравствена Организација (СЗО), бројот на новозаболени од туберкулоза кај населението во Битола е стабилен (6,7,8), а вкупниот број на лица болни од туберкулоза, е со тенденција на благо опаѓање. И покрај големото достигнување во подобрување на епидемиолошката состојба со туберкулозата, кое се спомнува скоро во

сите објавени студии (1 - 10), таа сеуште претставува сериозен проблем, со тешко предвидлив тренд на морбидитетот и морталитетот на болеста во иднина. Seu{te postoi evidentna zavisnost na tuberkuloznoto zaboluvawe so socijalno - ekonomskata polo`ba na naselenieto vo op{tinata i vo zemjata kako posledica na ekonomskata kriza, pad na `ivotniot standart, se pogolemata nevrabotenot i skromnite materijalni mo`nosti na zdravstvenite slu`bi. Потребно е подобрување на сисемот за прибирање податоци, сега порепрезентативни и достапни во повеќе земји отколку во претходните години. Во последните неколку години остварен е значителен напредок како во борбата против ХИВ инфекцијата, така и во борбата против туберкулозата. Но, туберкулозата се уште убива повеќе лица инфицирани со ХИВ отколку било која друга болест. Финансиската криза не смее да ја попречи имплементацијата на Глобалниот план за спречување на туберкулозата. Сега е време кога треба да се зголеми финансирањето на ефикасни интервенции за превенција и третман на туберкулозата низ целиот свет (4, 5, 8, 9, 10).

ЗАКЛУЧОК

Посебен ентузијазам и надеж во успешното совладување на туберкулозата овозможува спречувањето на појавата на инфекција кај здрави лица, да се спречи појавата на болеста кај веќе инфицираните лица, да се открие болеста во ран стадиум, сето ова е дел од активноста на националните програми за успешна контрола на ТБЦ, која секоја година ја подготвуваат Министерството за здравство и Институтот за белодробни заболувања и туберкулоза. Под превентивни мерки спаѓаат: BCG вакцина, превентивна терапија и хемиопрофилакса, флуографско снимање, испитување на ризични групи, испитување на контактите и контрола на инфекцијата. Со еден збор, правилното едуцирање на популацијата е со цел да се постигне навремено реагирање и откривање на туберкулоза. Лекарствата да се земаат под контрола на здравствени работници, односно примена на ДОТС стратегијата советувана од Светската здравствена организација.

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SUMMARY

PULMONARY TUBERCULOSIS AT THE AREA BITOLA MUNICIPALITY FOR PERIOD 2007-2011 YEAR

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It requires long and expensive treatment, causes lengthy absences from work and a large percentage of disability, and despite the financial burden on the community, the patient is a huge financial and emotional burden for its family and for himself. ***The purpose*** *of this work is to show the prevalence of active pulmonary tuberculosis in ambulance policlinic and dispensary activity at the area of Bitola municipality. Material and method. The data obtained from the Clinic hospital - Bitola and relate to the period of 2007-2011 year.* ***Results and discussion.*** *The number of patients with active pulmonary tuberculosis has been reduced from 31 in 2007 to 12 in 2011. Participation of pulmonary tuberculosis in the total number of tuberculous disease is 95,9%. From the results visible is gradually declining trend of morbidity, with hesitation of amlitude of incidence and prevalence alternated from year to year and ranges from 28.5 per 100 000 population in 2007 to 11.3 in 2011. Similarly is also with the reducing the number of newly registered patients. Mortality from pulmonary tuberculosis is also declining from 4.9 per 100,000 population in 2007 to 2.9 in 2011.* ***Conclusion.*** *This parameters indicate successful implementation of measures for the prevention and suppression of pulmonary tuberculosis in Bitola municipality. Further the fight against this disease involves even greater engagement of the health service and the community in general.*

Keywords*: pulmonary tuberculosis, health, social, economic, problem.*

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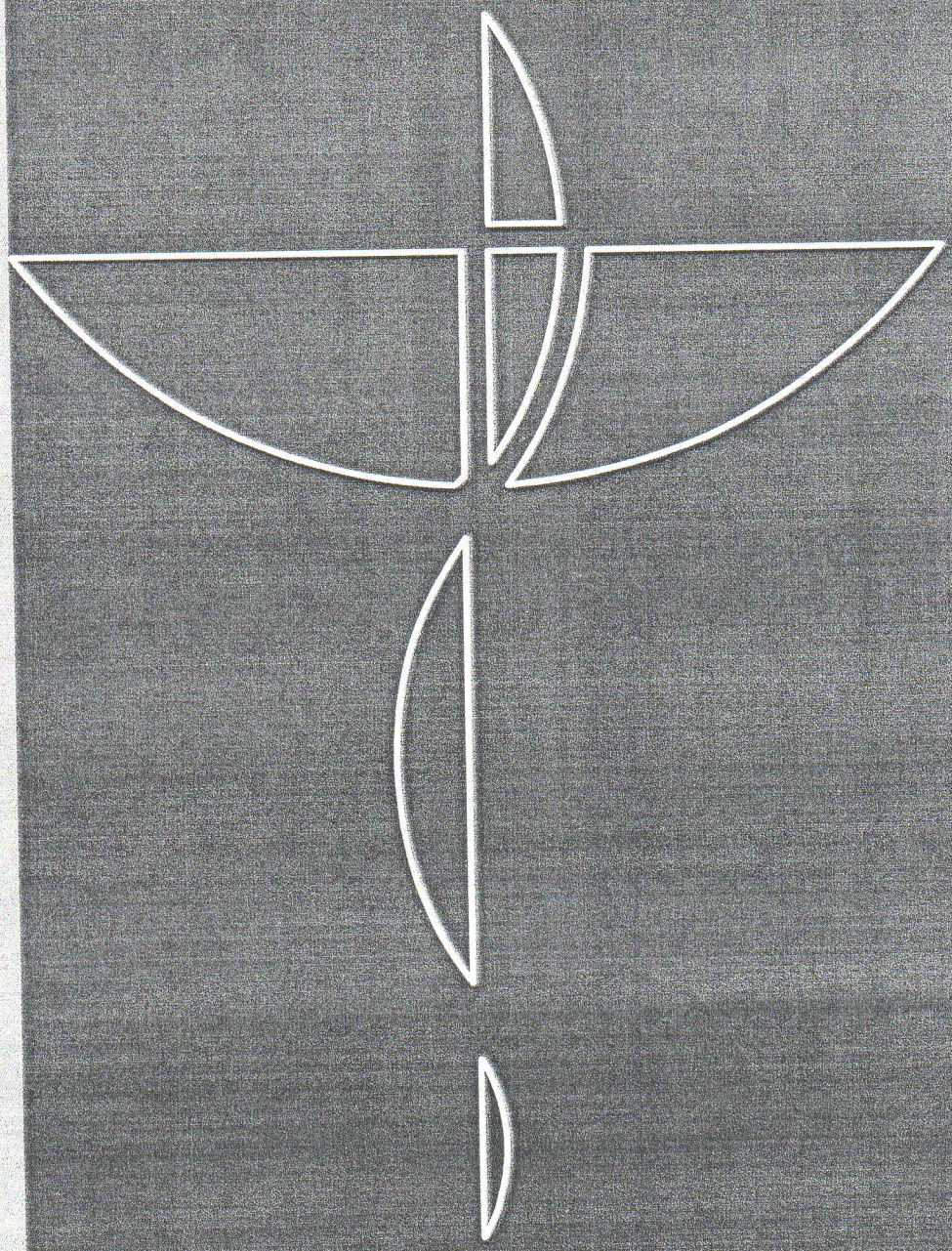

UDK: 616-056.52-053.2:616.43(4

LEPTIN LEVEL, BODY MASS INDEX AND LIPID PARAMETERS IN CHILDREN OF REPUBLIC OF MACEDONIA

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Abstract

Objective. Leptin, an important signal in the regulation of adipose-tissue mass and body weight, operates by inhibiting food intake and stimulating energy expenditure. Overweight and obese youth are at increased risk for premature cardiovascular disease. It's well known that the development of atherosclerosis begins in childhood and is accelerated in the presence of obesity. Leptin plays an important role in the pathogenesis of obesity.

Results. Serum leptin levels were higher in overweight boys and girls compared to normal-weight boys and girls respectively ($p< 0.001$; $p< 0.001$). Serum leptin positively correlated with BMI in all examined groups. Triglycerides were statistically significant higher in overweight boys compared to normal-weight boys ($p<0.08$). Overweight girls had significantly higher LDL cholesterol ($p<0.006$) and significantly lower HDL cholesterol ((p,004) than normal-weight girls.

Conclusion: Overweight boys and girls had higher leptin levels. The BMI showed a high correlation with leptin levels in all examined groups which suggests that BMI is the main indicator for the variations of leptin level. The concentration of LDL and HDL cholesterol differed only in the group of girls and triglycerides in the group of boys.

Keywords. Leptin, BMI, lipid parameters, children

ВРЕДНОСТИ НА ЛЕПТИН, ИНДЕКС НА ТЕЛЕСНА МАСА И ПАРАМЕТРИ НА ЛИПИДЕН СТАТУС КАЈ ДЕЦА ОД РЕПУБЛИКА МАКЕДОНИЈА

Апстракт

Цел. Лептинот, значаен сигнал во регулирањето на количината на масното ткиво и на телесната тежина, делува преку инхибиција на земањето на храна и преку стимулација на потрошувачката на енергијата.

Младите лица со прекумерна тежина и оние кои се гојазни се изложени на зголемен ризик за предвремени кардиоваскуларни болести. Добро е познато дека атеросклерозата започнува во детството и се забрзува во присуство на дебелината. Лептинот игра важна улога во патогенезата на дебелината.

Резултати. Нивото на лептинот беше повисок во серумот на момчињата и девојчињата со во поголема тежина во споредба со момчињата и девојчињата со нормална тежина ($p> 0,001$; $p <0.001$ соодветно). Нивото на лептинот позитивно

корелира со БМИ во сите испитани групи. Триацилглицеролите беа статистички значајно повисоки кај момчињата со поголема тежина во однос на момчињата со нормална тежина (p <0.08).Кај девојчињата со поголема тежина утврдивме статистички значително повисоко ниво на LDL- холестерол (p <0.006) и статистички значително пониско ниво на ХДЛ –холестерол (p< 0.004), во однос на девојчињата со нормална тежина.

Заклучок: Момчињата и девојчињата со прекумерна тежина имаат повисоко ниво на лептин. БМИ покажа статистички значителна висока корелација со нивото на лептинот во сите испитани групи, што значи дека БМИ е главен показател за варијациите на нивото на лептинот. Од липидните параметри , концентрацијата на LDL и на HDL холестеролот се разликува само во групата на девојки со прекумерна тежина во однос на оние со нормална тежина , а во групата момчиња разлика беше утврдена само во концентрацијата на триацилглицерглицеолите

Клучни зборови . Лептин, БМИ, липидни параметри, деца

Introduction:

Leptin, discovered in 1994, is a 167- amino acid protein produced by the leptin gene (LEP), whose name is derived from the Greek word "leptos", which means "thin." Leptin, an important signal in the regulation of adipose-tissue mass and body weight, operates by inhibiting food intake and stimulating energy expenditure. Leptin is produced by the white adipose tissue, the most frequent form of adipose tissue in mammals. It is also produced in several other places including placenta, bone marrow, stomach, muscle, and perhaps brain, thus increasing the number of potential regulatory roles for this hormone. (1) The amount of body fat is the main determinant of the leptin circulating levels. (2) Leptin is transported across the blood-brain barrier. In the brain, leptin stimulates or inhibits release of several neurotransmitters such as neuropeptide Y, melanin-concentrating hormone, orexins alpha-melanocyte-stimulating hormone etc. (3) Defects in leptin production cause severe obesity. (2)

Obesity in child and adolescent population is a growing problem worldwide. Also, overweight and obese youth are at increased risk for premature cardiovascular disease. It is well known that the development of atherosclerosis begins in childhood and is accelerated in the presence of obesity. (4)

The aim of this study is to examine the concentration of serum leptin, BMI, and lipid parameters in normal- and overweight children of the Republic of Macedonia.

Materials and Methods

This research was part of the cross-border project that was co-funded by the European Union and by the national funds of the participating countries under the IPA cross-border program "Greece – The Former Yugoslav Republic of Macedonia 2007-2013 "Using new technologies to promote children health in the cross-border region".

The study included 172 children (age 10.94±1.56 years) - 86 boys (age 10.92±1.47) and 86 girls (age 10.77±1.53) - with body mass index (BMI) ranging from 20 to 41 kg/m^2. A written informed consent was obtained from the parents of each child included in the study. Exclusion criteria were history of renal, hepatic, endocrine, and respiratory (with the exception of asthma) disease.

First, the parents filled out a questionnaire about the physical activity of their child. The children had light indoor clothes and were barefooted during the measurement of their height and weight. Their standing height was measured with stadiometer to the nearest 0.1 cm. Weight was measured using a digital weight scale with a precision of 0.1 kg. BMI was calculated by dividing the weight by height squared (kg/m^2). The authors used World Health Organization (WHO) growth reference data for children 5-19 years of age, which included weight, height, and BMI for these children. According to the BMI, the subjects were divided into four groups: normal weight girls normal weight girls boys with BMI < 25 kg/m^2, and overweight boys and overweight girls with BMI > 25 kg/m^2. (5)
The blood samples were taken after overnight fast (12 hours). Lipid parameters, such as total cholesterol, HDL cholesterol, LDL cholesterol, and triacylglycerols were measured in fresh sera by enzymatic methods, using biochemical analyzer Cobas Integra 400. Leptin was determined by ELISA kit (Mercodia Leptin ELISA, Sweden).
The data are presented as mean ± standard deviation (SD) and $p \leq 0.05$ is considered statistically significant. The results were done with the SPSS version 16; statistical significance was test with paired Student's t-test, and Pearson correlation coefficient was used for correlation of BMI and leptin levels.

Results

The concentration of leptin in all normal-weight children ranged from 2.07 ng/ml to 40.45 ng/ml (mean 8.04± 9.57). However, statistical analysis showed that normal-weight girls had statistically higher leptin levels than normal-weight boys ($p<0.001$). Therefore, all 172 participants were divided in 4 groups: normal-weight (NW) girls, normal-weight (NW) boys, overweight (OW) girls and overweight (OW) boys (n=43 each group).
The anthropometric characteristics of each group are shown in Table 1.

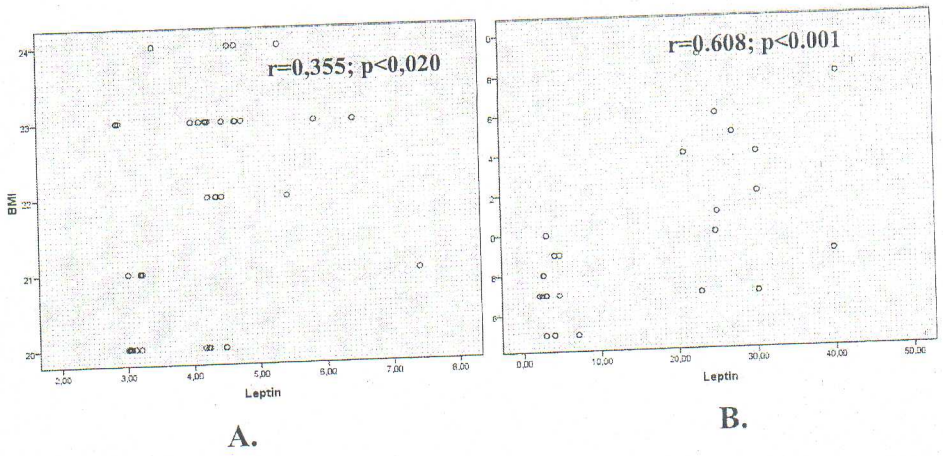
Table 1 Anthropometric characteristic of participants according to their sex and weight

Variables	NW-girls n=43	OW-girls n=43	p	NW- boys n=43	OW-boys n=43	p
Age (years)	10,58±0,98	10,95±1,94	ns	10,51±1,30	11,33±1,54	ns
Body mass (kg)	43,28±5,17	73,24±17,20	0.001	42,97± 5,28	70,21±15,73	0.001
Height (m)	1.47±1.20	1.49±1.26	ns	1.51±1.39	1.52±1.48	ns
BMI(kg/m^2)	21,84±1,41	29,70±3,79	0.005	21,98±1,47	27,88±4,35	0.005
Waist circumference (cm)	60,48 ±9,91	76,3±7,21	0.001	59,51±8,91	73,61±6,32	0.005
Hip circumference (cm)	76,78± 8,91	83,42 ± 9,36	0.001	72,79± 8,37	80,15±8,96	.002

NW –normal weight; OW- overweight ; p.> 0.05-statistical significant

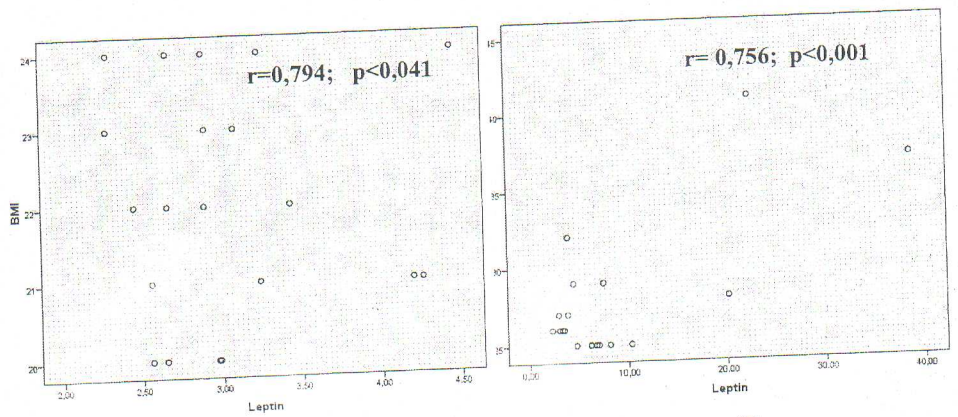
Table shows that BMI, waist and hip circumference are statistically significantly higher in overweight girls ($p<0.005$; $p<0.001$; and $p< 0.001$) and overweight boys ($p< 0.005$; $p< 0.005$; $p< 0.00$), compared to normal-weight girls and boys, respectively.

Serum leptin levels significantly correlate with BMI in normal-weight and overweight girls (Fig. 1 A and B) and in normal-weight and overweight boys (Fig. 2 A and B), respectively.



A. B.

Figure 1 Correlation between leptin level and BMI in normal weight girls (r=0,355; p<0,020) (A) and overweight girls (r=0.608;p< 0.001) (B)



A. B.

Figure 2 Correlation between leptin level and BMI in normal weight boys (r= 0.794; p< 0.041) **(A)** and overweight boys (r=0.756;p< 0.001) **(B)**

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Sjournals
Scientific Journals
Scientific Journal of Review (2013) 2(11) 279-295
ISSN 2322-2433
doi: 10.14196/sjr.v2i11.1039

Contents lists available at Sjournals
Scientific Journal of REVIEW
Journal homepage: www.Sjournals.com

Review article

The role of adipokines and gut hormones in the pathogenesis of obesity, and recent findings for the future treatment of obesity

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ARTICLE INFO

Article history,
Received 06 November 2013
Accepted 20 November 2013
Available online 29 November 2013

Keywords,
Dipose tissue
Adipokines
Gut hormones
Appetite
Body mass index
Obesity

ABSTRACT

Obesity has become one of the leading public health concern. Over one billion people are overweight or obese and the prevalence of these conditions is growing constantly. This review presents an overview of the endocrine functions of adipose tissue, the role of gut hormones and their associated neuronal networks (the gut-brain axis) in appetite control. Recent studies have improved our understanding of energy homeostasis by identifying sophisticated neurohumoral networks that transmit signals between the brain and gut to control food intake. Key adipokines, such as, leptin, adiponectin, interleukin-6, plasminogen activator inhibitor-1, resistin, tumor necrosis factor-α, adipsin and acylation stimulating protein, macrophages and monocyte chemoattractant protein-1, plasma renin, plasma angiotensin - converting enzyme and angiotensinogen linked with pituitary neuropeptide system began to clarify. Gut hormones, such as, cholecystokinin, ghrelin, peptide YY, pancreatic polypeptide, glucagon-like peptide-1, oxyntomodulin, ghrelin, insulin, glucagon, obestatin, amylin are modulated by acute food ingestion. This article highlights some of the recent findings and their implications for the future treatment of obesity, but there

are currently no effective pharmacological interventions for obesity.

1. Introduction

At the moment one of the greatest global problems is obesity. It represents a condition of increased fat tissue, or excessive accumulation of triglycerides in the fatty tissues, as a consequence of the increased food intake than the actual energy consumption and the lowered physical activity from the mostly sitting life-style (Paracchini et al., 2005). In 1985 the World Health Organization (WHO) defined obesity as a condition with body mass index (BMI) larger than 30.0 for men and larger than 28.6 for women (World Health Organisation, 1985). The Body Mass Index is calculated according to the standard formula – mass in kg divided with the square of height in m. Although, women have less bone and muscular tissue, they usually have a little more subcutaneous fatty tissue, but these subtleties are usually ignored during the standardized approach. Similarly, the muscle and bone weight are lowered with age, but these characteristics are not taken into account when defining the term. It should be emphasized that the waist – hips ratio (normally under 0.95 for men and under 0.85 for women) is generally a better prognostic indicator of the disease than the Body Mass Index (Paracchini et al., 2005). Never before in history has there been such an abundance of energy-rich, highly processed foods, as it has today (Sunye et al., 2002). The epidemic of obesity is a consequence of the economic, social and technological progress achieved during the previous few decades (Guyton et al, 2003). This so called nutritional transition combined with the more inactive life style promotes an environment which creates obesity (Sunye et al., 2002). More at risk of increased body mass and obesity are women, older people, members of minority groups and people with low socioeconomic status (Guyton et al, 2003). Obesity is not only an esthetical problem, but health problem also because it represents a risk factor for occurrence and development of diabetes mellitus type 2, vascular diseases, osteoarthritis, sleep apnoea and malignity (Silva et al., 2012). Obesity can be obtained as a result of a specific hormonal imbalance (Cushing's disease), and usual reasons are the mutations of specific genes. It can be monogenic or polygenic, but it is concluded that the polygenic forms are more common (Srivastava et al., 2007). For some genetic syndromes as Prader – Willi syndrome, Angelman syndrome and Wilson – Turner syndrome, obesity is only one symptom of many manifestations that are within the relating syndromes. Obesity might even be psychogenic. Testing of obese patients has shown that the vast percentage of obesity is consequence of psychological factors, and the reasons are stressful situations, and it is considered that food intake usually lowers tension. Obesities pathogenesis is complex and has interactions between, sex, age, race, socio-economic status, living environment, behaviour, ethnicity, genetic factors and others (Guyton et al, 2003). According to World Health Organization there are around one billion overweight people (Body Mass Index over 25 kg/m2), and 300 million of them are considered clinically obese (World Health Organization., 2000). It is supposed that 40% of adults might be obese by 2025 nationwide (Silva et al., 2012). Overweight and obesity are fifth leading risk factor for global mortality. At least 2,8 million people die each year as a result of overweight and obesity. Around 44% of diabetics, 23% of patients with ischemic heart disease and between 7% - 41% of cancer patients are obese.

In the thesis we will summarize our understandings of the pathophysiology of obesity, we will provide integrated perspective of how the metabolic signals which are synthesized in the

gastrointestinal tract, fatty tissue and other peripheral organs affect the brain and feeding centre, energy consumption and body weight.

2. Selection of studies

Electronic data base MEDLINE and PubMed were searched (research, 01. November 2013). Research strategy was conducted for all results of interest. The researched terms were, "obesity", "fatty tissue", "adipocytes", "intestine hormones", "appetite", "Body Mass Index". Mesh titles were used, where it was possible. The research was limited on articles published in English language. The research was limited on people, date of publish was limited to 10 years. We limited the review on healthy individuals and overweight children. The extracted references are verified by the title and abstracts for inclusion and exclusion, according to the following criteria. Inclusion criteria, fatty tissue, adipocytes, red hormones, appetite, body mass index, obesity, hips size, waist – hips ratio, every ethnic group and each population of people.

Exclusion criteria, studies that do not meet inclusion criteria; studies that included research of animal models. All inclusion studies were extracted as a complete text and were evaluated again for inclusion and exclusion. The process of inclusion and exclusion was carried according to predetermined criteria by two independent reviewers and trough discussion a consensus was achieved. Total of 175 340 theses were found during the research in the electronic data bases. Forty nine studies met the criteria for inclusion and exclusion and were available as documents with a complete text. Twenty seven of them were included in the preparation of this revial thesis.

3. Central nervous system and appetite regulation

Appetite means desire for a specific type of food and helps in the choice of quality food. If the desire for food is satisfied, feeling of satiation appears (Guyton et al, 2003). The gastrointestinal tract – brain axis controls appetite through neural and hormonal signals. With the entry of the nutrients, the small intestine releases peptides that act as negative feedback for lowering the size of the meal and feeding discontinuation (Figure 1).

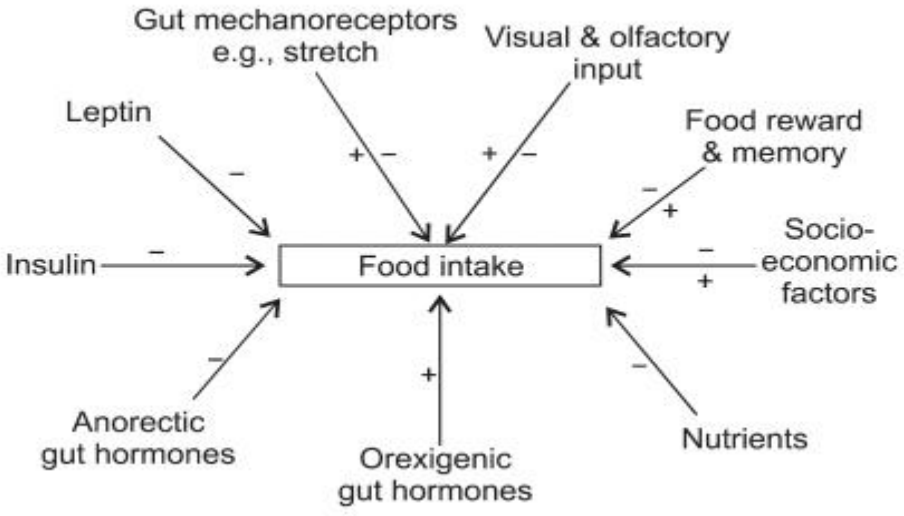

Fig. 1. The major determinants of appetite control (Silva et al., 2012).

Hormones and cytokines excreted from the peripheral organs have long-lasting effects upon the energy balance with control upon the reception of food and energy consumption. Neurons that are included in the regulation of homeostasis of nutrition are mainly located in the hypothalamus and the brainstem. The nucleus which is arc shaped located in the hypothalamus receives signals from the periphery. These signals act on two different neuronal population coding. First population that expresses agouti-related peptide (AgRP) and neuropeptide Y (NPY), stimulates the food intake; and the second neuron population, that contains pro-opiomelanocortin (POMC), cocaine – amphetamine regulated transcript (CART), inhibits the appetite (Figure 2).

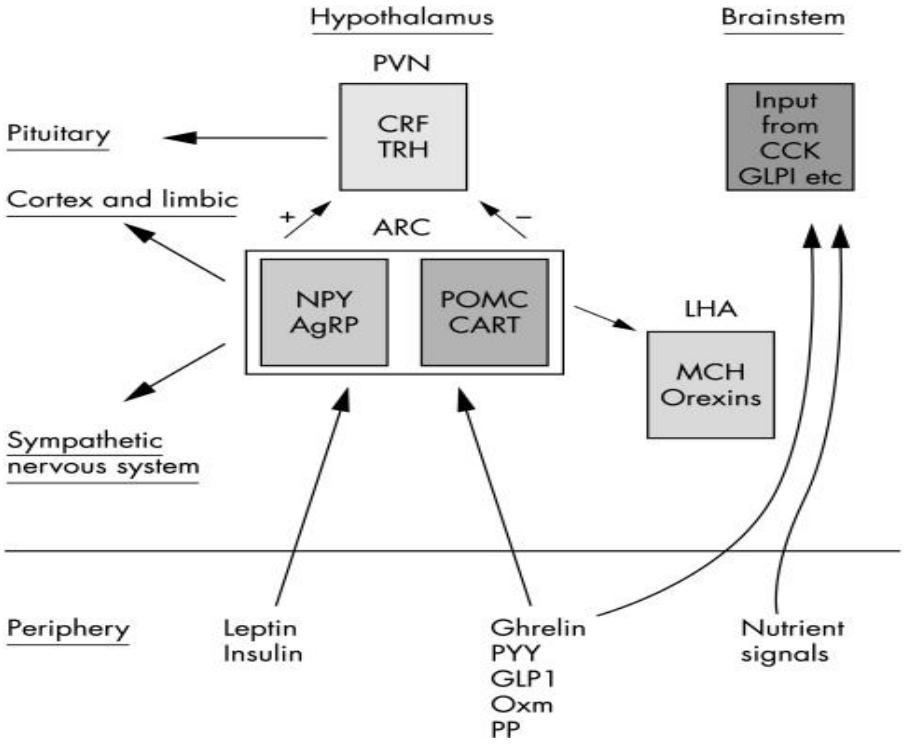

Fig. 2. Schematic representation of appetite control—actions of gut hormones and long term adiposity signals on neuronal populations in the arcuate nucleus and the integrated response of anorexigenic and orexigenic populations of neurones in the hypothalamus. ARC, arcuate nucleus; PVN, paraventricular nucleus; LHA, lateral hypothalamic area; CRF, corticotroph releasing factor; TRH, thyrotropin releasing factor (form part of integration with energy expenditure); NPY, neuropeptide Y; AgRP, agouti related peptide; POMC, proopiomelanocortin; CART, cocaine and amphetamine regulated transcript; MCH, melanin concentrating hormone; CCK, cholecystokinin; GLP-1, glucagon-like peptide 1; PYY, peptide YY; Oxm, oxyntomodulin; PP, pancreatic polypeptide (Druce et al., 2006).

Pro-opiomelanocortin is a precursor of alfa-melanocyte-stimulating hormone (α-MSH) that influences upon the receptor of melanocortin (MC4R), which decreases food consumption (Schwartz et al., 2000).

4. Adipose tissue, active endocrine organ and obesity

Adipose tissue is highly active metabolic and endocrine organ, which is essential for regulation of body weight (Kershaw et al., 2004). It is composed of cells filled with triglycerides (adipocytes), preadipocytes – adipocytes precursor, stromal cells, immune cells, collagen

network, fibroblasts, macrophages and blood vessels. These components work together as one integrated unit (Frayn et al., 2003). Except for the basic function of storage and release of energy, fatty tissue contains a metabolic apparatus that enables communication with further organs including CNS. Through this interactive network, fatty tissue is integral included in coordinating with different biological processes, including energy metabolism, neuroendocrine function and functions of the immunological system (Tartaglia et al., 1995). The endocrine function of the fatty tissues is particularly emphasized in unwanted metabolic consequence – excessive fatty tissue or obesity. Excessive fatty tissue, particularly in the visceral parts is associated with insulin resistance, hyperglycaemia, dyslipidemia, elevated blood pressure and with prothrombotic and proinflammatory state (Grundy et al., 2004). Fatty tissue secretes great number of different peptides, also known as adipokines which operate on local (autocrine / paracrine) and systemic (endocrine) level and have a wide biological activity. They control feeding, energy balance, neuroendocrine and other functions (Table 1).

Table 1
Examples of adipocyte-derived proteins with endocrine functions (Kershaw et al., 2004).

Cytokines and cytokine-related proteins	**Leptin**
	TNFα
	IL-6
Other immune-related proteins	MCP-1
Proteins involved in the fibrinolytic system	PAI-1
	Tissue factor
Complement and complement-related proteins	Adipsin (complement factor D)
	Complement factor B
	ASP
	Adiponectin
Lipids and proteins for lipid metabolism or transport	Lipoprotein lipase (LPL)
	Cholesterol ester transfer protein (CETP)
	Apolipoprotein E
	NEFAs
Enzymes involved in steroid metabolism	Cytochrome P450-dependent aromatase
	17βHSD
	11βHSD1
Proteins of the RAS	AGT
Other proteins	Resistin

Protein and cytokine molecules which are created in the fatty tissue are, leptin, adiponectin, angiotensine, atrial natriuretic peptide, cholesteryl ester transferase, estrogen, fatty-acid-binding-proteins ap2 FFA / Glicerol, IGF – binding proteins 3 (IGFBP – 3), insulin as growth factor – 1 (IGF – 1) interleukin – 1 beta, interleukin – 6 (IL – 6), interleukin – 8 (IL – 8), lipoprotein lipase – Monobutyrin, PAI – 1, resistin, retinol binding protein – 4, sterol regulatory elements, TNF-α, visfatin and others (Kershaw et al., 2004).

4.1. Leptin

Discovery of leptin by Friedman and his colleagues in 1994 was an event that was groundbreaking in the research field of obesity. Leptin (Greek leptos, meaning thin) is a 16-kDa polypeptide that contains 167 amino acids and is similar to cytokines structurally (Kershaw et al., 2004). LEP gene, which encodes leptin, is localized in chromosome 7aplpha31.3 and is composed of three exons and two introns (Paracchini et al., 2005). It is manufactured primarily in adipocytes of white fatty tissue and is proportional with (to) the total amount of fat tissue, and with nutrition status (Fain et al., 2004). Leptin, in small amounts, is synthesized in other tissues of the human body, stomach, heart, epithelium in the mammary gland and the placenta. Leptin manifests its effects through receptors (OBR) which are mostly localized in the hypothalamus and cerebellum, but it can be also found in the placenta, vascular tissue, stomach and brain (El-Atat et al., 2003). Leptin crosses the blood-brain barrier, connects to the receptor localized in the hypothalamus, inhibits the hunger feeling and increases energy consumption level and thus plays a major role in the establishment of energy balance (El-Atat et al., 2003). It lowers obesity through lowering appetite and increasing thermo genesis (Schwartz et al., 2000). Leptin, acting upon its receptor in the hypothalamus, activates the neurons of pro – opiomelanocortin (POMC), cocaine – amphetamine regulated transcript (CART) and inhibits neurons on neuropeptide Y (NPY) and agouti – related protein (AgRP). These pathways are interacting with other brain centre to coordinate appetite (Bils et al., 2009). Leptin signals are transported by JAK/STAT mechanism which takes place as it follows, the leptin receptor phospohrylates under the influence of Janus kinase enzyme (JAK), bonts with STAT – proteins (Signal transducers and activators of transduction)) which further phosphorylate under the influence of the same enzyme. Phospholytatet STAT – proteins enter the nucleus bonding with specific DNA sequences thus regulating the expression of specific genes. Leptin functions upon the principle of negative feedback, causing stimulation of hunger sense upon low concentrations and its inhibition upon high concentrations (Tanaskoska et al.,2009). It is assumed that increased body weight results with reduction of leptin activity upon leptin receptors in hypothalamus otherwise known as “leptin resistance” i.e. the effect of leptin in reducing the appetite and increasing the energy consumption is obstructed. Because of these, for obese individuals, despite high concentrations of leptin, it is observant positive energetic balance accompanied with increase of body weight (Tanaskoska et al.,2009). Deficiency of leptin receptor is rare, thereby the person is born with normal birth weight but shows quick weight gain in the first few months of life, which results with overweight obesity. It is characterized with expressed hyperphagia with needs for food and aggressive behaviour when food is not given. Energy input during meal by personal choice is increased significantly for persons with leptin and leptin receptor deficiency (Bils et al., 2009). According to the newest studies, leptin is recognized as one of the most sensitive adipokines markers for prediction of accumulation of risk factors for cardiovascular diseases and metabolic syndrome on adolescents (Arslan et al., 2010). Some other endocrine functions of leptin include regulation of the function of the immune system, haematopoiesis, angiogenesis, creation of bone tissue and wound healing (Margetic et al., 2002).

4.2. Adiponectin

Adiponectin is discovered in 1995 and 1996 by four groups of scientists using different methods, hence the different names, apM1 (adipose most widespread gene transcript 1), Acrp30 (adipocytes related to proteins of 30 kDa), adipoQ and GBP28 (gelatine binding protein of 28 kDa (Maeda et al., 1996, Scherer et al., 1995, Nakano et al., 1996, Hu et al., 1996). It is one of a kind specific protein of fatty tissue, with MM 30-kDa, that has a structural homology to collagen type VIII and X complement C1q factor. Adiponectin is composed of N-terminal signal

sequence, variable domain, domain similar to the collagen and C-terminal tail domain. It circulates in the human plasma in great amounts (Diez et al., 2003). Adiponectin expression and secretion is increased by activators PPAR-γ (Stefan et al., 2002). Adiponectin has powerful anti-inflammatory and anti-atherosclerotic influence (Goldstein et al., 2004), including inhibition of expression of TNF-α that induces endothelial adhesion, transformation of macrophages to foam cells, TNF-α expression in macrophages and fat tissue and smooth muscle cell proliferation (Ouchi et al., 2003). Producing is lowered by insulin resistance, showing that hypoadiponectinemia level is more related to the level of insulin resistance and hyperinsulinemia than to the level obesity and glucose intolerance (Weyer et al., 2001). Two adiponectin receptors are identified – AdipoR1 and AdipoR2 (Yamauchi et al., 2003). A strong and consistent inverse relation is determined between adiponectin and insulin resistance and inflammatory conditions. The concentration of adiponectin is reduced during obesity and is increased during hunger. Further more several polymorphisms in the adiponectin gene are associated with obesity and insulin resistance (30). Mechanisms of influence of adiponectin (Diez et al., 2003, Chandran et al., 2003) in the liver - adiponectin improves insulin sensitivity, reduces the intake of non-esterified fatty acids, increases the oxidation of fatty acids and reduces the export of glucose from the liver. In muscles, adiponectin stimulates glucose utilization and fatty acid oxidation. In vascular walls adiponectin, inhibits monocyte adhesion by reducing the expression of adhesion molecules thus inhibiting macrophageal cells transformation into foam cells and reduced proliferation of smooth muscle cells migrating in response to growth hormone. Adiponectin increases synthesis of nitric oxides in endothelial cells and stimulates angiogenesis. These effects are mediated with increased phosphorylation of the insulin receptor, activation of AMP-activated protein kinase and modulation of core factor κB(Diez et al., 2003, Chandran et al., 2003). Considering the above effects it can be concluded that adiponectin is the only hormone secreted by adipocytes with anti-diabetes, anti-inflammatory and anti-atherogenic effects(Chandran et al., 2003) .

4.3. Interleukin-6

Interleukin - 6 (IL–6) is a cytokine associated with obesity and insulin resistance in adults and children (Yeste et al., 2007). Secretion of IL-6 is two to three times higher in visceral compared to subcutaneous fat. It circulates in blood in multiple glycosylated forms, sized from 22 to 27 kDa. The receptor for IL–6 in fatty tissue is homologous to the leptin receptor (Arslan et al., 2010).

Circulating level of IL-6 positively correlates obesity and impaired glucose tolerance and insulin resistance; it has been noticed that loss of body weight decreases levels of concentration of IL-6. Moreover, plasma concentration of IL-6 predicts the development of type 2 diabetes and cardiovascular disease. IL-6 inhibits adipogenesis and reduces the secretion of adiponectin. These effects of IL-6 confirm its role in the development of obesity and insulin resistance (Fernandez-Real et al., 2003).

4.4. Tumour necrosis factor alpha, TNF – α

The 26-kDa is a transmembrane protein that manifests its effects through type I and type II TNFα receptors. It is a cytokine originally described as endotoxin induced factor that causes necrosis of tumors, and later it was proved to be identical with cachexin, secreted factor by macrophages (Ruan et al., 2003). TNFα is secreted by adipocytes and stromal vascular cells. Although, initially it was suspected to play a role in cachexia, today it is known that it correlates positively with obesity and insulin resistance and inflammatory changes in vascular tissue

resulting in endothelial disfunction and the development of atherosclerosis and elevated blood pressure (Hotamisligil, 2003, Hotamisligil, et al., 1993)

4.5. Monocyte and macrophages chemoattractant protein (MCP) – 1

Obesity is associated with increased infiltration of macrophages in adipose tissue. Activated macrophages secrete mediators of inflammation, such as IL-6 and TNFα, which contribute to insulin resistance (Wellen et al., 2003, Weisberg et al., 2003, Xu et al., 2003). Adipose tissue secretes MCP-1, chemokine that attracts monocytes to the inflamed area (Wellen et al., 2003). Moreover, it is demonstrated on cultured, in vitro, cells that MCP-1 reduces glucose intake stimulated by insulin and tyrosine phosphorylation of the insulin receptor to induce insulin. Thus MCP-1 contributes to insulin resistance in adipose tissue (Sartipy et al., 2003).

4.6. Plasminogen activator inhibitor – 1, PAI-1

Adipose tissue secretes several proteins involved in the coagulation system or fibrinolysis, including tissue factor and plasminogen activator inhibitor-1, PAI-1 (Mertens et al., 2002). Secretion of PAI-1 is greater than visceral compared to subcutaneous adipose tissue (Fain et al., 2004). PAI-1 is an inhibitory member of the family of serine proteases and is the primary physiological inhibitor of fibrinolysis by deactivating urokinase and tissue plasminogen activator in blood and is known to contribute to thrombus formation and the development of acute and chronic cardiovascular diseases. Plasma levels of PAI-1 are regulated upon genetic basis and the accumulation of visceral fat is considered as a major regulator of PAI-1. Plasma level of PAI-1 are elevated in obesity and insulin resistance and positively correlate with the occurrence of metabolic syndrome and also they are predictors of risk of diabetes type -2 and cardiovascular disease (Mertens et al., 2002, Juhan-Vague et al., 2003). It is assumed that PAI-1 may not only be increased in response to obesity and insulin resistance, but may also have a direct role in the occurrence of obesity and insulin resistance (Sikaris, 2004). It is established that TNF-α contributes to elevated PAI-1 levels in obese people and people with insulin resistance (Fain et al., 2004). PAI-1 is also involved in other biological processes, including angiogenesis and atherogenesis (Kershaw et al., 2004).

4.7. Resistin

Resistin (insulin resistance) is a 12-kDa polypeptide (Sikaris, 2004) that belongs to the only protein family that is cysteine-rich residues of C-ends (Kershaw et al., 2004). It is considered that resistin has pro-diabetogenesis ability. Although evidence exists that (that)circulating levels of resistin are proportional to the degree of obesity (Sikaris, 2004) and the distribution of fat, the levels are not in proportion to the degree of insulin resistance (Steppan et al., 2004, Courten et al., 2004, Heilbronn et al., 2004).

4.8. Adipsine and Acylation stimulating protein

Adipsine (complement D), serine protease, is one of the complement components that are obtained in adipose tissue that is required for enzymic creation of protein that stimulates acylation (ASP). Adipsine and ASP, have an impact on the metabolism of lipids and carbohydrates (Cianflone et al., 2003). They positively correlate with obesity, insulin resistance, dyslipidemia and cardiovascular diseases (Cianflone et al., 2003)

4.9. Proteins of renin-angiotensin system (RAS)

Several proteins of the classical renin-angiotensin system are produced in adipose tissue, such as renin, angiotensinogen (AGT), angiotensin I, angiotensin II, angiotensin receptor type 1 (AT1) and type 2 (AT2), angiotensin-converting enzyme (ACE) and other proteases capable of producing angiotensin II. Plasma renin activity, plasma angiotensin-converting enzyme activity and adipose tissue angiotensinogen are positively correlated with obesity (Engeli et al., 2003, Goossens et al., 2003)

5. Role of the hormones of gastrointestinal tract in pathogenesis of obesity

Gastrointestinal tract function is not only as a conduit for food, but also is essential for digestion and absorption of nutrients. Visual, olfactory and taste signals stimulate exocrine and endocrine secretions and stomach motility even before food enters the mouth. Swallowing stimulates mechanoreceptors, resulting in a coordinated series of distension, digestion and absorption of nutrients (Ahima et al., 2008). When the stomach and duodenum are stretched during the food intake, strain causes transmitting signals through the nervus vagus, in order to block the centre for food intake and reduce the desire for food (Guyton et al, 2003).

Appetite is controlled by intestinal hormones also. The gastrointestinal tract is the largest endocrine organ in the body that secretes over 30 different regulatory peptide hormones (Table 2).

Content of imported food stimulates intestinal secretion of many of these intestinal hormones which interact with receptors located at various points in the "stomach – brain axis" and thus affects the short-term and long-term sense of hunger and satiety (Silva et al., 2012). Hormones that are secreted by the gastrointestinal tract and affect the appetite centre in the hypothalamus and satiety are the following.

Table 2
The Major Gut Hormones Involved in Appetite Regulation (Silva et al., 2012).

	Hormone	Site of secretion	Major receptors	Major actions
Anorectic	PYY	Gastrointestinal L cells	Y_2	Delays gastric emptying Vagal and CNS effects
	GLP-1	Gastrointestinal L cells	GLP-1	Glucose dependant insulin release Delays gastric emptying Vagal and CNS effects
	Oxyntomodulin	Gastrointestinal L cells	GLP-1/? other	Glucose dependant insulin release Delays gastric emptying Vagal and CNS effects
	Glucagon	Pancreatic α cells	Glucagon	Gluconeogenesis Glycogenolysis
	Cholecystokinin	Intestinal I cells	CCK 2	Gall bladder contraction Delays gastric emptying Pancreatic enzyme secretion
	Pancreatic polypeptide	Pancreatic PP cells	Y_4	Delays gastric emptying
	Amylin	Pancreatic β cells	AMY_{1-3}	Inhibits gastric secretion Delays gastric emptying Decreases blood glucose
Orexigenic	Ghrelin	Gastric fundal A cells	GHS-R	Increases gastric motility Growth hormone release

Ghrelin is the first known hormone that stimulates appetite (Tschop et al., 2000). Ghrelin is acylated 28-amino-acid peptide, which is mainly secreted by oxyntic stomach glands, circulates in the blood and activates NPY / AgRP neurons in arcuate nucleus (Inui, 2001). Gastrectomy resulted in 80% reduction in plasma levels of ghrelin, and the remaining 20% is excreted from the small intestine, pancreas, pituitary gland and colon (Hosoda et al., 2006). Ghrelin is included in short-term and long-term regulation of the appetite and the body weight. Circulating levels rise sharply before feeding and decrease after meals (Cummings et al., 2001).

In humans, ghrelin has a diurnal rhythm, which is identical with the daily rhythm of leptin, so that both hormones are elevated during the day to reach its peak at 13 pm, then their level decreases, so the minimum occurs around 21 h (Cummings et al., 2001). In adults, the level of ghrelin is inversely proportional to body mass index (Druce et al., 2006). The concentration of ghrelin is influenced by pubertal development. It causes positive energy balans, stimulates food intake and reduces energy consumption. Levels are reduced in obese individuals, with the exception of patients with Prader-Willi syndrome, and increased in anorexia nervosa (Otto et al., 2000), low-calorie food (Nakazato et al., 2001), in cachexia caused by malignant diseases (Wisse et al., 2001).

5.2. Peptide YY (PYY)

Peptide YY is a hormone that suppresses appetite. Peptide YY (PYY, peptide tyrosine tyrosine) belongs to the PP family that includes neuropeptide Y (NPY) and pancreatic polypeptide (PP). Peptide YY is built of 36 amino acids; it is secreted by L-cells of the gastrointestinal tract, with maximum concentrations in the terminal ileum, colon and rectum. Its name comes from its tyrosine (Y) residues in both the N and S ends. PYY is released after a meal and mediates postprandial satiety, reducing appetite and (lose) weight loss (Srivastava et al., 2007). Two endogenous forms, PYY1-36 and PYY3-36 are released postprandially into the circulation. After the meal, circulating levels of PYY3-36 are elevated within 15 minutes, the peak is around 90 minutes, and remains elevated up to 6 hours. The size of the elevated PYY3-36 is in proportion to calories (Silva et al., 2012). Obese people have lower basal levels of PYY and reduced postprandial levels but remain sensitive to the inhibitory effects on appetite after the exogene administration. Thus PYY 3-36 could represent candidate targets for therapy for obesity (Batterham et al., 2003).

5.3. Glucagon-like peptide – 1 (GLP-1)

Proglucagon is 160 amino acidic prohormone which is generated from α-cells of the pancreas and L-cells of the distal parts of the digestive tract and CNS. Selective post-translational proteolysis of proglucagon from prohormone convertase 1 and 2 results in specific tissue synthesis of many biologically active fragments. GLP-1 is released after eating, by L-cells of the stomach in proportion to the amount of food consumed and affects the pancreas to release insulin (Ghatei et al., 1983). GLP-, manifests its influence through G-protein bonded GLP-1 receptor located in the pancreatic islets where GLP-1 acts as an incretin hormone. It amplifies the postprandial excretion of insulin, inhibits glucagon secretion, and slows gastric emptying (Gutniak et al., 1992). Intravenous intake of GLP-1 inhibits food intake in healthy individuals, diabetics and obese individuals (Verdich et al., 2001). Secretion of GLP-1 is reduced in obese people and by reducing body mass, levels are normalized. Decreased secretion of GLP-1 may contribute to the pathogenesis of obesity (Verdich et al., 2001, Naslund et al., 2004)

5.4. Oxyntomodulin (Oxm)

Oxyntomodulin is a 37-amino acid peptide (Bataille et al., 1981), obtained by processing preproglucagon in the intestines and brain and is released after a meal in proportion to the amount of ingested food (Druce et al., 2006). OXM has a suppressive effect on appetite and GLP-1 but with a much weaker effect, at the same time, it inhibits gastric secretion of hydrochlorid acid and delays gastric emptying (Schjoldager et al., 1989).

5.5. Cholecystokinin (CCK)

Cholecystokinin is the first intestinal hormone that was discovered in the role of control of appetite (Gibbs et al., 1973). Plasma levels of CCK rise within 15 minutes after intaking the meal. It has a short plasma half-life - a few minutes. Its effect manifests through two subtypes of CCK receptors - CCK1 and CCK2 receptors, previously classified as CCK A and CCK B. CCK 1 and 2 receptors are widely distributed in the brain including the brain stem and hypothalamus. Appetite – suppressive effect of CCK appears to be partly mediated by CCK1 receptors on vagal nerve (Suzuki et al., 2012).

It is dominantly released in response to fats intake in duodenum and has a strong direct impact upon the centre for food intake reducing the further food intake (Guyton et al, 2003). Stimulates contractions of the gallbladder, pancreatic secretion and peristalsis of the intestines (Schjoldager et al., 1989), stimulates and delayed gastric emptying (Suzuki et al., 2012).

5.6. Pancreatic polypeptide (PP)

Pancreatic polypeptide is secreted from PP-cells in Langerhans islets as a response of food intake. It was/is built from a chain of 36 amino acids (Michel et al., 1998). PP has appetite-suppressive effect and its levels are lower in obese individuals (Adrian et al., 1976). Plasma levels of PP have diurnal variation, thus, the lowest levels are seen in the early morning and highest in the evening (Suzuki et al., 2012).Circulating PP levels are inversely proportional to obesity; higher values of PP are published for people with anorexia nervosa (Uhe et al., 1992). Some, but not all studies have shown a significant reduction in circulating levels of PP in obese individuals (Suzuki et al., 2012). Moreover, it is known that obese individuals with Rrader-Willi syndrome have a reduced release of PP in the basal and postprandial conditions (Zipf et al., 1983).

5.7. Obestatin

Obestatin is a 23 amino acid peptide hormone produced by posttranslational split of preproghrelin and is released from the stomach (Zhao et al., 2008). In rodents is found that, unlike ghrelin that stimulates appetite, obestatin suppresses the appetite by reducing food intake, delayed gastric emptying and decrease in body mass (Lacquaniti et al., 2011). However, the potential of appetite suppression remains controversial because other investigators have failed to confirm this effect (Gourcerol et al., 2007, Lacquaniti et al., 2011, Zhao et al., 2008)

5.8. Amilin

Amilin functions as a suppressive hormone of appetite. Circulating levels of amilin are higher for obese persons compared to skinny individuals (Reinehr et al., 2007, Reda et al., 2002). It lowers the food intake and lowers the body weight (Lenard et al., 2008).

6. Hormones secreted by the pancreas

6.1. Insulin

It is synthesized in the beta cells of the pancreas, it is secreted rapidly after feeding and has hypoglycemic effect (Suzuki et al., 2012). Insulin, together with leptin acts as an appetite suppressive signal in the arc shaped core (Suzuki et al., 2012), reducing food intake (Lenard et al., 2008). It participates in long-term regulation of energy balance (Suzuki et al., 2012). Circulating levels of insulin and leptin are positively correlated with body fat mass (Suzuki et al., 2012).

6.2. Glucagon

It is produced by the alpha cells of the pancreatic islets and increases the concentration of glucose in response to hypoglycemia. Glucagon enhances the physiological response of the body during stress by increasing energy consumption. It reduces food intake and body weight, but causes hyperglycemia (Suzuki et al., 2012).

7. Obesity treatment

7.1. Physical activity

Loss of body mass in many obese persons/people may be increased with the intensification of physical activity. More exercise means better daily energy consumption and quicker decrease of obesity (Guyton et al, 2003).

7.2. Drug treatment

Different medicines to reduce hunger are used in the treatment of obesity. Some of them are, Amphetamine-that directly inhibits eating centre in the brain; Sibutramine – it is a sympathicomimetic which reduces food intake and increases energy consumption (Guyton et al, 2003), but has several side effects such as tachycardia and hypertension (Druce et al., 2006), and that is why it was recently withdrawn from the market (Silva et al., 2012). Currently the only licensed pharmacological treatment for obesity is Orlistat, an inhibitor of intestinal lipase; it works by altering the lipid metabolism reducing the intestinal digestion of fats. This causes part of the input to lose fat in the faeces and also reduces the absorption of energy. However, the loss of fat through the faeces can cause unpleasant gastrointestinal side effects as (are) loss of liposoluble vitamins through faeces(Guyton et al, 2003).

The role of intestine hormones in appetite control is studied for over 30 years, with a clear demonstration that they have a role in mediating the postprandial satiety. Appetite suppressive intestinal hormones, such as PYY and GLP-1 play an important role in reducing the food intake, but still do not have application (Schwartz et al., 2000).

7.3. Surgical treatment of obesity

Three surgical procedures are in use at the moment, gastric restriction, gastric bypass and biliopancreatic diversion (Druce et al., 2006). Bariatric surgeries are procedures that are based on malabsorption, include Jejunoileal bypass, resulting in reduced absorption of nutrients by shortening the length of the functioning large intestine and allowing nutrients to pass directly from the proximal jejunum to the terminal ileum. Roux-en-Y gastric bypass (RYGB) is a combined restrictive and malabsorption procedure that provides long-term weight loss with an acceptable level of risk. RYGB achieves its beneficial effects through BRAVE effects, change in bile flow, reducing the size of the stomach, anatomical rearrangement of the stomach, changes the flow

of nutrients, vagal manipulation and subsequent modulation of enteric gastrointestinal hormones (Suzuki et al., 2012).

7.4. Stomach microflora

The potential link between stomach microflora and pathogenesis of obesity has been recently discovered. The stomach contains 1000-1150 bacterial species called stomach microflora. In a randomized, double-blind, parallel, placebo-controlled study to evaluate the effect of probiotics on plasma levels of intestine hormones, 10 healthy subjects received either 16 g probiotics /a day or 16 g maltose dextrin /a day for 2 weeks. On the people treated with probiotics, increased gastric microflora was noticed, increased fermentation, decreased appetite, improved postprandial response to glucose and increased plasma levels of GLP-1 and PYY. Adjustment to probiotics led to certain modulations of gastric mikroflora. Studies suggest (suggest) that gastric microflora may be associated with development of obesity and probiotics are the new treatments for obesity. These observations may help to develop new pharmacological strategy for patients with overweight (Suzuki et al., 2012).

8. Conclusion

Overweight as a phenomenon has a growing trend in modern society, especially in developed countries. Body mass index, although it is not an ideal parameter, nevertheless is accepted as an indicator of obesity. The control of obesity involves large number of hormones, proteins, adipokines, cytokines and other substances suppressing the appetite, in contrast, the number of appetite stimulators is reduced to one - ghrelin. It is important to have a practical approach to the investigation and treatment of patients with obesity because they are at greater risk of morbidity and mortality. The need to treat excessively obese patients in recognized centres will increase the need to cooperate with academic centres with expertise and experience in this field, to make laboratory research more accessible to patients in need. Treatment for excessive obese patients is becoming more sophisticated and requires the development of new biochemical and molecular genetic diagnostics.

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АјДи Дизајн 2012/ДООЕЛ Скопје
Македонско медицинско електронско списание
Волумен 2015; Статија ИД 50010, 14 страници
http://dx.doi.org/10.3889/mmej.2015.50010
eISSN: 1857-9809
Ревиски труд

Современи погледи на хомеостазата на железо со основен акцент на хепцидинот – новиот хормон, регулатор на метаболизмот на железо

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Цитирање: Илковска Б, Котевска Б, Трифунов Ѓ. Современи погледи на хомеостазата на железо со основен акцент на хепцидинот – новиот хормон, регулатор на метаболизмот на железо. Макед Мед Електр С. 2015 Авг 12; 2015; 50010:14. http://dx.doi.org/10.3889/mmej.2015.50010

Клучни зборови: железо; феритин; трансферин; хепцидин.

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Примено: 07-Јул-2015; **Ревидирано** 30-Јул-2015; **Прифатено:** 31-Јул-2015; **Објавено:** 12-Авг-2015

Конкурентски интереси: Авторите изјавуваат дека немаат конкурентски интереси.

Извадок

Железото е есенцијален елемент за скоро сите живи организми. Тој е клучен функционален дел на кислородните транспортери, депонирачките молекули и многу ензими кои ја катализираат редокс реакцијата неопходна за генерирање на енергија, продукти на различни метаболички интермедиери и за одбрана. Истражувањата покажаа дека клучен регулатор во хомеостазата на железото е хепцидинот и го поставија црниот дроб за централен орган во системската хомеостаза на железото. Хепцидинот е катјонски пептид составен од 25 амино киселини и 4 дисулфидни врски. Неодамна беше откриено дека циркулирачкиот хепцидин со релативно висок афинитет е врзан за α2-макроглобулин и со релативно низок афинитет со албуминот. Во прилог на својата улога во регулирањето на системскиот метаболизам на железо, хепцидинот може да придонесе за одбраната на домаќинот. Хепцидинот првично беше идентификуван како антимикробен пептид и беше откриено дека може индиректно да придонесе за одбраната на домаќинот преку намалување на концентрацијата на железо во плазмата.

Contemporary Views of Iron Homeostasis with Main Focus of Hepcidin - New Hormone Regulator of Iron Metabolism

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Citation: Ilkovska B, Kotevska B, Trifunov G. [Contemporary Views of Iron Homeostasis with Main Focus of Hepcidin - New Hormone Regulator of Iron Metabolism]. Maced Med Electr J. 2015 Aug 12; 2015;50010:14. [Macedonian] http://dx.doi.org/10.3889/seejim.2015.50010

Key words: iron; ferritin; transferrin; hepcidin.

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Received: 07-Jul-2015; **Revised:** 30-Jul-2015; **Accepted:** 31-Jul-2015; **Published:** 12-Aug-2015

Competing Interests: The authors have declared that no competing interests exist.

Abstract

Iron is an essential element of almost all living organisms. It is key functional part of oxygen transporters, depositary molecules and many enzymes which catalyze redox reactions necessary to generate the energy, products of various metabolic intermediates and defense. Studies have shown that a key regulator of iron homeostasis is hepcidin and set the liver as the central authority in the system of iron homeostasis. Hepcidin is cationic peptide composed of 25 amino acids and four disulfide bonds. Recently it was revealed that circulating hepcidin with a relatively high affinity is bound to α2-macroglobulin and with relatively low affinity is bound to albumin. In addition to its role in regulation systemic metabolism of iron, hepcidinot can contribute to host defense. Hepcidinot was originally identified as an antimicrobial peptide and found that it could indirectly contribute to host defense by reducing the concentration of iron in plasma.

Зовед

Микроелементите се хемиски елементи, коишто се содржат во организмите во илјадити делови од процентот (железо, бакар, цинк, молибден, бром, флуор, јод и др). Тие се неопходни за нормалната животна функција. Влегуваат во составот на ензими, витамини и хормони. Влијаат врз растот, размножувањето и образувањето на крвта. Нивниот недостаток или вишок води до нарушен метаболизам на материите.

Железото е есенцијален елемент за скоро сите живи организми [1]. Тој е клучен функционален дел на кислородните транспортери и депонирачките молекули (пр. хемоглобин, миоглобин) и многу ензими кои ја катализираат редуктивната реакција неопходна за создавање на енергија (пр. цитохромите), продукти на различни метаболички интермедиери и за одбрана (никотин амид динуклеотин фосфат оксидаза [NADPH]) [2].

Историја на железо

Од античко време, човекот ја препознал посебната улога на железото во здравјето и болестите [3]. Железото имало рана употреба во медицината на Египјаните, Хундите, Грците и Романците [4, 5].

За време на 17^{ти} век, железото се користело за лекување на хлороза (зелена болест), состојба која настанувала од недостаток на железо [6]. Веќе во 1930 год. McCance и Widdowson ја предвидуваат цревната апсорпција на железо со одземање на концентрацијата на железо од изметот и урината од внесената концентрација на железо преку храната. Тие истакнале дека апсорпцијата на железо е зголемена кај лица со недостаток на железо.

Hahn и Whipple ја анализирале кинетиката на цревната радиоактивно обележена железна апсорпција и користењето на човечки и анимални модели и потврдиле дека апсорпцијата е регулирана и нема значајно излачување на железо. Во 1932 год. беше откриена важноста на железото со убедливи докази дека неорганското железо е потребно за синтеза на хемоглобинот [7, 8]. Во 1950 год. Finch и Saylor докажуваат дека апсорпцијата на железо се стимулира од зголемана еритропоетинска активност, а се потиснува од хипертрансфузија.

Рециклирање на хемоглобин (од оштетени еритроцити обележани со радиоактивно железо) во железо беше измерено од Noyes, Bothwell и Finch. Тие открија дека повеќе железо се ослободува од ретикулоендотелниот систем кај пациенти или експерименталните модели животни кои имаат недостаток на железо, што покажува дека ослободувањето на железо од макрофагите е регулирано од резервите на железо.

Freireich, Wintrobe, Cartright, Finch и други покажаа дека воспалението поттикнува секвестрација на железо во макрофагите од хепар и слезина (ретикуло ендотелен систем) и го инхибираат снабдувањето со железо на еритропоезата, предизвикувајќи анемија. Beutler и сор. очекуваа во 1960 год. дека хуморални супстанции учествуваат во апсорпцијата на железо според потребите од железо на еритропоезата но Krantz и сор. докажаа дека оваа супстанција не е еритропоетин.

Во 1960 год. Manis, Schachter, Wheby и сор. покажаа во изолирани цревни петелки дека апсорпцијата на железо се одвива во проксималниот дел од дванаесетпалачно црево и се регулира во 2 етапи: навлегување на железо во ентероцитите (мукозно навлегување), потоа следи складирање на железо во форма на феритин во цитоплазмата или ослободување на железо во циркулацијата (мукозен трансфер). Бидејќи животниот век на еритроцитите е неколку дена, судбината на железото од исхраната кое е преземено од ентероцитите ќе биде утврдена од страна на базолатералниот транспорт на железо: или апсорпцијата на железо ќе дозволи влез во крвотокот или ќе биде вратено во цревниот лумен со смрт на ентероцитите и исфрлање преку изметот.

Во 1970 год. истражувачите ја преиспитале патогенезата на наследната хемохроматоза (НН), синдром при кој вишокот на железо се натрупува во црниот дроб и други ткива, резултирајќи со оштетување на ткивата, нарушена функција на органите и црнодробна карциногенеза [9]. Во 1990 год. имаше ренесанса на патологијата на железо. Испитувањата на пациенти и животински модели со генетски нарушувања на железо доведе до идентификување на гени кои се одговорни за транспортот на железо, оксидоредуктази поврзани со транспортот и железо регулаторни молекули [10].

Историја на феритин

Феритинот беше откриен во 1937 год. од францускиот научник Laufberger, кој изолира нов протеин од слезинка на коњ, која содржела околу 23% од сувата тежина, железо [11]. Присуството на феритин во човечки серум е документирано неколку години подоцна [12].

Во 1972 год. со користење на имунорадиометриски методи, Addison и сор.

уверливо демонстрираа дека феритинот може сигурно да се открие во човечки серум [13]. За да се открие поврзаноста помеѓу серумските нивоа на феритин и тоталните резерви на железо во организмот, авторите ја измериле концентрацијата на феритин во серумот кај здрави лица, кај пациенти со недостаток на железо и кај пациенти со вишок на железо. Тие докажале дека серумскиот феритин е покачен кај пациенти со вишок на железо и намален кај пациенти кои имаат недостаток на железо [14]

Во 1975 год. Jacobs и Worwood сугерираат дека методата на определување на феритин во серумот може да обезбеди "корисна и конвенционална" метода за определување на статусот на резервите на железо [15].

Историја на хепцидин

За прв пат за поврзаноста помеѓу хепцидинот и метаболизмот на железо говорат Pigeon и сор. во студијата за одговорот на црниот дроб на оптеретувањето со железо. Тие откриле mRNA на глувчешки хепцидин со субтракциска хибридизација при преоптеретување со железо наспроти нормалните глувци и покажаа дека mRNA е доминантно експресирана во хепатоцитите.

Во 2001, обсервациите на зголемена експресија на хепцидин во одговор на вишок на железо во храната водат до хипотезата дека хепцидинот може да делува како централен регулатор на хомеостазата за железо [16]. За време на истражувањето на антимикробните својства на различни човечки течности, Park и сор. изолирале нов пептид богат со цистеин од човечка урина и го именувале како хепцидин заради неговото црнодробно потекло и антибактериските ефекти (името произлегува од неговата синтеза (во црн дроб, hep-) и антимикробните својства in vitro (-cidin)) [17].

Независно, Krause и сор. трагајќи за пептид богат со цистеин со антибактериски карактеристики, во 2000 год. изолирале ист пептид како Park и сор. од ултрафилтрат на плазма и го именувале како антибактериски пептид кој се синтетизира во црн дроб (анг. liver expressed antibmicrobial peptide-1, LEAP-1) [18].

Дневни потреби на железо

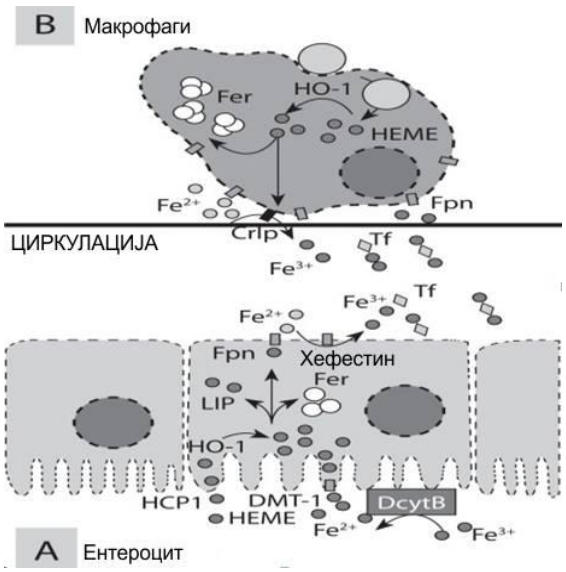
Возрастен организам, просечно има 3-5 g железо (~ 45 mg/кг жена, ~ 55 mg/kg за мажи). Поголем дел од железото во организмот е вградено во хемоглобинот на циркулирачките еритроцити (60-70%). Околу 20 - 30% железо е форма на феритин и хемосидерин во хепатоцитите и РЕС макрофагите, како резервно железо. Додека возрастен маж има 0.5-0.2 g складирано железо, децата, адолесцентите и жените во репродуктивниот возраст речиси немаат резерви на железо. Една мала количина, од преостанато железо во телото, е во форма на миоглобин во мускулите или инкорпорирано во ензими [19]. Околу 1–2 mg од железото се губи секој ден преку: кожата, со десквамација на цревата и минимални крвозагуби [20].

Дистрибуција на железо

Апсорпција на железо во тенкото црево

Железото во храната е присутно во две форми – како неорганско или нехем железо и хем железо. Неорганското железо е доминантно во секојдневната исхрана (~90%), додека пак органското (~10%) од вкупната количина на железо во нашата исхрана. Хем железото потекнува од хемоглобинот, миоглобинот и други хем протеини во храната од животинско потекло.

Апсорпцијата на железо се одвива во дуоденум и горните делови на јејунум. Процесот вклучува протеини кои го транспортираат железото низ апикалната мембрана (импортери), протеини кои го транспортираат низ базолатералната мембрана (експортери) и протеини кои ја менуваат неговата редуктивна состојбата, па се вклучени во неговиот транспорт [21] (Слика 1).



Слика 1: Апсорпција и транспорт на железото низ ентероцитите: фериредуктаза; транспортер за двовалентен метал-1 (DMT-1); протеин за транспорт на хем-1 (HCP1); хем оксигеназа (HO-1); изнесувач на железо - феропортин; хефестин; рецептор за трансферин -1 (TfR1) [22]

Апсорпција на неорганско железо

Најголем дел од нехем железото од храната пристигнува до трепчестиот епител во фери форма (Fe^{3+}). Во дуоденум под дејство на фериредуктаза, дуоденален цитохром B (DcytB) ензим, железото се редуцира во феро форма (Fe^{2+}). Аскорбатот (витамин Ц), е коензим на ензимите вклучени во редукцијата на фери форма (Fe^{3+}) во феро (Fe^{2+}) [23].

Железото се транспортира низ апикалната мембрана во цитоплазмата на дуоденалните ентероцити со посредство на транспортерот за двовалентен метал-1 (Divalent metal transporter-1 DMT1) (слика 1-А). DMT1 не е специфичен само за транспорт на железо, тој исто така транспортира и други двовалентни метали вклучувајќи цинк, магнезиум и бакар [1, 24]. Овој транспортен протеин се наоѓа и на мембраната на ендозомите каде што посредува при транспортот на железо од ендозомите во цитоплазмата за време на трансферинскиот циклус [25]. DMT-1 се чини дека има важна улога во транспортот на железото кое не е врзано за трансферинот (NTBI), особено при вишок на железо [26].

Некои истражувања покажале дека транспортот на железо низ дуоденумот се врши со посебен, досега недоволно откриен пат. Додека феро железото користи DMT-1, фери железото користи интегрин-мобилферински пат (IMT) кој транспортира исклучиво фери железо [27]. Овој пат вклучува неколку протеини: мобилферин, бета-3-интегрин и флавин-монооксигеназа. Флавин-монооксигеназата има улога на фериредуктаза. Во цитоплазмата на клетката овие протеини се интегрирани во голем протеински комплекс наречен параферитин [28]. Western Blott анализа на параферитин покажа дека тој содржи и бета-2-микроглобулин и DMT-1. Присуството на DMT-1, мобилферитин и хефестин во цитоплазмата на клетките укажува на можната интраклеточна функција на овие протеини (29) [29].

Апсорпција на хем железо

На култивирани клетки, експериментално е покажано дека интактниот хем, низ апикалната мембрана на ентероцитите се апсорбира со протеински носач на хем-1 (Heme-carier protein 1, HCP1) (слика 1-А). HCP1 е транспортен протеин кој во големи количини е присутен во дванаесет-палачно црево [30, 31]. Последните истражувања посочуваат дека овој протеин можеби има улога на транспортер и на фолати [32].

Интраклеточен транспорт на железо

Дел од железото, внесено во интестинал-ните епителни клетки преку апикалната мембрана се вградува во феритинот или се транспортира циркулацијата минувајќи низ базолателарната мембрана [33].

Железото задржано во феритинот се губи со десквамација на старите мукозни клетки – процес кој се одвива на 3 - 4 дена, колку што е животниот век на мукозните клетки. Другиот дел од железото, преку базолатералната мембрана со помош на феропортинот (експортер), се транспортира во циркулацијата. Според тоа феропортинот, одредува дали железото ќе се испорача во циркулација или ќе се отстрани од организмот со десквамација [21].

Транспортот на железото низ цитоплазмата на ентероцитите е сеуште неразјаснет дел од процесот на апсорпција на железото; се смета дека постојат два механизми: транспорт потпомогнат со некои протеини, шаперони или со трансцитоза [34].

Феропортинот е единствен, сега за сега, познат експортер на железото [35] (слика 1-А). Феропортинот се среќава во сите ткива кои изнесуваат железо во плазмата: базолатералната мембрана на ентероцитите во дуоденумот, мембраните на ретикулоендотелните макрофаги (вклучени во складирање и рециклирање на железото), хепатоцитите, клетките на плацентата [36].

Слично како кај апикалната апсорпција на железо, базолатералното изнесување на железото е потпомогнато од ензим, кој ја менува оксидативната состојба на железото. Во овој случај, феро (Fe^{2+}) железото, кое ја напушта клетката мора да биде оксидирано во фери форма (Fe^{3+}) за да се врзе со трансферинот. Оваа оксидација, во дуоденумот, ја врши хефестинот, а кај сите останати клетки во организмот, церулоплазминот (слика 1-А) [37].

Транспорт на железо во плазмата

Во плазмата, железото се транспортира со трансферинот. Се смета дека околу 20 mg железо, секојдневно се пренесува со трансферинот [38]. Трансферинот е железо - врзувачки гликопротеин, со молекулска тежина од приближно 80 kDa [39]. Генот за трансферин е лоциран на хромозом 3q21 блиску до гените за лактоферин и церулоплазмин. Трансферинот се синтетизира во хепатоцитите [40]. Само фери формата (Fe^{3+}) има способност да се врзе со трансферинот. Трансферинот е завиткан во форма на два глобуларни домени; секој од домените содржи по едно специфично место за врзување на еден атом на тровалентно железо (Fe^{3+}). При физиолошки pH, трансферинот има многу висок афинитет за железото, па скоро целото нехем железо во циркулацијата е врзано за трансферинот. Утврдено е дека со намалување на pH, се намалува и афинитетот на железото за

трансферинот, така да при pH под 4.5 не можеле да се детектираат мерливи врзани количини на железо за трансферинот [41].

Трансферинот го испорачува железото во клетките со процес на ендоцитоза посредувана со трансферинот во т.н. трансферински циклус. При физиолошки услови, овој циклус овозможува контролиран пристап на железото во клетките бидејќи поедините клетките ефикасно можат да го регулираат влезот на железото преку регулација на експресијата на трансферински рецептор-1 на површината, врз основа на нивните потреби за железото [21].

До денес, се опишани два типа на функционални различни рецептори за трансферин: трансферин рецептор 1 и 2 (TfR1 и TfR2). Најдено е дека TfR1 се наоѓа на површината на сите клетки кои имаат потреба од железото, но нивото на неговата експресија се разликува во огромна мерка од типот на клетките [42].

TfR1 претставува трансмембрански гликопротеин, со молелуларна маса ~180 kDa, изграден од две идентични субединици поврзани со дисулфиден мост. Секоја субединица има по едно место за врзување на трансферин [21].

Експериментално на култура од клетки е утврдено дека TfR2, воглавно е присутен во црн дроб, хематопоетски клетки и дуоденалните криптогени клетки [43].

Депоа на железо во организмот

Железото се складира во црниот дроб во хепатоцитите и Купферовите клетки во две форми: феритин и хемосидерин [21].

Во клетките, феритинот, има двојна улога - како депо за железото и како молекула за детосикација на клетките од вишокот на железо и на тој начин ги штити од тосичното дејство на вишокот на железо [44].

Феритинот е изграден од 24 субединици, организирани така да има изглед на шуплива школка, во која се складираат приближно 4500 Fe^{3+} атоми како неорганski комплекс. Феритинот изолиран од рбетниците е изграден од два типа на субединици: H (heavy - тешки или heart – срце) и L (light – лесни или liver – црн дроб). Количинскиот сооднос меѓу двата типа на субединици се разликува од ткиво до ткиво. Вградувањето на железото во феритинот, бара фероксидативна активност која се препишува на H-субединиците, додека пак L-субединиците учествуваат во минерализацијата [45].

Железото, депонирано во феритинот, може да се користи кога ќе се намали неговото ниво во клетката. Сеуште не е докрај разјаснет механизмот според кој железото се ослободува од феритинот [46].

Иако, главно феритинот се наоѓа во цитоплазмата на клетката, мала фракција е најдена и во јадрото на некои клетки. Се смета дека феритинот во јадрото го испорачува железото потребно за ензимите кои зависат од присуството на овој метал или за факторите за транскрипција [47].

Најновите истражувања покажале дека феритинот е присутен и во митохондриите (MtF). Митохондриите се органели кои имаат висок обрт на железото потребен за биосинтеза на хем и ензими кои содржат Fe-S групи. И митохондријалниот недостаток на железо и вишокот на железо ја нарушуваат метаболната и респираторната активност на митохондриите, затоа хомеостазата на железо во овие органели мора да биде строго контролирана. Се претпоставува дека феритинот има важна улога во депонирањето на железо, заштитувајќи ги митохондриите од оксидативен стрес [48].

MtF се наоѓа во исклучително ниски количини во повеќето клетки. Студиите покажале дека зголеменото присуство на MtF значително влијае врз интрацелуларната хомеостаза на железо и води до брза прераспределба на железо од цитоплазмата во митохондриите, каде што се депонира во форма која не е достапна за метаболичка употреба [49, 50].

Друга форма на складирано железо во клетката е хемосидерин, нерастворлив деградационен производ од нецелосна деградација на феритинот во лизозомите. При преоптоварување со железо, хемосидеринот станува протеин во кој доминантно се складира железо. Во физиолошки услови хемосидеринот не е ефективен донатор на железо, но има заштитна улога. При воспаление и хипоксија тој може да стане донатор на железо и да придонесе за создавање на слободни радикали и оштетување на ткивата и клетките кои се преоптеретени со железо [51].

Регулација на системската хомеостаза на железо

Коскената срцевина е основниот потрошувач на железото од циркулацијата и најголем дел од дневните потреби за железо се користат за синтеза на хемоглобинот во 200 милјарди нови еритроцити. За рамнотежа, макрофагите рециклираат 10–20 пати повеќе железо од железото кое се апсорбира во цревата (кое се користи за задоволување на дневните потреби од железо). Макрофагите од RES во слезината и другите органи ги фагоцитираат и лизираат старите и оштетени еритроцити. Хем оксигеназата (HO-1) го разградува хемот, се ослободува железото од протопорфиринот и со

помош на феропортин се враќа во плазмата и се врзува за трансферинот (Слика 1-Б) [52, 53].

Дневно, човековиот организам губи околу 1-2 mg железо и исто толку количество на железо се ресорбира во тенкото црево, за да се обезбеди доволно, но не и премногу железо, за да бидат полни резервите на железо. Според тоа, системската хомеостаза на железо ја регулира цревната апсорпција на железото, неговото навлегување и мобилизирање од депоата се со цел да се задоволат потребите на еритропоезата. Таа, исто така обезбедува стабилна средина каде што секоја клетка го регулира навлегувањето на железото во зависност од сопствените потреби.

Истражувањата покажаа дека клучен регулатор во хомеостазата на железото е хепцидинот. и го поставија црниот дроб за централен орган во системската хомеостаза на железото [54, 55].

Хепцидин

Хепцидинот е негативен хормонски регулатор на метаболизмот на железо. Генот за хепцидин (HAMP; OMIM 606464), е лоциран на хромозом 19q13.1 [56].

Човечкиот хепцидин доминантно се синтетизира во црниот дроб како 25 амино киселински пептид (2789.4 Da) кој се секретира во циркулацијата [16, 18]. Хепцидинот се синтетизира како пре-пропептид составен од 84 амино киселини. Со ензимско каталитичко отцепување, пре-пропетидот поминува во прохепцидин изграден од 64 амино киселини. Зрелиот, активен, 25-аминокиселински хормон хепцидин, најверојатно се добива со отцепување на прорегионот (н =39 аминокиселини) под дејство на пропротеин конвертазата, фурин [57].

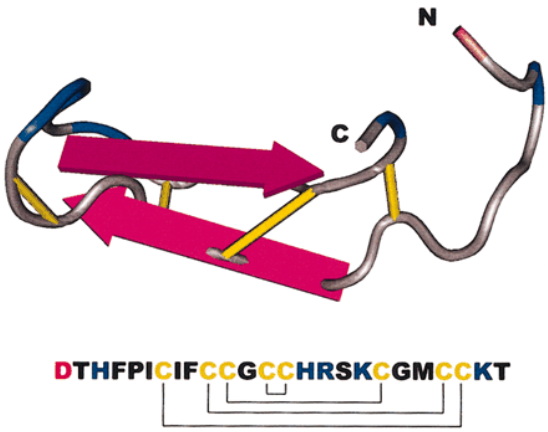
Во плазмата, исто така, циркулира и како 20 и 22-аминокиселински пептид и како про-хепцидин кој нема хормонско дејство [58].

Последните студии укажуваат на експресија на хепцидин и во други клетки, покрај хепатоцитите, иако во многу мали количини. Тие вклучуваат: срце, ретина, моноцити, неутрофили, масни клетки, алвеоли, панкреасни клетки и миокардни клетки [59-65]. Синтезата на хепцидин од овие клетки, неможе да направи сигнификантно значење во концентрацијата на системската циркулација, но може да има локален ефект во тие ткива. Преку автокрина интеракција со феропортинот, хепцидинот локално може да ги заштити околните клетки од недостаток на железо, превенира екстрацелуларен оксидативен стрес, влијае на инфламаторниот одговор и/или ги осиромашува екстраклеточните резарви на желе... кои се достапни за екстрацелуларните патогени [65-68].

Структура на хепцидинот

Со нуклеарно магнетна резонансна спектроскопија е утврдено дека хепцидинот има структура која личи на шнола, стабилизирана со 4 дисулфидни мостови [69].

Хепцидинот е катјонски пептид составен од 25 амино киселини и 4 дисулфидни врски. Hunter и сор., ја анализирале синтетската форма со нуклеарно магнетна резонантна спектрометрија и ги потврдија неговите врски и структура (слика 2).



Слика 2: Амино киселински секвенции во модел на човечки хепцидин. Амино и карбокси краевите се именувани N и C. Дисулфидните врски се во жолто, базните амино киселини се во сино и киселите амино киселини се во црвено. Моделот на дисулфидните врски помеѓу 8-те цистеина е покажан на аминокиселинските секвенци [79]

Тесно поврзани гени за хепцидин, исто така, се пронајдени кај стаорци и неколку видови на риби [17] (слика 3). Глувчешкиот геном содржи два гена за хепцидин, но се смета дека само еден хепцидин има улога во метаболизмот на железото [70].

Слика 3: Секвенци на хепцидин кај вертебрати: човек (hHEP), прасе (pHEP), зајак (rHEP), глушец (mHEP) и куче (dHEP). Хепцид кај риба зера (zHep) [71]

Анализа на секвенците на хепцидин (DTHFPICIFCCGCCHRSKCGMCCKT) откриваат

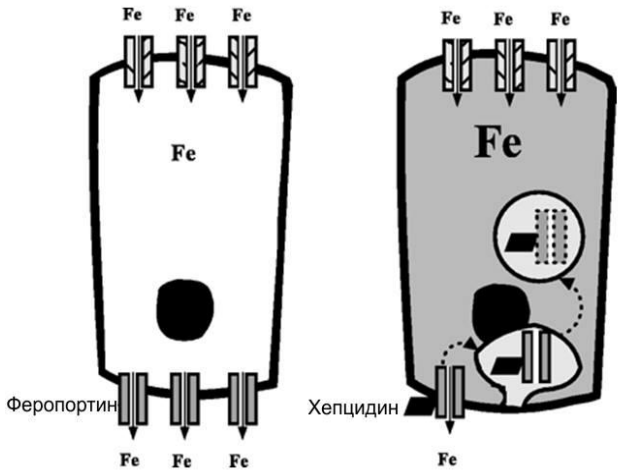
голем процент на цистеин – 8 цистеина [69]. Ова е необично висока содржина на цистеин, кога се споредува со други антимикробни пептиди богати со цистеин, слично на дефензин [72], тахиплезин [73], протегрин [74] и снакин [75]. Со масена спектроскопија и хемиска анализа е откриено дека сите цистеини се поврзани во секвенци, со што овој пептид е мошне ограничени пептид [76].

На кружна спектрометрија со дихроизам на човечки уринарен хепцидин се покажа дека тој е богат со β-вериги и NMR потврди дека хепцидинот претставува едноставна цефка стабилизирана со три дисулфидни врски и соседна дисулфидна врска на кривината [69].

Функција на хепцидинот

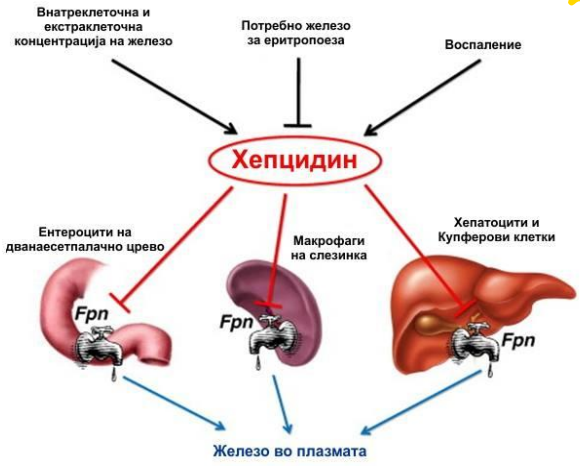
Хепцидин-25 се претпоставува дека е главен регулатор на апсорпцијата на железо со храната и клеточното ослободување на железо. Хепцидинот игра клучна улога во хомеостазата на железото. Тој го регулира депонирањето и преземањето на железото преку врзување за феропортинот-експортер на железото. Хепцидинот предизвикува внесување на феропортинот во клетката каде што тој, феропортинот, се разградува и на тој начин го спречува излегувањето на железото од клетките во кој е депонирано [36].

Преку овој механизам, хепицидинот ја инхибира: (1) апсорпција на железото во тенкото црево (слика 2), (2) отпуштањето на железото од неговото депо во хепатоцитите и (3) отпуштањето на железото од макрофагите вклучени во рециклирање на остарените клетки [57].



Слика 4: Хепцидинот ја регулира синтезата на феропортин на базолатерална мембрана на ентероцитите

Преку сите горенаведени дејствија, хепцидинот го контролира нивото на железото во крвта (слика 4).



Слика 5: Хепцидинот има централна улога во одржување на хомеостазата на железо

Во прилог на својата улога во регулирањето на системскиот метаболизам на железо, хепцидинот може да придонесе за одбраната на домаќинот. Хепцидинот првично беше идентификуван како антимикробен пептид [17, 18]. Иако in vitro студиите укажуваат бактерициден ефекти на хепцидин, овие ефекти бараат концентрации повисоки од оние забележани во циркулацијата. Таквите концентрации може да се постигне на локално ниво, на пример, во фагозоми на инфицираните макрофаги [77].

Хепцидинот исто така, може индиректно да придонесе за одбраната на домаќинот преку намалување на концентрацијата на железо во плазмата. Железото е неопходно за микробен раст, исто така намалување на железото во плазмата делува бактериостатски. Покрај тоа, беше пронајден хепцидин за моделирање на липополсахаридно индуцирана транскрипција и во култивирани макрофаги и ин виво во глувчешки модел. Последново набљудување укажува на улогата на хепцидин во модулирање на акутен воспалителен одговор на бактериска инфекција [78].

Регулација на синтезата на хепцидин

Синтезата на хепцидин е регулирана од физиолошки и патолошки процеси. Концентрацијата на хепцидин се намалува во ситуации кои бараат зголемена концентрација на циркулирачко железо. Синтеза на генот за хепцидин е регулиран на транскрипциско ниво [79].

До денес се откриени два главни патишта од клеточна сигнализација кои се вклучени во транскрипцијата на хепцидин.

1. Првиот пат е поврзан со активација на цитоплазматскиот протеин за транскрипција Stat3

(сигнален трансдјусер и активатор на транскрипција). После активирање, овој протеин се пренесува во јадрото каде што активира транскрипција на генот за хепцидин, врзувајќи се со адекватен сегмент на DNA [80].

2. Втор механизам на контролата на транскрипција на хепцидин (сигнален пат зависен од BMP/Smad) вклучува Smad протеини (нивното име е комбинација од името на два хомологни протеини Sma и MAD) и коскен морфоген протеин (BMPs). BMPs се плеотропни сигнални молекули од фамилијата на растечки фактори [80]. BMP - сигнализирачки пат се иницијализира по врзување на BMP со BMP рецепторскиот комплекс на површината на клетката, кој ја активира рецепторната киназа за да ги фосфорилира цитоплазматските протеини SMAD1, SMAD5 и SMAD8. Овие фосфорилирани, рецепторски-регулирани SMADs, формираат комплекс со SMAD4, кој се состои од рецепторски-регулиран SMADs и SMAD4, кои се пренесуваат во јадрото и индуцираат транскрипција на целниот ген како хепцидин [81, 82].

Во регулација на хепцидинот, се претпоставува дека се вклучени најмалку 4 одделни и различни патишта:

1) регулација со статусот на железото, железото од храната и депонираното железо;

2) регулација со процес на воспаление;

3) регулација со хипоксија/аноксија; и

4) регулација со еритроидните фактори [83,84].

Регулација со статусот на железото (четири хипотези слични, но различни)

Во случај на зголемена еритропоеза, како на пример во одговор на недостаток на железо, намалена концентрација на хепцидин ќе резултира со ослободување на депонирано железо и со зголемана апсорпција на железо од храната [85-88].

Резервите на железо во црниот дроб и циркулирачкиот трансферин врзан за железо (Tf-Fe2) даваат различни сигнали за синтеза на хепцидин од хепатоцитите на глувчешки модели [89-91]. Циркулаторниот трансферин се чини дека е во хепатоцелуларен комплекс, кој вклучува трансферински рецептор-1 (TfR1) трансферински рецептор-2 (TfR2) и хемохроматозен железен протеин (HFE). Дефекти во TfR2 и HFE водат до намалена концентрација на хепцидин со екстраклеточна сигнално-регулаторна киназа: митогенски активиран протеин киназен пат (ERK/MAPK) и/или коскен морфоген протеин/против мајчин декапентаплегичен хомологен пат (Drosophila) (BMP/SMAD). Интраклеточните резерви на железо преку BM... се во комуникација со хепцидинот, на паракрин или автокрин начин. Овие екстраклеточни сигнални молекули делуваат на хепатоцелуларниот BMP рецептор за активација на интраклеточниот SMAD сигнален пат и ја зголемуваат транскрипцијата на хепцидин. Хемојувалинот (HJV) и BMP корецепторот [92], се основни за синтеза на хепцидин бидејќи различни хепцидин регулаторни патишта се во конверзија со протеините врзани за мембраната. Во случаи на ниско железо, мембрански врзаниот HJV се расцепува од матриптаза-2, трансмембрански протеазен серин 6 [93, 94]. Ова одвојување од матриптаза-2 ја ослабнува BMP сигнализацијата.

Регулацијата на хепцидин при оптеретување со железо е посредувана од коскен морфогенетски протеински (BMP) рецепторен комплекс врз површината на хепатоцитите [92, 95, 96]. Овој комплекс се состои од два протеини: HFE и хемојувалин. Иако точните молекуларни механизми се уште не се целосно разјаснети, овој коскен морфогенетски протеински (BMP) рецепторен – комплекс, се поврзува со трансферински рецептори 1 и 2, најверојатно поврзувајќи ја чувствителноста за серумското железо со синтезата на хепцидин [97, 98].

Основниот механизам со кој железото стимулира синтеза на хепцидин е со активација на BMP6-HJV-SMAD сигнален пат. BMP6-SMAD сигналниот пат во црниот дроб се активира од железо и способноста на железото да стимулира синтеза на хепцидин зависи од BMP-SMAD сигнали. Механизмот со кој нивото на железо води до зголемени BMP6-HJV-SMAD сигнали, сеуште не е добро разјаснет. Беше откриено дека HFE, TFR2 и трансферин рецептор-1 (TFR1) може да се вклучени во овој процес [97, 99].

Регулација со еритроидните фактори (две хипотези слични, но различни)

Беше откриено дека аплицирање на агенси стимулатори на еритропоезата (ESA) ја намалуваат хепатоцитната синтеза на хепцидин кај глувци, луѓе и in vitro студии [100-103]. Еритропоезата бара значителни количини на железо, затоа намалена синтеза на хепцидин во црниот дроб од еритропоетските сигнали е од голема физиолошка важност. Сепак, сеуште не е јасно како еритропоезата го регулира хепцидинот. Хипотезата дека еритропоетинот (EPO) делува директно врз хепатоцитните рецептори во клеточни култури [101] не можеше да биде потврдено кај животински модели за анемија, што покажа дека намалена синтеза на хепцидин зависна од еритропоезата не е дирекно медиирана од EPO [88,103]. Веројатно дополнителни еритропоетски фактори ја регулираат синтеза на хепцидин и истите допрва треба да бидат

откриени.

Регулацијата на производството на хепцидин од страна на еритропоезата останува слабо разбрана. Еден или повеќе неидентификувани сигнали од коскената срцевина, генерирани во текот на зголемена еритропоеза, водат до намалено произведување на хепцидин [88, 103]. Зголемената потреба од вградување на железо во хемоглобинот е посредувана со зголемена цревна апсорпција на железо и ослободување на депонираното железо во ретикулоендотелниот систем. Овој механизам на сигнална трансдукција, се чини многу стабилен и способен е да го задржи нивото на хепцидин многу ниско, дури и при состојби на системско преоптеретување со железо како што има при таласемија [104]. Во оваа конкретна подгрупа на пациенти, раст на факторот 15 на диференцијација, се смета како сигнал на коскена срцевина кој предизвикува супресија на хепцидинот [105].

Регулација со хипоксија (неколку хипотези слични, но различни)

Во случај на зголемена еритропоеза, како на пример во одговор на хипоксија, намалена концентрација на хепцидин ќе резултира со ослободување на депонирано железо и со зголемана апсорпција на железо со храната [85-88].

Намалена синтеза на хепцидин беше откриена во одговор на хипоксија in vivo [85, 106]. Овој ефект може да се должи на ефектот на хипоксија, на EPO експресија, на еритропоетска активност и/или евентуално директна интеракција со хепатоцитните рецептори.

Намалена синтеза на хепцидин при хипоксија може да биде препишана на хепар-специфична стабилизација од фактор кој индуцира хипоксија (HIF)-1 [107] со ефект врз BMP/SMAD сигналниот пат [108, 109]. Дали HIFs директно се врзува со хепцидинскиот промотор во моментот сеуште е контроверзно. Сепак, постојат индиректни механизми со кои HIFs може да ја регулира синтеза на хепцидин. Зголемена активност на HIF е поврзана со зголемено расцепување на хемојувелинот медиирано од матриптаза, а со тоа и намалена синтеза на хепцидин [109].

Хипоксијата е потенцијален инхибитор на синтеза на хепцидин дури и при отсуство на анемија. Механизмите не се комплетно разјаснети, но се смета дека се поврзани со фактор кој индуцира хипоксија HIFs. HIFs се хетеродимерни транскрипциони фактори кои содржат алфа регулаторна субединица (HIF-1α, HIF-2α, or HIF-3α) и бета субединица која се експресира на повеќе места (HIF-1β, исто така позната како ARNT).

При нормоксични состојби, со нормална концентрација на железо, HIF-α субединицата хидроксилира од кислородот и железо зависниот 2-оксоглутарат зависна оксигеназа, застапена како Hippel–Lindau (VHL) протеин и се разградува.

При хипоксија или состојби со недостиг на железо, хидроксилазната активност се инхибира во HIF-α субединица, транслоцирајќи се во јадрото, хетеродимеризирајќи со ARNT, и врзувајќи се со промоторни елементи одговорни за хипоооксија (HREs) од целни гени за да модулира транскрипција на гени [110].

Иако еден извештај сугерира дека HIF-1α се врзува директно со HREs во хепцидинскиот промотор и врзувањето со HIF ја потискнува транскрипцијата на хепцидин [107], во други студии се потврдени спротивставени мислења [111,112].

Алтернативни патишта сугерирани од други студии вклучуваат други HIF-1 независни, 2-оксоглутарат-зависни патишта [112] или патишта кои вклучуваат реактивни кислородни видови [111].

Интересно, фурин кој ги расцепува HJV и TFR1 исто така се кодирани од HIF целни гени [113, 114]. HIF може да ја потисне синтезата на хепцидин индиректно со редукција на BMP-SMAD–медиирана индукција на хепцидин и/или редуциран HFE/TFR2-посредувана хепцидинска индукција [113-115].

Регулација со воспаление

Воспалението предизвикува зголемена синтеза на хепцидин [85, 106, 118], резултирајќи со намалена достапност на циркулирачко железо, што се претпоставува дека претставува одбрамбен механизам на човечкиот организам кон екстраклеточните пролиферирачки патогени (кои се зависни од железо).

При хронични инфламаторни состојби (од низок степен), ова во крајна линија води до намалена достапност на железо за еритропоезата, наречена анемија на хроничната болест [119].

Воспалението и оптеретување со железо предизвикаат производство на хепцидин. Во случај на воспаление, примарниот медијатор е со зголемено ново на IL-6, што пак предизвикува врзувањето на сигналниот трансдјусер и активатор на транскрипција (STAT) 3 со промоторот на хепцидин, зголемувајќи ја неговата активност [120, 121].

Студии на луѓето со хронични инфекции и тешка воспалителна болест покажаа значително зголемување на нивото на хепцидин, што силно сугерира дека покачени нивоа на хепцидин играат клучна улога во анемија на воспаление и

ретикулоендотелна блокада [122].

Најдобро карактеризираниот механизам е директна транскрипциска активација на црнодробната синтеза на хепцидин со интерлеукин 6 (IL-6) врзувајќи се со неговиот рецепторен комплекс кој содржи gp130 активирајќи ја Јанушовата киназа (JAK) и активаторот на транскрипција 3 (STAT3), кој се врзува за DNA во проксимален хепцидин промотор [122-124]

Други проинфламаторни цитокини како IL-1, исто така може да учествуваат во индукцијата на хепцидин ([125].

Индукцијата на хепцидин од IL-6 се чини дека бара непроменети BMP-SMAD сигнални патишта поради загуба на хепато-специфичен заеднички медијатор Smad4 [126], присуство на растворлив HJV [96] или присуство на мал молекулен инхибитор од BMP тип I рецептор киназа активност [127], сите нарушувачи на индукција на IL-6 синтеза на хепцидин. Поврзаноста на овие патишта се чини дека делумно влијае врз нивото на хепцидинскиот промотор, каде проксималниот BMP одговорен елемент и STAT3 врзувачки елемент се во близина, бидејќи мутација на BMP одговорниот елемент сериозно ја нарушува индукцијата на хепцидин од IL-6.

Многу скоро, втор механизам беше окарактеризиран со кој проинфламаторните цитокини и бактериски липополисахариди (LPS) можат да индуцираат синтеза на хепцидин. Проинфламаторните цитокини и бактериски липополисахариди (LPS) активираат стрес на ендоплазматски ретикулум (ER) и одговор на протеините, зголемена експресија на CREBH (циклична AMP одговор елемент – врзувачки протеин H), кој ја активира транскрипција на гените одговорни за акутна фаза во црниот дроб [128]. ER стрес исто води до зголемена синтеза на хепцидин со врзување со CREBH и трансактивација на хепцидинскиот промотор. ER стрес е сугерирано дека со транскрипција регулира синтеза на хепцидин со CCAAT/ врзувачки протеин (C/EBP) хомологен протеин (CHOP) и C/EBPα, иако ова неможе директно да се поврзи со воспаление [129].

Кинетика на хепцидинот

Неодамна беше откриено дека циркулирачкиот хепцидин со релативно висок афинитет е врзан за α2-макроглобулин и со релативно низок афинитет со албуминот. На база на теоретски пресметки, беше проценето дека 11% од хепцидинот слободно циркулира [130].

Екскрецијата на хепцидин се одвива преку бубрезите, што е докажано со наод на хепцидин – 20 и хепцидин – 22 формите, кои се скратени изоформи на хепцидин–25, во урината [17].

Клиренсот на хепцидин се претпоставува дека се одвива: - преку клеточна разградба заедно со феропортинот на местата каде што дејствува и - со екскреција преку бубрезите.

Поради неговата ниска молекулска тежина и малиот радиус, неврзаниот хепцидин може слободно да помине низ гломерулскиот филтер. Во мали студии на луѓе, делумна екскреција на хепцидин била пресметана дека е помалку од 0%–5% [131, 132], дел поради неговата реапсорпција, слично на други мали пептиди или поради тоа што не се филтрирал слободно.

Доказ за овие објаснувања се наодите на зголемена концентрација на хепцидин во серумот за 1 до 6 пати кај пациенти со гломерулска дисфункција [133-137] споредено со 20 до 30 пати зголемен β2-микроглобулин во серумот. Екскрецијата на протеините со ниска молекулска тежина е речиси целосно регулирано од гломерулската филтрација. Можно е врзувањето со α2-макроглобулин или друг врзувачки протеин да превенира циркулирачкиот хепцидин да биде слободно филтриран.

Во заклучок, постојат евидентни докази дека железото претставува есенцијален микроелемент за правилен раст и развој на сите клетки во човечкиот организам. Тоа е предизвик за многу научни работници и бројот на научни студии кои се поврзани со метаболизмот на железо е во постојан пораст.

Благодарност

Неизмерна благодарност до мојот ментор проф. д-р Даница Лабудовиќ за помошта која ми ја пружа при пишувањето на овој труд, кој е дел од мојата докторска дисертација.

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Sjournals
Scientific Journals
Scientific Journal of Review (2014) 3(11) 965-972
ISSN 2322-2433
doi: 10.14196/sjr.v3i11.1729

Contents lists available at Sjournals
Scientific Journal of REVIEW
Journal homepage: www.Sjournals.com

Original article

Correlation between serum levels of hepcidin and ferritin in patients with metabolic syndrome in R. Macedonia

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ARTICLE INFO

Article history:
Received 03 October 2014
Accepted 24 October 2014
Available online 28 November 2014

Keywords:
Hepcidine
Ferritine
Metabolic syndrome
Diabetes mellitus type 2

ABSTRACT

The metabolic syndrome (MS) is a complex of interrelated risk factors for cardiovascular disease and diabetes. These factors include hyperglycemia, hypertension, high triacylglycerol levels, low HDL-cholesterol (HDL-c) levels, and abdominal obesity. Evidence suggests that iron influences glucose metabolism, even in the absence of significant iron overload. Iron stores, expressed as serum ferritin concentration, have been proposed to be a component of the insulin-resistance syndrome. In 1997, Moirand et al. first reported the presence of histologically proven liver iron overload in overweight subjects with abnormal glucose metabolism and dyslipidemia. The aim of this study was to evaluate the correlation between serum levels of hepcidin and ferritin in patients with metabolic syndrome in R.Macedonia. The study included 240 subjects - 60 males are with MS and 60 males as control group. 60 females are with MS and 60 females as control group. Individuals aged 18 years or older were eligible to participate in the study. In this analysis we included subjects with available complete data allowing their classification according to established criteria for MetS. In detail, the following features were considered: 1) abdominal obesity, defined as the presence of waist circumference ≥102 cm in men or ≥88 cm in women; 2) fasting plasma glucose ≥ 6.1

mmol/l or drug treatment for elevated blood glucose; 3) serum triglycerides ≥ 1.69 mmol/l or drug treatment for elevated triglycerides; 4) serum HDL cholesterol in men< 1.03 mmol/l and <1.29 mmol/l in women or drug treatment for low HDL-C; 5) blood pressure ≥130/85 mmHg or drug treatment for elevated blood pressure. Statistical analysis showed that males and females with MS had statistically higher ferritin levels than control group. Statistical analysis showed that males and females with MS had statistically higher hepcidin levels than control group. Serum ferritin levels significantly correlate with hepcidine in all participants with MS excluded females control group.Body mass, BMI, waist circumference, hip circumference, and WHR are statistically significant higher in subjects with MS compared to control groups. Concentrations of lipid parameters for all examinated groups. The concentrations of HDL-cholesterol, triglycerides and apo A are significantly increased in subjects with MS compared to control groups. It has been demonstrated that the prevalence of MS is increasing worldwide, largely the result of greater obesity and sedentary lifestyles. The concentration of serum hepcidin is associated with gender. Males hepcidine levels are higher than females levels. We found a statistically higher hepcidin levels in both groups with MS, compared to control groups, and males hepcidine levels are almost twice higher then females hepcidine levels in bout groups (control group and group with MS). The authors found a strong positive relationship between increased iron stores measured by the concentration of plasma ferritin and risk of type 2 diabetes, impaired glucose tolerance and metabolic syndrome in middle age and older people. The average concentration of ferritin in men is almost twice higher than in postmenopausal women, and three times higher than in premenopausal women with metabolic syndrome.

1. Introduction

The metabolic syndrome (MS) is a complex of interrelated risk factors for cardiovascular disease and diabetes. These factors include hyperglycemia, hypertension, high triacylglycerol levels, low HDL-cholesterol (HDL-c) levels, and abdominal obesity (de Carvalho et al., 2013, Alberti et al., 2009) . Separately the MS components increase the risk of diabetes, cardiovascular disease and all-cause mortality, but the full syndrome is associated with risk increases that are greater than the sum of the risk of each feature (Gami et al., 2007). It has been reported that the association of MS with cardiovascular disease increases total mortality 1.5 times and cardiovascular death 2.5 times (Sociedade Brasileira., 2005). People with MS also have a 5-fold higher risk of developing type 2 diabetes (de Carvalho et al., 2013). It has been demonstrated that the prevalence of MS is increasing worldwide, and for the adult population is estimated to be about 20 to 25%, largely the result of greater obesity and sedentary lifestyles (de Carvalho et al., 2013, Alberti et al., 2009).

Evidence suggests that iron influences glucose metabolism, even in the absence of significant iron overload (Fernandez-Real et al., 2002,Sam et al., 2013). Mildly elevated body iron stores are associated with increased fasting serum insulin and blood glucose (Sam et al., 2013, Tuomainen et al., 1997). The

underlying mechanism for the increased body iron stores in conditions of insulin resistance is unclear. Iron stores, expressed as serum ferritin concentration, have been proposed to be a component of the insulin-resistance syndrome. Indeed, the concentration of circulating ferritin was significantly associated with centrally distributed body fatness as well as with several other measurements of obesity (Fernandez-Real et al., 2002, Gillum, 2001). In the apparently healthy general population, serum levels of ferritin were also positively correlated with baseline serum glucose and with the area under the curve for glucose during the glucose oral tolerance test (Fernandez-Real et al., 2002, Tuomainen et al., 1997, Fernandez-Real et al., 2002). Ferritin levels also correlated with diastolic arterial blood pressure, even after adjustment for BMI (Fernandez-Real et al., 2002).

In 1997, Moirand et al. first reported the presence of histologically proven liver iron overload in overweight subjects with abnormal glucose metabolism and dyslipidemia (Nicola et al., 2012, Moirand et al., 1997). Nevertheless, the complex pathophysiological links between iron and metabolic derangements remain poorly understood (Dongiovanni et al., 2011). In the last ten years, hepcidin has emerged as the key iron-regulatory hormone (Ganz, 2011). It is a 25-amino-acid peptide predominantly synthesized in the liver (Park et al., 2001, Pigeon et al., 2001). Hepatic secretion of hepcidin in response to iron overload negatively regulates iron homeostasis. Hepcidin prevents iron efflux from enterocytes, macrophages and hepatocytes into the plasma by inducing internalization and degradation of the iron exporter ferroportin in these cells (Nemeth et al., 2004).

The aim of this study was to evaluate the correlation between serum levels of hepcidin and ferritin in patients with metabolic syndrome in R.Macedonia.

2. Materials and methods

The study included 240 subjects - 60 males are with MS and 60 males as control group. 60 females are with MS and 60 females as control group. Individuals aged 18 years or older were eligible to participate in the study. In this analysis we included subjects with available complete data allowing their classification according to established criteria for MetS. In detail, the following features were considered: 1) abdominal obesity, defined as the presence of waist circumference ≥102 cm in men or ≥88 cm in women; 2) fasting plasma glucose ≥ 6.1 mmol/l or drug treatment for elevated blood glucose; 3) serum triglycerides ≥ 1.69 mmol/l or drug treatment for elevated triglycerides; 4) serum HDL cholesterol in men< 1.03 mmol/l and <1.29 mmol/l in women or drug treatment for low HDL-C; 5) blood pressure ≥130/85 mmHg or drug treatment for elevated blood pressure. Subjects were considered to have MetS when they had at least three of the above-mentioned five traits.

Exclusion criteria were history of: cirrhosis or chronic hepatitis B and C, clinical evidence of bleeding in the previous 6 months, anemia (hemoglobin <120 g / L), treatment with iron in the previous year, alcohol consumption - women with daily consumption of alcohol > 40 g / day and men with daily alcohol consumption > 60 g / day, donation of blood in the previous 6 months, haemochromatosis, concomitant infections, malignant disease, chronic diseases other than diabetes mellitus type 2, immunosuppressive therapy, acute infections or invasive procedures (operations, catheterization) in the previous 6 months, neurological, endocrine or other systemic diseases, cardiovascular incident in the previous 6 months and pregnancies.

A written informed consent was obtained for all the subjects included in the study. All subjects filled out a questionnaire about the family history, physical activity and alcohol consumption. Subjects had light indoor clothes and were barefooted during the measurement of their height and weight. Their standing height was measured with stadiometer to the nearest 0.1 sm. Weight was measured using a digital weight scale eith a precision of 0.1 kg. Waist and hip were measured with the tape measure. Waist-to-hip ratio (WHR) was calculated by dividing the circumference of the waist by dividing of the hip. The blood samples were taken after overnight fast (12 hours). Blood pressure was measured using a mercury manometer. Lipid parameters, glucose, ferritin and transferine were measured in fresh sera by enzymatic methods, using biochemical analyzer Biosystems A25. Hepcidin was determined by ELISA kit (DRG Hepcidin-25 bioactive ELISA, Marburg).

The data are presented as mean± standard deviation (SD) and $p \leq 0.05$ is considered statistically significant. The results were done with the SPSS version 16, statistical significance was test with paired

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Student's t – test, and Pearson correlation coefficient was used for correlatin of hepcidin and ferritin levels.

3. Results

All 240 participants were divided in 4 groups: males control group, females control group, males with MS, females with MS.

Table 1
Concentrations of transferine, ferritine and hepcidine in 4 groups : males control group, females control group, males with MS, females with MS.

Variables	Males control group	Males with MS	p	Females control group	Females with MS	p
Transferin	279.97±56.28	264.4±61.47	0.151	232.86±35.43	246.62±40.95	0.053
Ferritin	120.17±70.66	197.95±142.57	0.000	69.02±49.36	118.98±70.31	0.000
Hepcidin	12.33±7.37	25.54±1.33	0.000	6.16±3.20	11.22±5.30	0.000

The concentration of ferritin in males control group was ranged from 9 to 309 (mean 120, 17 ± 70,669) and in females control group was ranged from 10 to 257 (mean 69,02 ± 49,36). The concentration of ferritin in males with MS was ranged from 34 to 668 (mean 197,95 ±142,57) and in females with MS was ranged from 11 to 456 (mean 118,98± 70,311). Statistical analysis showed that males and females with MS had statistically higher ferritin levels than control group.

The concentration of hepcidin in males control group was ranged from 3 to 36 (mean 12,337 ± 7,37) and in females control group was ranged from 1,235 to 14,748 (mean 6,163± 3,202). The concentration of hepcidin in males with MS was ranged from 2,474 to 85,98 (mean 25,54 ± 18,33) and in females with MS was ranged from 2,933 to 24,055 (mean 11,228± 5,302). Statistical analysis showed that males and females with MS had statistically higher hepcidin levels than control group.

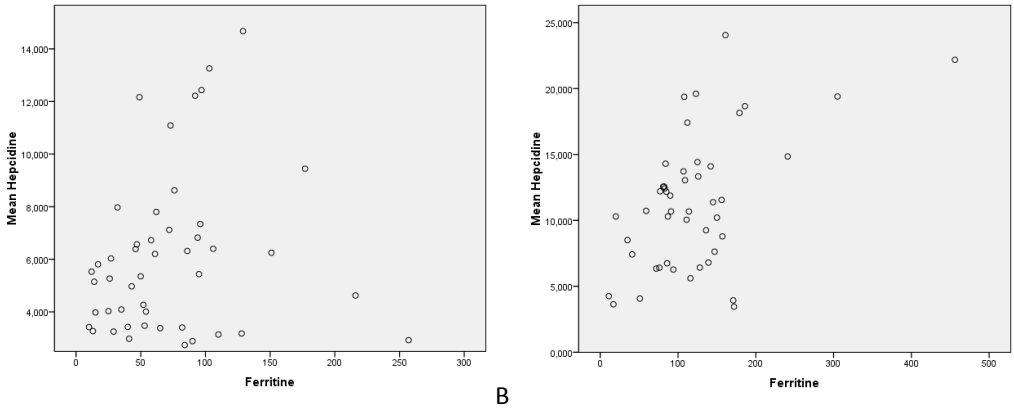
The anthropometric characteristics of each group are shown in Table 2.

Table 2
Anthropometric characteristic of participants.

Variables	males control group	males with MS	p	females control group	females with MS	p
Age (years)	39.73±12.256	51.03±7.94	0.000	43.57±12.183	54.70±6.426	0.000
Body mass (kg)	81.6±10.65	96.35±16.57	0.000	74.47±17.766	84.90±14.451	0.001
Hight (m)	174.83±7.547	174.18±9.19	0.67	164.18±6.299	160.85±7.424	0.009
BMI (kg/m2)	26.75±3.543	31.67±4.298	0.000	27.577±6.329	32.796±5.030	0.000
Waist circumference (cm)	91.33±11.419	109.68±13.25	0.000	92.002±14.702	107.45±12.222	0.000
Hip circumference (cm)	99.52±10.186	110±9.789	0.000	107.52±14.571	116.43±12.636	0.000
WHR	0.919±0.067	1.002±0.0683	0.000	0.855±0.072	0.932±0.067	0.000

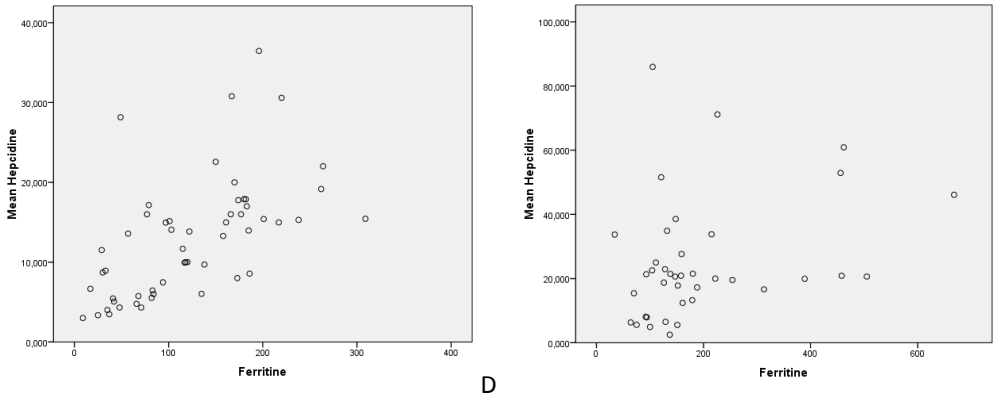
Table show that body mass, BMI, waist circumference, hip circumference, and WHR are statistically significant higher in subjects with MS compared to control groups.

Serum ferritin levels significantly correlate with hepcidine in all participants with MS excluded females control group.



A B

Fig. 1. Correlation between hepcidin level and ferritin in females control group (r =0,205 p>0,01) (A) and females with MS (B) (r =0,439 ; p <0,01).



C D

Fig. 2. Correlation between hepcidin level and ferritin in males control group (r =0,591; p <0,01) (C) and males with MS (D) (r = 0,416 ; p < 0,01).

Table 3

Concentrations of lipid parameters in 4 groups: males control group, females control group, males with MS, females with MS.

Variables	Males control group	Males with MS	p	Females control group	Females with MS	p
Total cholesterol (mmol/l)	5.17±0.6	5.57±1.26	0.028	4.93±0.9	5.2±1.15	0.145
HDL-cholesterol(mmol/l)	1.41±0.33	1.19±0.29	0.000	1.57±0.42	1.32±0.27	0.000
LDL-cholesterol(mmol/l)	3.02±0.55	2.94±1.49	0.711	2.83±0.82	2.86±1.09	0.852
Triglycerides (mmol/l)	1.49±0.53	2.59±1.26	0.000	1.18±0.47	1.97±0.83	0.000
Apo A	112.62±57.61	89.42±30.78	0.007	136.47±48.4	99.93±28.66	0.000
Apo B	150.6± 33.29	179.22±30.78	0.000	148.04±39.41	161.22±31.13	0.044

Table 3 displays the concentrations of lipid parameters for all examinated groups. The concentrations of HDL- cholesterol and triglycerides are significantly increased in subjects with MS compared to control groups.

4. Discussion

It has been demonstrated that the prevalence of MS is increasing worldwide, largely the result of greater obesity and sedentary lifestyles. This is a problem because MS increase the risk of diabetes, cardiovascular disease and mortality. The concentration of serum hepcidine is associated with gender. Males hepcidine levels are higher than females levels.

We found a statistically higher hepcidin levels in both groups with MS, compared to control groups, and males hepcidine levels are almost twice higher then females hepcidine levels in bout groups (control group and group with MS).

In the recent years, a bulk of evidence, particularly from epidemiological studies (Bozzini et al., 2005, Sheu et al., 2003, Jiang et al., 2004, Jehn et al., 2007) have established a link between iron metabolism and insulin resistant states, including type 2 diabetes mellitus and the MetS (Rajpathak et al., 2009). On the other hand, some prospective studies (Jiang et al., 2004), (Jehn et al., 2007) have shown a positive association between baseline levels of ferritin, i.e. the best available serum marker of body iron stores (Cook et al., 2003), and development of type 2 diabetes. The authors found a strong positive relationship between increased iron stores measured by the concentration of plasma ferritin and risk of type 2 diabetes, impaired glucose tolerance and metabolic syndrome in middle age and older people (Liang et al., 2008).

The authors found the average concentration of ferritin in men is almost twice higher than in postmenopausal women, and three times higher than in premenopausal women with metabolic syndrome (Istvan et al., 2007). The authors found ferritin concentration in serum is positively related to dyslipidemia (Halle et al., 1997, Williams et al., 2002). The authors found ferritin is positively associated with increased triglycerides in females and males with MS (Megan et al., 2004).The authors found ferritin is connected to one or more of the characteristics of MS (Festa et al., 2002, Toumainen et al., 1997, Jehn et al., 2004,Bozzini et al., 2005).

Of note, when women with or without MS were stratified by ferritin levels, MS women with ferritin in the lower range had hepcidin levels significantly higher than non-MS counterpart. Since this was particularly evident in women with ferritin levels indicating true iron deficiency where hepcidin is generally almost completely suppressed (Traglia et al., 2011), this suggests that some MS-related factors may affect hepcidin in this subgroup. On the other hand, the influence of MS per se on hepcidin levels appears limited when iron stores are abundant.

Our results may warrant further studies on adults in this direction, particularly focusing on differences by gender.

5. Conclusion

The concentration of serum hepcidine is associated with gender. Males hepcidine levels are higher than females levels. The concentration of hepcidin was higher in males and females with MS compared to the control groups. The ferritine showed a high correlation with hepcidine levels in all examinated groups excluded females control group.

Acknowledgements

This research is part of PhD that is co-funded by the Farmahem diagnostics.

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Volume : 4 | Issue : 8 | Aug 2015 ISSN : 2250-1991

Research Paper Medical Science

Hepcidin levels in patients with metabolic syndrom and healthy subjects in R. Macedonia

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ABSTRACT

In the last ten years, hepcidin has emerged as the key iron-regulatory hormone. Given its central role in iron homeostasis, hepcidin represents an appealing candidate to be investigated in subjects with metabolic syndrom features, but until now methodological difficulties have hampered large epidemiological studies. The aim of this studie is to investigate the relationships between hepcidin and metabolic syndrom. This study was carried out from January 2014 to August 2014 at the Department of medical biochemistry and Diabetes Center of Public Health Organization Clinical hospital d-r Trifun Panovski in Bitola (R.Macedonia). The total number of patients with metabolic syndrom was 120, and the remaining 120 patients we have been chosen healthy people, blood donors. Hepcidin was determined by ELISA kit (DRG Hepcidin-25 bioactive ELISA, Marburg). We found the concentration of hepcidin in males and females with metabolic syndrom had statistically higher than control group.

KEYWORDS hepcidin, metabolic syndrome, healthy subjects

Introduction

The metabolic syndrom (MetS) is a condition highly prevalent in western countries, involving near one fourth of the adult population [1]. The components of the MetS are: abdominal obesity given as waist circumference for men >102 cm (>40 in) and for women >88 cm (>35 in); triglycerides ≥150 mg/dL; HDL cholesterol for men <40 mg/dL and for women <50 mg/dL; blood pressure ≥130/≥85 mm Hg; fasting glucose ≥110 mg/dL. This components are defined by the third report of the U.S. National Heart, Lung and Blood Institute's Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (ATP III) (U.S. National Cholesterol Education Program Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults, 2001) [2].

In 1997, Moirand et al. first reported the presence of histologically proven liver iron overload in overweight subjects with abnormal glucose metabolism and dyslipidemia [3].

This condition, later designated as dysmetabolic iron overload syndrome (DIOS) [4], is now known to occur in about one third of subjects with NAFLD and represents the most severe counterpart of the so-called dysmetabolic hyperferritinemia (DHF) [5]. Nevertheless, the complex pathophysiological links between iron and metabolic derangements remain poorly understood [5].

In the last ten years, hepcidin has emerged as the key iron-regulatory hormone [6]. Hepcidin was initially isolated from plasma ultrafiltrate [7] and named liver-expressed antimicrobial peptide (LEAP-1). Around the same time, it was isolated from human urine and named hepcidin after its hepatic origin and bactericidal effect in vitro [8].

The development of severe iron overload [9] by knocking out the gene in mice suggested that hepcidin is involved in iron metabolism, whereas this key role in regulation was underlined by the discovery of hepcidin mutations in patients [10]. Hepcidin was found to be regulated by inflammation, iron stores [11], hypoxia and anemia [12].

The human hepcidin gene (HAMP; OMIM 606464), located on chromosome 19q13.1, encodes a precursor protein of 84 amino acids (aa). During its export from the cytoplasm, this full-length preprohepcidin undergoes enzymatic cleavage, resulting in the export of a 64 aa pro-hepcidin peptide into the ER lumen [13]. Next, the 39 aa pro-region peptide is probably posttranslationally removed by a furin-like proprotein convertase7 resulting in mature bioactive hepcidin-25 (25 aa form). In human urine, Park et al. also identified hepcidin-22 and hepcidin-20, which are N-terminally truncated isoforms of hepcidin-25. It was confirmed that in addition to hepcidin-25, the 20 aa isoform is detectable in both human urine and serum, while the 22 aa isoform can only be detected in urine [14]. These results support the hypothesis that the 22 aa peptide is merely an urinary degradation product of hepcidin-25 [15].

Given its central role in iron homeostasis, hepcidin represents an appealing candidate to be investigated in subjects with MetS features, but until now methodological difficulties [16] have hampered large epidemiological studies. The aim of this studie is to investigate the relationships between hepcidin and MetS.

Methods

This study was carried out from January 2014 to August 2014 at the Department of medical biochemistry and Diabetes Center of Public Health Organization Clinical hospital d-r Trifun Panovski in Bitola (R.Macedonia). All biochemical parameters were measured in a biochemistry laboratory at the Department of medical biochemistry of Public Health Organization Clinical hospital d-r Trifun Panovski in Bitola (R.Macedonia), just hepcidin was measured in Deparmtent of Serology in of Public Health Organization Center for Public Health in Bitola(R.Macedonia). The study was approved by the Ethics Committee of Health Organization Clinical hospital d-r Trifun Panovski, and all of the procedures were performed in accordance with ethical approval institutional guidelines. The study protocol followed the ethical guidelines of the most recent Declaration of Helsinki. Written consent was obtained from

e parti[illegible]nts prior to the start of the study.

The [illegible] number of patients with MetS was 120, recruited in Diabetes Research Centre of Health Organization Clinical hospital d-r Trifun Panovski, Bitola, R.Macedonia. Individuals aged 18 years or older were eligible to participate in the study. In this analysis we included subjects with available complete data allowing their classification according to established criteria for MetS. In detail, the following features were considered: 1) abdominal obesity, defined as the presence of waist circumference ≥102 cm in men or ≥88 cm in women; 2) fasting plasma glucose ≥ 6.1 mmol/l or drug treatment for elevated blood glucose; 3) serum triglycerides ≥ 1.69 mmol/l or drug treatment for elevated triglycerides; 4) serum HDL cholesterol in men< 1.03 mmol/l and <1.29 mmol/l in women or drug treatment for low HDL-C; 5) blood pressure ≥130/85 mmHg or drug treatment for elevated blood pressure. Subjects were considered to have MetS when they had at least three of the above-mentioned five traits.

Exclusion criteria were history of: cirrhosis or chronic hepatitis B and C, clinical evidence of bleeding in the previous 6 months, anemia (hemoglobin <120 g / L), treatment with iron in the previous year, alcohol consumption - women with daily consumption of alcohol > 40 g / day and men with daily alcohol consumption > 60 g / day, donation of blood in the previous 6 months, haemochromatosis, concomitant infections, malignant disease, chronic diseases other than diabetes mellitus type 2, immunosuppressive therapy, acute infections or invasive procedures (operations, catheterization) in the previous 6 months, neurological, endocrine or other systemic diseases, cardiovascular incident in the previous 6 months and pregnancies.

The remaining 120 patients we have been chosen healthy people, blood donors from the Department of Transfusion Medicine of Health Organization Clinical hospital d-r Trifun Panovski, Bitola, R.Macedonia. Patient had light indoor clothes and were barefooted during the measurement of their height and weight. Their standing height was measured [illegible]th stadiometer to the nearest 0.1 sm. Weight was measu[illegible]g a digital weight scale eith a precision of 0.1 kg. Waist [illegible] hip were measured with the tape measure. Waist-to-hip [illegible] (WHR) was calculated by dividing the circumference of the waist by dividing of the hip. The blood samples were taken after overnight fast (12 hours). Blood pressure was measured using a mercury manometer. Hepcidin was determined by ELISA kit (DRG Hepcidin-25 bioactive ELISA, Marburg).

The data are presented as mean± standard deviation (SD) and $p \leq 0.05$ is considered statistically significant. The results were done with the SPSS version 16.

Results

All 240 participants were divided in 4 groups: males control group, females control group, males with MetS group and females with MetS group.

Participants were age 18 to 60 years, for males control group (mean 39,73 ± 12,25), females control group (mean 43,57 ± 12,2), males MetS group (mean 51,03 ± 7,94) and females MetS group (mean 54,7 ± 6,43). There is not statistical significant difference between age of patients.

The concentration of hepcidin in males control group was ranged from 3 ng/mL to 36 ng/mL (mean 12,34 ± 7,37) and in females control group was ranged from 1,235 ng/mL to 14,748 ng/mL (mean 6,163± 3,202). The concentration of hepcidin in males with MetS was ranged from 2,474 ng/mL to 85,98 ng/mL (mean 25,54 ± 18,33) and in females with MetS was ranged from 2,933 ng/mL to 24,055 ng/mL (mean 11,228± 5,302).

Statistical analysis showed that males and females with MetS had statistically higher hepcidin levels than control group. Also, males hepcidine levels are higher compared to females in bouth groups – control group and MetS group.

Table 1. Present statistical analyzes of correlation between hepcidin levels in two groups - control and MetS group

Variable	Contol groups N = 120		MetS groups N = 120	
	males n= 60	females n= 60	males n=60	females n=60
Hepcidin mean±SD, (median- ng/mL)	12,34 ± 7,37 (10,99) ng/mL	6,16 ± 3,2 (5,6) ng/mL	25,54 ± 18,33 (20,75) ng/mL	11,23 ± 5,3 (10,81) ng/mL
Difference	males control group vs MetS group females control group vs MetS group		t = 5,18 p=0,000001** t = 6,3 p=0,000**	

The hepcidin concentrations in 4 groups: males control group, females control group, males with MetS, females with MetS are shown in Table 1.

Discussion

It has been demonstrated that the prevalence of MetS is increasing worldwide, largely the result of greater obesity and sedentary lifestyles. This is a problem because MetS increase the risk of diabetes, cardiovascular disease and mortality.

In the recent years, a bulk of evidence, particularly from epidemiological studies have established a link between iron metabolism and insulin resistant states, including type 2 diabetes mellitus and the MetS [5,17].

Some prospective studies have shown a positive association between elevated circulating ferritin concentrations and risk of type2 diabetes and MetS independent of obesity, inflammation, adipokines, and other risk factors [18]. View of iron overload disorders has radically changed with discovering of hepcidin [6], which has been demonstrated to be inappropriately low in genetic hemochromatosis [19].

Our results establish for the second time at population level that subjects with MetS have increased serum levels of hepcidin, the first one this was establish in 2013 year by Martinelli [20].

We found that concentration of serum hepcidine is associated with gender. Males hepcidine levels are higher than females levels.

We found a statistically higher hepcidin levels in both groups with MetS, compared to control groups, and males hepcidine levels are almost twice higher than females hepcidine levels in bout groups (control group and group with MetS).

Recent experimental studies have found that leptin is able to stimulate hepatic hepcidin production [21], and a positive correlation has been found between serum levels of leptin and hepcidin in obese children [22].

Since the discovery of hepcidin 15 years ago, multiple studies have contributed insights into the regulation of hepcidin and its functional properties. The first reliable assays to quantify hepcidin in human body fluids have recently been developed, and proof-of-principle studies in human iron disorders highlight hepcidin as a promising novel tool in diagnostic medicine [23].

Conclusions

In summary, the rapid progress in the understanding of how hepcidin controls iron homeostasis and the intense research as

[illegible]how [illegible]cidin levels can be altered promise new therapies in the f[illegible]e for diseases exacerbated by iron overload or iron [illegible].

Our data support the crucial role of iron overload for metabolic diseases, even in a country with relatively high prevalence of iron deficiency.

In conclusion, hepcidin is a promising diagnostic tool but efforts must be undertaken to assess the relevance of specifically measuring hepcidin-25, to harmonize assay outcomes throughout the world, to define clinical decision limits, and to make assays available to clinical laboratories before hepcidin assays can be fully included in clinical practice.

Competing interests
None of the authors have professional, personal, or financial conflicts of interest to report.

Authors' contributions
All the authors collected information, designed and organized the structure of the contents and wrote the manuscript, reviewed literature, discussed and suggested the contents as well as edited the manuscript. Also, all the authors read and approved the final manuscript.

Acknowledgements
I would like to express my special appreciation and [illegible]s to my advisor Professor Dr. Danica Labudovic have be[illegible] tremendous mentor for me. I would like to thank you f[illegible] couraging my research and for allowing me to grow as a research scientist. Your advice on both research as well as on my career have been priceless. Thanks to Pharmachem for ELISA kits that sponsored me And many thanks to my husband Sasho Ilkovski for support and technical help.

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RCH PAPER | Medical Science | Volume : 5 | Issue : 8 | August 2015 | ISSN - 2 -555X

Elevated Serum Hepcidin and Ferritin Levels in Patients With Metabolic Syndrom in Macedonian Population

KEYWORDS: metabolic syndrome, hepcidin, ferritin

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ABSTRACT *The metabolic syndrome is a condition highly prevalent in western countries. The serum ferritin concentration reflects iron stores in the body. In the last fifteen years, hepcidin was established as the key iron-regulatory hormone, closely related with metabolic syndrom. Aim of this study is to find elevated serum hepcidin and ferritin levels in patients with metabolic syndrom in Macedonian population.The analysed group consisted of 240 subjects – 50% of them with metabolic syndrome and 50% control group. Hepcidin levels were measured with ELISA kit (DRG Hepcidin-25 bioactive, Marburg). Metabolic syndrome subjects have significantly higher serum levels of both ferritin and hepcidin as compared to subjects in control group.The concentration of serum hepcidine and ferritin are associated with gender, males had higher levels compared to females for both groups-control and group with metabolic syndrom.*

Introduction

The metabolic syndrome (MetS) is a condition highly prevalent in western countries, involving near one fourth of the adult population (1). Although definitions vary, the essential features of MetS are represented by the deadly quartet of hyperglycemia, dyslipidemia, hypertension, and obesity (2), leading to a substantial cardiovascular risk, but also to risk of hepatic diseases, namely nonalcoholic fatty liver disease.

Iron is a ubiquitous metal of vital importance to the normal physiologic processes of many organisms (3). The first evidence linking iron to MetS was the observation that patients with hereditary hemochromatosis were at higher risk of developing type II diabetes (4,5). Hemochromatosis is an inherited disorder commonly associated with European ancestry. Patients with type 2 diabetes have a high frequency of the C282Y mutation of the hemochromatosis gene (6). The prevalence of diabetes (23%) and impaired glucose tolerance (IGT) (30%) increased in hemochromatosis compared with matched control subjects (0% diabetes and 14% IGT) (5).

The serum ferritin concentration reflects iron stores in the body (7). Ferritin is a large hollow, symmetrical protein, usually comprised of mixtures of two kinds of paired homologous but not identical subunits (heavy and light; H and L; about 20 kDa) expressed from separate genes, for a total 24 subunits and a molecular weight of about 480 kDa (8-10).

In the last fifteen years, hepcidin was established as the key iron-regulatory hormone (11). Human hepcidin is predominantly produced by hepatocytes as a 25 amino acid peptide (2789.4 Da) (12,13). At the molecular level, hepcidin acts by binding and inactivating its cell membrane receptor ferroportin, the only known cellular iron exporter. Ferroportin is particularly expressed by cells critical for iron homeostasis, like absorbing duodenal enterocytes, reticuloendothelial macrophages (involved in iron storage and recycling), and hepatocytes (involved in iron storage and endocrine regulation) (14). Given its central role in iron homeostasis, Martinelli et al. (15) in 2012 for the first time reported increased serum hepcidin levels in subjects with the MetS.

Aim of this study is to find elevate serum hepcidin and ferritin levels in patients with metabolic syndrom in Macedonian population.

Material & Methods,

The study included 240 subjects - 60 males with MetS and 60 males as control group; 60 females with MetS and 60 females as control group. The present study only analyzed data on adults, aged ≥19 years old. In this analysis we included subjects with available complete data allowing their classification according to established criteria for MetS. In detail, the following features were considered: 1) abdominal obesity, defined as the presence of waist circumference ≥102 cm in men or ≥88 cm in women; 2) fasting plasma glucose ≥ 6.1 mmol/l or drug treatment for elevated blood glucose; 3) serum triglycerides ≥ 1.69 mmol/l or drug treatment for elevated triglycerides; 4) serum HDL cholesterol in men< 1.03 mmol/l and <1.29 mmol/l in women or drug treatment for low HDL- cholesterol; 5) blood pressure ≥130/85 mmHg or drug treatment for elevated blood pressure. Subjects were considered to have MetS when they had at least three of the above-mentioned five traits.

Exclusion criteria were as followed: cirrhosis or chronic hepatitis B and C, clinical evidence of bleeding in the previous 6 months, anemia (hemoglobin <120 g / L), treatment with iron in the previous year, alcohol consumption - women with daily consumption of alcohol > 40 g / day and men with daily alcohol consumption > 60 g / day, donation of blood in the previous 6 months, haemochromatosis, concomitant infections, malignant disease, chronic diseases other than diabetes mellitus type 2, immunosuppressive therapy, acute infections or invasive procedures (operations, catheterization) in the previous 6 months, neurological, endocrine or other systemic diseases, cardiovascular incident in the previous 6 months and pregnancies.

Ethics approvals for the study protocol and analysis of the data were obtained from the institutional review board of Gen. Hosp. Trifun Panovski Clinical Hospital. Written informed consent was obtained from all subjects.

Clinical and laboratory measurements

Study data included a medical history, a physical examination, information provided by a questionnaire, anthropometric measurements, and laboratory measurements. The medical and drug prescription history were assessed by the examining physicians. All of the participants were asked to respond to a health-related behavior questionnaire, which included the topics of alcohol consumption, smoking, and exercise.In addition, the participants were asked about the frequency per week of physical activities they engaged in that lasts long enough to produce perspiration such as jogging, bicycling, and swimming (≥1 time/ week).

Blood samples were collected after 12 h of fasting and drawn from an antecubital vein. Serum levels of enzymes, lipid profile, CRP, fasting serum glucose, urea, creatinine, iron, ferritin wer measured by automated chemistry analyzer (Biosystems, Spain). Hepcidin levels were measured with ELISA kit (DRG Hepcidin-25 bioactive ELISA, Marburg).

The data are presented as mean± standard deviation (SD). The results were done with the SPSS version 13.

Results

The analysed group consisted of 240 subjects – 120 patients with MetS and 120 subjects in control group.

The values of the age in control group are present in Table 1.

Control group / Control group	**Descriptive Statistics –** age			p-value
	N	mean ± SD	min – max	
Total	120	41,65 ± 12,3	18 – 60	
Male	60	39,73 ± 12,25	18 – 60	t = 1,7 p = 0,089 NS
Female	60	43,57 ± 12,2	23 – 60	

As we can see in Tab.1 there is no significant statistical difference between age of male and female participants in control group.

Table 2. Present mean values ±SD, median, rang of serum concentrations of: iron, ferritin, trensferin and hepcidine in control group.

Variable	Control group			p-value
	Total N = 120	Male n = 60	Female n = 60	
Iron (μmol/l) mean±SD, median, rang	13,97 ± 6,0 13,1 3,7 – 32,4	15,03 ± 5,77 13,85 4,5 – 32,4	12,91 ± 6,1 12,2 3,7 – 27,6	Z = 2,08 p = 0,037*
Ferritin (ng/ml) mean±SD, median, rang	94,59 ± 65,9 82,0 9,0 – 309,0	120,2 ± 70,67 116,0 9 – 309	69,01 ± 49,36 56,0 10 – 257	Z = 4,12 p = 0,00004**
Transferin (mg/dl) mean±SD, median, rang	256,6 ± 52,54 249,0 134,0 – 416,0	279,9 ± 56,28 276,5 185 – 416	232,86 ± 35,44 238 134 – 335	t = 5,45 p = 0,000**
Hepcidin (ng/mL) mean±SD, median, rang	9,25 ± 6,45 6,77 1,23 – 36,46	12,34 ± 7,37 10,97 3 – 36,5	6,16 ± 3,2 5,6 1,23 – 14,75	Z = 5,38 p = 0,000**

***p<0,05 **p<0,01**

In Tab. 2 we can see that there is a significant statistical difference between males and females serum concentrations of iron, ferritin, transferine and hepcidine in participants included in control group. We can see that concentration of iron, ferritin, transferine and hepcidine is higher in males control group in a correlation with female participants in control group.

The values of the age in subjects with MetS are present in Table 3.

	Descriptive Statistics – age			p-value
	N	mean ± SD	min – max	
Total	120	52,87 ± 7,42	32 – 60	
Male	60	51,03 ± 7,94	35 – 60	t = -2.78 p=0,006**
Female	60	54,7 ± 6,43	32 – 60	NS

p<0,01

In Tab. 3 we can see that there is not statistical significant difeference between age of patients with MetS.

Table 4. Present serum concentrations of iron, ferritin, trensferin and hepcidine in subjects with the MetS.

Variable	Patients with MetS			p-value
	Total N = 120	Male n = 60	Female n = 60	
Iron (μmol/l) mean±SD, median, rang	15,79 ± 5,28 14,95 5,7 – 28,8	17,23 ± 5,22 16,3 7,6 – 28,8	14,36 ± 4,99 13,5 5,7 – 24,4	t = 3,07 p = 0,0026**
Ferritin (ng/ml) mean±SD, median, rang	158,47 ± 118,75 129,0 11,0 – 668,0	197,9 ± 142,57 149,5 34 – 668	118,98 ± 70,31 111,5 11 - 456	Z = 4,04 p = 0,00005**
Transferin (mg/dl) mean±SD, median, rang	255,51 ± 52,77 244,0 172,0 – 582,0	264,4 ± 61,47 249 172 – 582	246,62 ± 40,96 239 175 - 405	Z = 1,89 p = 0,058 NS
Hepcidin (ng/mL) mean±SD, median, rang	18,38 ± 15,24 14,29 2,47 – 85,98	25,54 ± 18,33 20,75 2,47 – 85,98	11,23 ± 5,3 10,81 2,93 – 24,05	Z = 5,54 p = 0,000**

**p<0,

In Tab we can see that there is a significant statistical ence between males and females serum concentrations of iron, ferritin, and hepcidine in participants included in control group. We can see that serum concentrations of iron, ferritine, and hepcidine is higher in males control group in a correlation with female participants.

Table 5. Present statistical analyzes of correlation between two groups - control and MetS group

Variable	Contol groups N = 120		MetS groups N = 120	
	males n= 60	females n= 60	males n=60	females n=60
Iron mean±SD, median	15,03 ± 5,77 13,85	12,91 ± 6,1 12,2	7,23 ± 5,22 16,3	14,36 ± 4,99 13,5
Difference	males Control group vs MetS group t = 2,18 p=0,03*			
females Control group vs MetS group t = 1,4 p=0,16 NS				
Ferritin mean±SD, median	120,2 ± 70,67 116,0	69,01 ± 49,36 56,0	197,9 ± 142,57 149,5	118,98 ± 70,31 111,5
Difference	males Control group vs MetS group Z = 3,2 p=0,01**			
females Control group vs MetS group Z = 4,8 p=0,000002**				
Transferin mean±SD, median	279,9 ± 56,28 276,5	232,86 ± 35,44 238	264,4 ± 61,47 249	246,62 ± 40,96 239
Difference	males Control group vs MetS group t = 1,4 p=0,15 NS			
females Control group vs MetS group t = 1,96 p=0,053 NS				
Hepcidin mean±SD, median	12,34 ± 7,37 10,97	6,16 ± 3,2 5,6	25,54 ± 18,33 20,75	11,23 ± 5,3 10,81
Difference	males Control group vs MetS group t = 5,18 p=0,000001**			
females Control group vs MetS group t = 6,3 p=0,000**				

***p < 0,05 **p < 0,01**

In table 5 we can see that there is a statistical significant difference betwin males control group and MetS group (t = 2,18 p=0,03 for p<0,05). There is present difference betwin ferritin levels for males control group and MetS group (Z = 3,2 p=0,01for p<0,01) and for females control group and MetS group (Z = 4,8 p=0,000002 for p<0,01). Also there is present difference betwin hepcidin levels for males control group and MetS group (t = 5,18 p=0,000001for p<0,01) and for females control group and MetS group (t = 6,3 p=0,000 for p<0,01).

Discussion

In the recent years, a bulk of evidence, particularly from epidemiological studies (16-19) have established a link between iron metabolism and insulin resistant states, including type 2 diabetes mellitus and the MetS (20,21). Increased body iron stores predicted the development of MetS and diabetes in healthy individuals of European ancestry (22) and recently in healthy East Asians (23). Increasing evidence has shown that body iron excess is associated with one or more MetS components (16, 24-27).

In our study we found that the males hepcidin and ferritine levels are higher compared to females in bouth groups – control group and MS group. MetS subjects significantly higher serum levels of both ferritin and hepcidin as compared to subjects in control group, which is similar to previous studies of Martinelli. Martinelli et al. establish for the first time at population level that subjects with MetS have increased serum levels of hepcidin. In subjects of both sexes hepcidin increased linearly with increasing number of the five classical MetS features. These data indicate that the fundamental iron regulatory feedback is preserved in MetS, i.e. that hepcidin tends to progressively increase in response to a moderate increase of iron stores, likely in the attempt to counterbalance it by limiting intestinal iron absorptionIn view of the rapidly growing evidence for pleiotropic effects of hepcidin, this may have relevant implications for the MetS pathophysiology (15).

Statistical analysis showed that males had statistically higher hepcidin and ferritin levels than women. That means that concentration of serum hepcidine and ferritin levels are associated with gender what is comparable to the scarce data reported by a few other groups (28).

Galesloot TE et al (29) find variation in hepcidin concentration over age differed between men and women. Men showed a stable hepcidin concentration, although a non-significant trend for an age-related increase in serum hepcidin was previously reported based on 65 men. In women, serum hepcidin concentration was substantially higher for postmenopausal than for premenopausal women.

Elevated serum ferritin concentrations have recently been implicated in the pathogenesis of many chronic inflammatory diseases including the MetS (30). Cross-sectional studies have shown associations of elevated serum ferritin concentration with MetS (16, 26, 27).

Conclusion:

MetS subjects had significantly higher serum levels of both ferritin and hepcidin as compared to subjects without MetS. The concentration of serum hepcidine and ferritin levels are associated with gender. In our study we found that the males hepcidine and ferritine levels are higher compared to females in bouth groups – control group and MS group. Elevated serum ferritin concentration is implicated in the pathogenesis of MetS. We show associations of elevated serum ferritin and hepcidin concentration with MetS.

Conflict of interest

The authors state that no conflict of interest exists. The authors have not received any funding or benefits from industry to conduct this study.

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http://dx.doi.org/10.5272/jimab.2016222.1127

Journal of IMAB
ISSN: 1312-773X
http://www.journal-imab-bg.org

Journal of IMAB - *Annual Proceeding (Scientific Papers)* 2016, vol. 22, issue 2

SERUM HEPCIDIN REFERENCE RANGE, GENDER DIFFERENCES, MENOPAUSAL DEPENDENCE AND BIOCHEMICAL CORRELATES IN HEALTHY SUBJECTS

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ABSTRACT:

Background: Hepcidin has emerged as the central regulatory molecule of iron homeostasis. Iron deficiency and iron overload play a major role in molecular insights of many disease states and serum hepcidin normal values and biochemical correlations are of substantial importance.

Objective: The aim of this study is to examine the serum hepcidin reference range, gender and age differences, menopausal dependence and biochemical correlates in healthy subjects.

Methods: Serum hepcidin concentration was measured with a competitive enzyme-linked immunosorbent assay (DRG Hepcidin-25 ELISA Kit) together with hemoglobin, hematocrit, serum iron, transferrin and C-reactive protein in 120 healthy subjects both men and pre- and post-menopausal women.

Results: Normal serum hepcidin values were found in the range of 1,23 – 36,46 ng/mL (mean 9,25 ± 6,45 ng/mL).There were statistically significant differences in measured hepcidin levels between men (12,34 ± 7,37 ng/mL) and women (6,16 ± 3,2 ng/mL) ($p<0.01$) and between pre-menopausal (5,51 ± 2,8 ng/mL) and post-menopausal women (7,29 ± 3,59 ng/mL) ($p<0,05$). Strong correlations were found with serum ferritin and hemoglobin but not with serum iron, transferrin and CRP. No 5-year age interval differences were deemed significant.

Conclusion: Serum hepcidin concentration varied substantially between subjects, which is reflected in wide reference ranges. Serum hepcidin levels were gender and menopausal status related and were in correlation with hemoglobin and serum ferritin in healthy subjects.

Key words: hepcidin, range, gender, menopause, healthy subjects,

INTRODUCTION

Hepcidin has emerged as the central regulatory molecule of systemic iron homeostasis. It is predominantly produced by hepatocytes as a 25 amino acid peptide (2789.4 Da), that is secreted into the circulation [1]. It has been shown to bind in vitro to á2-macroglobulin and albumin in human plasma [2]. Hepcidin is produced as an 84-amino acid precursor that subsequently undergoes proteolytic cleavages to generate the mature form. Further hepcidin - 25 processing can result in the generation of two amino-terminal truncated isoforms, hepcidin-22 (hep-22) and hepcidin-20 (hep-20), which physiological role is still unclear [3]. Human gene named HEPC for hepcidin is constituted of 3 exons and 2 introns located on chromosome 19, in close proximity to USF2 gene [4]. Acute phase protein hepcidin is the master regulator of iron homeostasis [5]. Hepcidin binds to ferroportin, the only known iron export protein, which results in internalization and degradation of this transporter, which then blocks iron export from enterocytes and macrophages to the circulation [6].

Increased iron stores and inflammation decreases hepcidin production, whereas hypoxia, anaemia, iron deficiency, increased erythropoiesis and hepcidin synthesis. Thus, inflammation decreases the availability of iron, whereas hypoxia or anaemia increases iron release and absorption. Recent studies demonstrated that the hypoxia-inducible factor (HIF)-1 alpha contributes to (down-) regulation of hepcidin, which was suggested to be a direct transcriptional mechanism or mediated by muscle-derived soluble haemojuvelin, which may be increased by the HIF - dependent induction of furin activity. However, the molecular mechanisms of the hypoxic or anaemic regulation of hepcidin are far from being understood. Several studies demonstrated that the induction of erythropoiesis and not hypoxia or anaemia itself down-regulates hepcidin. The relationship between hepcidin production and erythropoiesis suggests presence of a regulator between the erythron and the liver, and several candidates for this role have been proposed, for example the soluble transferrin receptor (sTfR) and the growth differentiation factor (GDF)-15 [7]. Determination of serum hepcidin concentration may be a helpful tool in screening for hereditary hemochromatosis, thus preventing cumbersome procedures in the search for causative (rare) genetic variants. Furthermore, hepcidin concentrations have been suggested to negatively correlate with the severity of hemochromatosis and to determine the prognosis and need for stringency of the treatment protocol. Hepcidin concentrations may also be used in the management of patients with iron-loading anemias. In addition, hepcidin is a key in the diagnosis of iron refractory, iron deficiency anemia and might contribute for diagnosis of iron

ciency in patients with anemia of chronic diseases. Hepcidin might be a potential marker in the prediction of erythropoietin response and to guide treatment with erythropoietin and intravenous iron. Finally, measurement of serum hepcidin concentration is important for the monitoring of novel therapies for iron disorders that target hepcidin, its upstream regulators, or its downstream receptor ferroportin [8].

METHODS

Study population

The pilot study entailed a random sample of 120 male and female above or 18 years of age. The blood samples were taken between 8 AM and 10 AM; after overnight fast (12 hours). All participants gave written informed consent for participation in the study.

The following variables were extracted from the self-administered questionnaire: length, weight, age, use of iron supplements at time of blood donation for at least 6 months, presence of anemia determined by a physician, being a blood and/or plasma donor, pregnancy, and presence of a regular menstruation, cirrhosis or chronic hepatitis B and C, alcohol consumption - women with daily consumption of alcohol >40 g / day and men with daily alcohol consumption >60 g/day, haemochromatosis, concomitant infections, malignant disease, chronic diseases, immunosuppressive therapy, acute infections or invasive procedures (operations, catheterization) in the previous 6 months, neurological, endocrine or other systemic diseases, cardiovascular incident in the previous 6 months or pregnancies.

Laboratory methods

Serum hepcidin concentration was measured with a competitive enzyme-linked immunosorbent assay (DRG Hepcidin-25 ELISA Kit). It is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding. The micro titer wells are coated with a monoclonal (mouse) antibody directed towards an antigenic site of the hepcidin-25 molecule. Endogenous hepcidin-25 of a specimen sample competes with a hepcidin-25-biotin conjugate for binding to the coated antibody. After incubation, the unbound conjugate is washed off and a streptavidin - peroxidase enzyme complex is added to each well. After incubation, unbound enzyme complex is washed off and substrate solution is added. The blue color development is stopped after a short incubation time, turning the color from blue to yellow. The intensity of color developed is reverse proportional to the concentration of hepcidin in the specimen sample. Hemoglobin (HGB) and hematocrit (HCT) were measured using the Sysmex analyzer. Total serum iron was measured by colorimetric measurement using ascorbate / Ferrozine reagents (Biosystems) on an Biosystems analyzer. Serum ferritin causes agglutination of latex particles coated with anti-human ferritin antibodies. The agglutination of the latex particles is proportional to the ferritin concentration was determined by turbidimetry on Biosystems analyzer. Serum C-reactive protein (CRP) causes agglutination of the latex particles coated with antihuman C-reactive protein. The agglutination of the latex particles is proportional to the CRP concentration and it was measured by turbidimetry on Biosystems analyzer.

Statistical analysis

Statistical analysis was performed with SPSS for Windows release 16.0.2, using the standard descriptive methods (mean ± SD), and corresponding analytical tests. Levene's Test for Equality of Variances was performed to determine the equality of variances, and appropriate independent samples, while the Student's t-test was used to compare the means. The correlation between the results was tested with the Pearson´s Correlation Coefficient. The data are presented as mean± standard deviation (SD) and $p \leq 0.05$ is considered statistically significant

RESULTS

Characteristics of the study population

Study included 120 participants (age 41,65 ± 12,3), 50% of them were males (age 39,73 ± 12,25) and 50% women (age 43,57 ± 12,2). HGB and HCT were increased in males, compared with women. The concentration of iron was significant increased in males compared to females. Serum ferritin concentration was considerably lower in women than in men, which is in concordance with the lower median iron concentration and transferrin observed in women compared with men.

Table 1. The characteristics of the study population.

Variables	All	Males	Females	P
N	120	60	60	
Age (years)	41,65 ± 12,3	39,73 ± 12,25	43,57 ± 12,2	P=0,089 NS
Haemoglobin (g/dl)	139,76 ± 14,5	149,7 ± 11,75	129,82 ± 9,3	P=0,000 p<0,01
Hematocrit (L/L)	0,415 ± 0,04	0,43 ± 0,03	0,398 ± 0,03	p = 0,000 p<0,01
Ferritin(μg/L)	94,59 ± 65,9	120,2 ± 70,67	69,01 ± 49,36	p= 0,00004p<0,01
Serum hepcidin (ng/mL)	9,25 ± 6,45	12,34 ± 7,37	6,16 ± 3,2	p = 0,000 p<0,01
CRP(mg/l)	2,87 ± 2,8	2,69 ± 2,3	3,06 ± 3,2	p = 0,54 NS

Table 1 present characteristics of the study population (N = 120). Data are means with std. deviation and p-values of males *vs* females by t-test.

Age- and sex-specific reference ranges for serum hepcidin concentration in the reference set

The concentration of hepcidin in all participants was ranged from 1,23 – 36,46 ng/mL (mean 9,25 ± 6,45 ng/mL). Statistical analysis showed that males (3,493 - 30,471 ng/mL) had statistically higher hepcidin levels than women (2,740- 13,208 ng/mL). Reference values for hepcidin for all participants were 2,933 - 21,913 ng/mL. Reference ranges of serum hepcidin concentration in the reference per 5-year age group are given for all participants in Table 2. Serum hepcidin concentration in men was almost constant over age. Hepcidin concentrations in women trend upwards as they progress through menopause, with median serum hepcidin concentration of 4,71 ng/mL for premenopausal women and 6,39 ng/mL for menopausal women.

Table 2. Reference ranges for serum hepcidin (ng/mL) per 5-year age group for all participants

Age	N	Median	5%	95%
18-24	10	7,9	3	15,3
25-29	20	5,76	3,03	19,1
30-34	9	6,57	2,93	16
35-39	13	14,74	3,25	30,79
40-44	14	6,072	3,42	22,57
45-49	16	6,1	1,23	30,6
50-54	14	8,55	4	20
55-59	15	7,06	2,74	22,01
60	9	10,43	2,13	36,46

The lowest median hepcidin concentration (3- 15,3 ng/mL) was found in the category 18-24 years of age, whereas the highest median concentration was observed in the category of 60 years of age (2,13 - 36,46 ng/mL). Serum hepcidin concentration varied substantially between subjects, which is reflected in wide reference ranges.

The median hepsidin values in healthy premenopausal women is 4,71 ng / mL (rank 1,23 - 14,7 ng / mL), while the group in healthy women after menopause amounts to 6,39 ng / mL (rank 2 13 - 14,67 ng / mL). Statistical analysis confirmed the difference in values between hepsidin healthy women before and after menopause as significant for p <0.05 (Z = 1,94 p = 0,042).

Table. 3. Values of hepcidin in pre-menopausal and post-menopausal women

Parameter(units)	Females		p-value
	Pre-menopausal n = 38	**Post-menopausal** n = 22	
Hepcidin(ng/mL) mean±SD, range	5,51 ± 2,8 1,23 – 14,7	7,29 ± 3,59 2,13 – 14,67	Z = 1,94 p = 0,042*

p<0,05 **p<0,01

Biochemical correlates of serum hepcidin concentration

We found that hepcidin levels were strongly correlated with HGB and HCT (R = 0,428, p < 0,05; R = 0,347, p < 0,05) (Table 4). A positive association between iron and serum hepcidin concentration was observed (R = 0,189 p < 0,05) (Table 4).Additional statistically significant associations were found for increasing serum hepcidin concentration and ALT (R=0,186, p < 0,05) (Table 4).

These analyses revealed ferritin to be most strongly associated with serum hepcidin concentration. CRP and transferrin were not statistically significantly associated with serum hepcidin (Table 4).

Table 4. Results of correlations of hepcidin with: HGB, HCT, serum iron cocentrations , CRP, ferritin and t
ferrin.

Hepcidin with	Spearman Rank (R)	p – value
HGB	**R = 0,428**	p < 0,05
HCT	**R = 0,347**	p < 0,05
Iron	R = 0,189	p < 0,05
CRP	R = 0,146	p > 0,05
Ferritin	**R = 0,577**	p < 0,05
Transferin	R = 0,016	p > 0,05

DISCUSSION

In this study, we used a newly developed ELISA test from DRG (http://www.drg-diagnostics.de) with monoclonal antibodies against bioactive hepsidin-25, without detectable cross reactivity against pro hepcidin, á-fetoprotein, human chorionic gonadotropin, human placental HPL and follicle stimulating hormone. We set found hepcidin reference values in healthy population in the range 2,933 - 21,913 ng / ml. These results confirm data from the study in a Geert et al. [9] that use the same kit and receive reference values 20.5-66 ng / mL. Ashby et al. [10] are reference values for hepcidin in healthy volunteers in the range of 2-56 ng / ml, with a median of 11 ng / ml. In our study a statistically significant difference between sexes was found - namely hepcidin is significantly higher in men compared to women. A similar study of Ganz T et al. [11] shows that men have higher concentrations of hepcidin than women. It was also found that post-menopausal women have significantly higher levels of hepcidin compared to pre-menopausal women. These results confirmed the data of Galesloot TE et al. [8]. Still hepcidin was significantly higher in men compared to both pre-menopausal and post-menopausal women. These differences are almost double between men and women after menopause and triple between men and women in the period before menopause. When examining correlations serum concentration of hepcidin were strongly bound to ferritin, but not to transferrin. These results confirm published data from Galesloot TE et al, Roe MA et al. [8, 12]

CONCLUSION

ELISA method for hepcidin determination is characterized by high reliability and analytical selectivity for accurate quantification of hepcidin with relatively short analysis time and provide insights of hepcidin relationships with gender, age and menopausal status in women. Our study provides age- and sex-specific reference ranges of serum hepcidin concentration and indicates ferritin as the primary correlate of serum hepcidin concentration.

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Please cite this article as: Ilkovska B, Kotevska B, Trifunov G, Kanazirev B. Serum hepcidin reference range, gender differences, menopausal dependence and biochemical correlates in healthy subjects. *J of IMAB.* 2016 Apr-Jun;22(2):1127-1131. DOI: http://dx.doi.org/10.5272/jimab.2016222.1127

Received: 18/04/2016; Published online: 31/05/2016

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Volume : 6 | Issue : 6 | June 2016 ISSN - 2250-1991 | IF : 5.215 | IC Value : 77.65

Research Paper Medical Science

Impact of Lipid Status, Liver Enzymes and Iron Homeostasis on Metabolic Syndrome Among Adult People

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ABSTRACT

Metabolic syndrome is consisted of a set of metabolic disturbances which support the risk increasing of cardiovascular disease and diabetis mellitus. Aim of this study is to present impact of lipid status, liver enzymes and iron homeostasis on metabolic syndrome among adult people. The study included 240 subjects at the age of 18 to 65 who were divided in two groups(examined and control group). The total number of patients with Metabolic syndrom was 120. In our research it was confirmed that at patients with metabolic syndrome there are increased values of feritin and hepcidin compared to the control group. In our research difference in the values of cholesterol statically was confirmed as significant which is due to the significantly higher values of cholesterol in the group with metabolic syndrome compared to the group of healthy people. We discover that the tests for liver function are higher at women with metabolic syndrome compared to the control group of women.

KEYWORDS lipid status, iron, metabolic syndrome

Introduction

Metabolic syndrome does not represent new medical condition. In early 1920 Swedish doctor Kylin published interesting observations for aggregation of some metabolic risk factors (1). Still the term "metabolic syndrome" was not formilized until 1998 (2). Other terms which are used as synomims to metabolic syndrome are: syndrome X (3), deadly quarter (4) and syndrome of resistance of insulin (5).

Metabolic syndrome is consisted of a set of metabolic disturbances which support the risk increasing of cardiovascular disease and diabetis mellitus (6,7). In 2001 National program for education for cholesterol (NCEP:ATPIII) announced its definition which includes at least three of five criteria for metabolic syndrome (8).

Definition of metabolic syndrome according to ATP III Panel III for treatment of adults clinical identification of metabolic syndrome (8).

1) abdominal obesity, defined as the presence of waist circumference ≥102 cm in men or ≥88 cm in women;

2) fasting plasma glucose ≥ 6.1 mmol/l or drug treatment for elevated blood glucose;

3) serum triglycerides ≥ 1.69 mmol/l or drug treatment for elevated triglycerides;

4) serum HDL cholesterol in men< 1.03 mmol/l and <1.29 mmol/l in women or drug treatment for low HDL-C;

5) blood pressure ≥130/85 mmHg or drug treatment for elevated blood pressure.

Risk factors for appearance of metabolic syndrome are : overweight, physical inactivity, getting older, diabetis mellitus and etc.

Etiology of metabolic syndrome includes: resistance to insulin, increased size of the waist, dyslipidemy, intolerance of glucoses, hypertension, adiponectin and etc.

Aim of this study is to present impact of lipid status, liver enzymes and iron homeostasis on metabolic syndrome among adult people.

Material & Methods,

This study was carried at the Department of medical biochemistry and Diabetes Center of Public Health Organization Clinical hospital d-r Trifun Panovski in Bitola.

The study was approved by the Ethics Committee of Health Organization Clinical hospital d-r Trifun Panovski, and all of the procedures were performed in accordance with ethical approval institutional guidelines. The study protocol followed the ethical guidelines of the most recent Declaration of Helsinki. Written consent was obtained from the participants prior to the start of the study.

The study included 240 subjects at the age of 18 to 65 who were divided in two groups (examined and control group).

The total number of patients with metabolic syndrom was 120, recruited from Diabetes Research Centre of Health Organization Clinical hospital d-r Trifun Panovski, Bitola, R.Macedonia.

Individuals aged 18 years or older were eligible to participate in the study. In this analysis we included subjects with available complete data allowing their classification according National program for education for cholesterol (NCEP:ATPIII) which includes at least three of five criteria for metabolic syndrome.

Exclusion criteria were history of: cirrhosis or chronic hepatitis B and C, clinical evidence of bleeding in the previous 6 months, anemia (hemoglobin <120 g / L), treatment with

...on in the previous year, alcohol consumption - women with daily consumption of alcohol > 40 g / day and men with daily alcohol consumption > 60 g / day, donation of blood in the previous 6 months, haemochromatosis, concomitant infections, malignant disease, chronic diseases other than diabetes mellitus type 2, immunosuppressive therapy, acute infections or invasive procedures (operations, catheterization) in the previous 6 months, neurological, endocrine or other systemic diseases, cardiovascular incident in the previous 6 months and pregnancies.

The remaining 120 patients are healthy people, blood donors from the Department of Transfusion Medicine of Health Organization Clinical hospital d-r Trifun Panovski.

Clinical and laboratory measurements

Study data included a medical history, a physical examination, information provided by a questionnaire, anthropometric measurements, and laboratory measurements.

The medical and drug prescription history were assessed by the examining physicians. All of the participants were asked to respond to a health-related behavior questionnaire, which included the topics of alcohol consumption, smoking, and exercise.In addition, the participants were asked about their physical activities per week, duration of the same in order to produce perspiration such as jogging, bicycling, and swimming (≥1 time/ week).

Blood samples were collected after 12 h of fasting and drawn from an antecubital vein. Serum levels of enzymes, lipid profile, iron, ferritin wer measured by automated chemistry analyzer (Biosystems, Spain).

Hepcidin levels were measured with ELISA kit (DRG Hepcidin-25 bioactive ELISA, Marburg).

The data are presented as mean± standard deviation (SD). The results were done with the SPSS version 13.

Results

The average age of the examined group of patients with metabolic syndrome is 52,87±7,42, the youngest patient with metabolic syndrome is 32 years old and the oldest is 60 years of age. Men from the examined group with average age of 51,03±7,94 are significantly younger than women from the same group who are at the average age of 54,7±6,43 (t=2.78 p=0,006).

Analyses of lipid status

Average values of cholesterol in the whole examined group and in groups male and female respondents are 5, 39±1, 22, 5, 58±1, 26 и 5, 21±1, 16 correspondingly. The average value of cholesterol is insignificantly higher in the group of sick male respondents (p>0,05).

Insignificant differences are registered between men and women from the examined group and regarding the average values of HDL-cholesterol (1,19±0,29 вс 1,32±0,28 p>0,05).

Values of LDL-cholesterol in the examined group are in the range from 1,05 – 6,34, with average value that is medium of 2,95.In male examined group LDL-cholesterol has minimal value of 1,25, maximal 6,34, medium of 2,98, while in the female examined group values of LDL- cholesterol are in the range from 1,05 to 5,72, with medium of 2,94.Statistic analyses confirmed the values of LDL- cholesterol in the group of male with metabolic syndrome significantly higher from female with metabolic syndrome (Z=2,53 p=0,011).

Troglycerides in the examined group have medium from range (0,76 – 6,35). In group with metabolic syndrome men have medium of 2,33 (range 0,81 – 6,35), women have medium value of triglycerides 1,81 (range 0,76 – 4,35). Value of triglycerides is significantly higher at group of men with metabolic syndrome compared to the group women with metabolic syndrome (Z=3,16 p=0,0016).

Values of ApoA in the group with metabolic syndrome are in the range of 56,0 – 171,0, with medium of 87,5, while the group of examined men ApoA has medium of 78 (range 56 – 170), while in the group examined women values ApoA have medium of 82, 5 (range 57 – 171). Differences in values of ApoA between men and women with metabolic syndrome were also statistically confirmed as significant as a result of evidently higher values in the group of women with metabolic syndrome (Z=2,59 p=0,0096).

Average value of ApoB in the whole examined group in both male and female examined group is 170,22±32,13, 179,2±30,79 and161,22±31,13 consequently. Statistic analyses confirmed that the sex has significant influence to the values of ApoB at respondents with metabolic syndrome that is men with this disease have significantly higher average values of ApoB compared to women (t=3,18 p=0,0018).

Table 1. Present mean values ±SD, median, rang of serum concentrations of: cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, ApoA, apoB in patientrs with methabolic syndrom.

Variable (unit)	Total N = 120	Males N = 60	Females N = 60	p-value
Cholesterol (mmol/l) mean±SD, median, rang	5,39 ± 1,22 5,21 2,4 – 8,89	5,58 ± 1,26 5,23 2,91 – 8,89	5,21 ± 1,16 5,17 2,4 – 7,9	t=1,66 p=0,099 ns
HDL-cholesterol (mmol/l) mean±SD, median, rang	1,26 ± 0,29 1,2 0,6 – 2,0	1,19 ± 0,29 1,11 0,6 – 2,0	1,32 ± 0,28 1,3 0,63 – 1,96	t=0,334 p=0,74 ns
LDL–cholesterol (mmol/l) mean±SD, median, rang	2,91 ± 1,31 2,95 1,05 – 6,34	2,95 ± 1,5 2,98 1,25 – 6,34	2,87 ± 1,09 2,94 1,05 – 5,72	Z=2,53 p=0,011*
Triglycerides (mmol/l) mean±SD, median, rang	2,28 ± 1,11 2,11 0,76 – 6,35	2,59 ± 1,26 2,33 0,81 – 6,35	1,97 ± 0,84 1,81 0,76 – 4,35	Z=3,16 p=0,0016**
ApoA(mg/dl) mean±SD, median, rang	94,67 ± 30,09 87,5 56,0 – 171,0	89,42± 30,78 78 56 – 170	99,93 ± 28,67 82,5 57 – 171	Z=2,59 p=0,0096**
ApoB(mg/dl) mean±SD, median, rang	170,22 ± 32,13 167,79 78,0 – 240,35	179,2± 30,79 183,08 108,7 – 240,3	161,22± 31,13 155,42 78 – 232	t=3,18 p=0,0018**

***p<0,05 **p<0,01**

Analyses of enzyme status

All enzymes which were analyzed in the research (ALT, AST и GGT), have significantly higher serum concentrations in a group of men with metabolic syndrome compared to the group of women with metabolic syndrome. Values of the enzyme ALT in the group with methabolic syndrome have medium of 30,2(range 8,1 – 82,0), while in the group of male and female respondents ALT has medium of 39,23 (range

...,95 – ...,6) and medium of 23,7 (range 8,1 – 82) consequently ...4,47 p<0,000008).

...ium of the values of the enzyme AST in the group with methabolic syndrome is 26,65 (range 18,3 – 79,70), in male examined group is 28,3 (range 19,3 – 79,7), while in the female examined group the values of AST are considerably lower with medium 25,55 (range 18,3 – 79,0) (Z=2,17 p<0,029).

In the group of respondents with metabolic syndrome the measured serum concentrations of the enzyme GGT are in the range from 7,4 to 101,1, with average of 31,0. This enzyme also presents significantly higher values in the examined group of men compared to the examined group of women. (Z=5,49 p<0,001). GGT enzyme has medium of 40,0 in the group of men and 22,6 in the group of healthy women.

Table 2.Present mean values ±SD, median, rang of serum concentrations of: AST, ALT, gGT in patientrs with methabolic syndrom.

Variable (unit)	Total N = 120	Males n = 60	Females n = 60	p-value
ALT(U/L) mean±SD, median, rang	35,94 ± 19,75 30,2 8,1 – 82,0	43,59 ± 20,97 39,23 11,95 – 81,6	28,27 ± 15,09 23,7 8,1 – 82	Z = 4,47 p = 0,000008**
AST(U/L) mean±SD, median, rang	30,94 ± 13,6 26,65 18,3 – 79,70	33,55 ± 15,56 28,3 19,3 – 79,7	28,33 ± 10,84 25,55 18,3 – 79,0	Z = 2,17 p = 0,029*
gGT(U/L) mean±SD, median, rang	36,63 ± 22,26 31,0 7,4 – 101,1	45,86 ± 22,66 40,0 10,5 - 100	27,41 ± 17,68 22,6 7,4 – 101,1	Z = 5,49 p < 0,001**

***p<0,05 **p<0,01**

Analyses of iron, its transporters feritin, transferin and hormone regulator of iron hepcidin.
The average value of the serum iron in the examined group is 15,79±5,28, that is 17,23±5,22 in the examined group of men and 14,36±4,99 in the examined group of women. For p<0,01 there is significant difference in the average values of the serum iron between men and women from the examined group as a result of significantly higher average values in the group of men with metabolic syndrome (t=3,07 p=0,0026).

Men from the examined group have significantly higher values of feritin than women from the same group (Z=4,04 p=0,00005). Medium that is the average value of feritin in the group of examined men and women is 116,0 (range 34 – 668) and 11,5 (range 11 – 456) consequently.

In the group with methabolic syndrome values of transferin are registered in the range of 172,0 to 582,0, with average value of 244. Male and female respondents have insignificantly different values for (p>0, 05).

Results from our research showed that at the respondents with metabolic syndrome, sex has significant influence to the values of hepcidin (Z=5,54 p<0,001). Men with metabolic syndrome have significantly higher values regarding the respondents from female sex. Value of this hormone regulator of iron, in male examined group has medium of 20, 75 (range 2, 47 – 85, 98), while in the female examined group the medium of hepcidin is 10, 81 (range 2, 93 – 24, 05).

In the whole group of respondents with metabolic syndrome the values of hepcidin are in the range from 2,47 – 85,98, with medium 14,29.

Table 3.Present mean values ± SD, median, rang of serum concentration of: iron, ferritin and hepcidin in patients with metabolic syndrom

Variable (unit)	Total N = 120	Males N = 60	Females N = 60	p-val...
Iron (µmol/l) mean±SD, median, rang	15,79 ± 5,28 14,95 5,7 – 28,8	17,23 ± 5,22 16,3 7,6 – 28,8	14,36 ± 4,99 13,5 5,7 – 24,4	t=3,07 p=0,0026**
Ferritin (ng/ml) mean±SD, median, rang	158,47 ± 118,75 129,0 11,0 – 668,0	197,9 ± 142,57 149,5 34 – 668	118,98 ± 70,31 111,5 11 – 456	Z=4,04 p=0,00005**
Hepcidin(ng/mL) mean±SD, median, rang	18,38 ± 15,24 14,29 2,47 – 85,98	25,54 ± 18,33 20,75 2,47 – 85,98	11,23 ± 5,3 10,81 2,93 – 24,05	Z=5,54 p<0,001**

**** p <0,01**

Results from the comparative analyses of respondents from the control group and examined group
In this part of the research results are presented that were received by comparison of the healthy respondents and the respondents with metabolic syndrome.

At the same time parameters where the significant difference by sex has not been confirmed, only the difference between control and examined group has been tested while for those parameters for which there is significant difference regarding sex, the differences between men from the control group and men from the examined group have been compared as well as between women from the both groups.

Analyses of lipid status control group / the group with methabolic syndrome
The average value of cholesterol has value 5, 05±0, 8 in the control group of respondents, and 5,39±1,22 in the group with methabolic syndrome. Difference in the average values of 0, 3 statistically was confirmed as significant (t=2, 6 p=0, 01), which is based on significantly average values of cholesterol in the group with metabolic syndrome compared to the group of healthy respondents.

Results from our research showed that men from control group and the group with methabolic syndrome have significantly different values of LDL- cholesterol, and the remaining analyzed parameters of lipid status: HDL- cholesterol, triglycerids, ApoA and ApoB significantly differ between men from the control group and the group with methabolic syndrome.

Men from the control group have significantly higher average values of HDL- cholesterol compared to the men from the examined group (1,4±0,3 вс 1,19±0,29 t=3,8 p=0,0002).

Triglycerides have significantly lower values in the control group of men compared to the sick group of men (medium 1, 44 vs. 2, 33 Z = 5, 98 p<0,001).

Values of ApoA present significnaly higher values in the group of men without metabolic syndrome compared to the group of men with metabolic syndrome (medium 99 vs78 Z=2,33 p=0,02).

In the control group men significantly lower values ApoB are registered compared to the examined group of men (150, 6±33, 3 вс 179, 2±30, 79 t=4, 89 p=0, 000003).

Women from the control group and the group with metabolic syndrome as well as men insignificantly differ regarding the values of LDL- cholesterol, and significantly differ regarding HDL- cholesterol, triglyceride, ApoA and ApoB.

The average values of HDL- cholesterol in the group of healthy and the group of sick women are 1,58±0,4 и 1,32±0,28 consequently. Statistically seen the difference between two

...oups ...gnificant (t=3,87 p=0,00018), as a result of significantly ...er average values in the control group of women.

...s of triglycerides are significantly lower in the group without metabolic syndrome (medium 1, 05 vs. 1, 81 Z=5, 68 p<0,001).

Women from the control group have significantly higher values of ApoA compared to the women of the group with metabolic syndrome (medium 133, 5 vs. 82, 5 Z= 4, 47 p=0, 000008).

In the group of healthy women significantly lower values are registered of ApoB compared to the group of women with metabolic syndrome (148, 16 vs. 155, 42 t=2, 03 p=0,044).

Table 4. Present statistical analyzes of correlation between serum concentrations of cholesterol in two groups - control and group with metabolic syndrom

Variable (unit)	Control group N = 120	Group with metabolic syndrome N = 120
	Total N = 120	Total N = 120
Cholesterol (mmol/l) mean±SD, median	5,05 ± 0,8 5,235	5,39 ± 1,22 5,21
tested differences control group/group with metabolic syndrom t=2,6 p=0,01*		

***p < 0,05**

Table 5.Present statistical analyzes of correlation between serum concentrations of HDL-cholesterol, LDL-cholesterol, triglycerides, ApoA, ApoB in two groups - control group and group with metabolic syndrom

Variable (unit)	Control group N = 120		Group with metabolic syndrom N = 120	
	Males N= 60	Females N= 60	Males N=60	Females N=60
HDL-chol. (mmol/l) mean±SD, median	1,4 ± 0,3 1,39	1,58 ± 0,4 1,6	1,19 ± 0,29 1,11	1,32 ± 0,28 1,3
tested differences males control group / males with metabolic syndrom t = 3,8 p=0,0002** females control group / females with metabolic syndrom t = 3,87 p=0,00018**				
LDL-chol. (mmol/l) mean±SD, median	3,025 ± 0,55 3,07	2,88 ± 0,7 2,86	2,95 ± 1,5 2,98	2,87 ± 1,09 2,94
tested differences males control group / males with metabolic syndrom Z=0,5 p=0,58 ns females control group / females with metabolic syndrom Z=0,07 p=0,09 ns				
Triglycerides (mmol/l) mean±SD, median	1,49 ± 0,5 1,44	1,185 ± 0,5 1,05	2,59 ± 1,26 2,33	1,97 ± 0,84 1,81
tested differences males control group / males with metabolic syndrom Z=5,98 p<0,001** females control group / females with metabolic syndrom Z=5,68 p<0,001**				
ApoA(mg/dl) mean±SD, median	112,62± 57,6 99,0	136,47 ± 48,4 133,5	89,42± 30,7 78	99,93 ± 28,67 82,5
tested differences males control group /males with metabolic syndrom Z = 2,33 p=0,02* females control group/females with metabolic syndrom Z = 4,47 p=0,000008**				
ApoB(mg/dl) mean±SD, median	150,6 ± 33,3 150,775	148,04± 39,42 148,16	179,2±30,79 183,08	161,22±31,13 155,42
tested differences males control group/males with metabolic syndrom t=4,89 p=0,000003** females control group/females with metabolic syndrom t=2,03 p=0,044*				

***p < 0,05 **p < 0,01**

Analyses of enzyme status control group / the group with metabolic syndrome

The analyses of the enzyme status between men from the control group and the group with metabolic syndrome showed that both groups men have insignificantly different values of ALT and AST (Z=0,97 p=0,33 и Z=0,42 p=0,67 cconsequently). Significant difference in the values of GGT (Z=4, 49 p=0, 000007) between healthy and sick men is registered. Medium of GGT in the control group of men is 25, 15 and it is significantly lower than the medium from the examined group which has value 40.

Women from the control group and from the group with metabolic syndrome differ significantly regarding the values of ALT, AST и GGT.

In the control group of women significantly lower values are registered of ALT regarding the examined group of women. (medium 19,8 vs 23,7 Z=2,4 p=0,016).

Values of AST are significantly lower in the group of healthy women compared to the group of sick women. (medium 23,1 vs 25,5 Z=2,14 p=0,03).

GGT enzyme has significantly lower values in the group of women without metabolic syndrome compared to the group of owmen with metabolic syndrome (medium 13,95 vs. 22,6 Z=4,14 p=0,000006).

Table 6.Present statistical analyzes of correlation between serum concentrations of ALT, AST, gGT in two groups - control group and group with metabolic syndrome

Variable (unit)	Control group N = 120		Group with metabolic syndrom N = 120	
	Males N= 60	Females N= 60	Males N=60	Females N=60
ALT (U/L) mean±SD, median	39,67 ± 19,8 35,025	24,28 ± 13,3 19,8	43,59 ± 20,97 39,23	28,27 ± 15,09 23,7
tested differences males control group /males with metabolic syndrom Z=0,97 p=0,33 ns females control group/females with metabolic syndrom Z = 2,4 p=0,016*				

AST (U... mean...	31,26 ± 8,8 27,8	25,42 ± 7,9 23,1	33,55 ± 15,56 28,3	28,33 ± 10,84 25,55
tested differences males control group/males with metabolic syndrom Z=0,42 p=0,67 ns females control group/females with metabolic syndrom Z=2,14 p=0,03*				
gGT (U/L) mean±SD, median	39,63 ± 20,05 25,15	18,09 ± 14,0 13,95	45,86 ± 22,66 40,0	27,41 ± 17,68 22,6
tested differences males control group/males with metabolic syndrom Z=4,49 p=0,000007** females control group/females with metabolic syndrom Z = 4,14 p=0,000006**				

***p < 0,05 **p < 0,01**

Analyses of iron, its transporters feritin, and hormone regulator of iron hepcidin in control group / the group with metabolic syndrome

There is significant difference between men from the control and examined group in the values of serum iron, feritin and hepcidin.

The average values of the serum iron in the group of healthy men is 15,03±5,77 and it is significantly (t=2,18 p=0,03) lower than the average value in the group of men with metabolic syndrome which is 17,23±5,22.

Feritin has significantly lower values in the control group of men compared to the group of sick men (medium116 vs. 149,5 Z=3,2 p=0,01).

For p<0,01 significant difference is confirmed in the average values of hepcidin between men from the group of healthy repsodnents and the group with metabolic syndrome (t=5,18 p=0,000001). The average values of hepcidin in the control and group of sick men are 12, 34±7, 37 и 25, 54±18, 33 consequently that is the same are significantly lower than in the group of healthy men.

Between women from control and examined group there is significant difference of values of feritin and hepcidin, and insignificant difference regarding the values of serum iron.

Women in the control group have insignificantly lower average serum values of iron than women in the group with metabolic syndrome (12, 91±6, 1 vs. 14, 36±4, 99 p>0, 05).

In the group of healthy women significantly lower values are registered of feritin compared to the women from the group with metabolic syndrome (medium 56 vs. 111,5 Z=4,8 p=0,000002).

Values of hepcidin in the control group and the group with metabolic syndrome of women are average 6,16±3,2 и 11,23±5,3 consequently. Difference in the average values between two groups of the respondents from 5,07 was statistically confirmed as significant that is important (t=6, 3 p<0,001), that is healthy women have significantly lower values of hepcidin compared to women with metabolic syndrome.

Table 7.Present statistical analyzes of correlation between serum concentrations of iron, ferritin and hepcidin in two groups - control group and group with metabolic syndrom

Variable (unit)	Control group N = 120		Group with metabolic syndrom N = 120	
	Males N= 60	Females N= 60	Males N=60	females N=60
Iron (μmol/l) mean±SD, median	15,03 ± 5,77 13,85	12,91 ± 6,1 12,2	17,23 ± 5,22 16,3	14,36 ± 4,99 13,5
tested differences males control group/males with metabolic syndrom t=2,18 p=0,03* females control group/females with metabolic syndrom t=1,4 p=0,16 ns				
Ferritin (ng/ml) mean±SD, median	120,2 ± 70,67 116,0	69,01 ± 49,36 56,0	197,9 ± 142,57 149,5	118,98 ± 70,31 111,5
tested differences males control group/males with metabolic syndrom Z=3,2 p=0,01** females control group/females with metabolic syndrom Z=4,8 p=0,000002**				
Hepcidin (ng/mL) mean±SD, median	12,34 ± 7,37 10,97	6,16 ± 3,2 5,6	25,54 ± 18,33 20,75	11,23 ± 5,3 10,81
tested differences males control group/males with metabolic syndrom t=5,18 p=0,000001** females control group/females with metabolic syndrom t=6,3 p<0,001**				

***p < 0,05 **p < 0,01**

Discussion

Metabolic syndrome was described for the first time in the first half of the 20th century (6), and the world epidemy of oversize and obesity are the basic reasons for its identification. The central adiposity is the basic characteristic of the syndrome which reflects the fact for the strong bond between the waist and the increased adioposity which influence the distribution of metabolic syndrome (9).

The distribution of metabolic syndrome through the world is different and partially is a reflexion of the age and the ethnicity of people and used diagnostic criteria.

As a whole the distribution of the metabolic syndrome is increased with the age of the population. According to the data of the examination of the national health and nutrition of the USA, the distribution of the metabolic syndrome grows from 7% at respondents at the age of 20-29., 44% at the age of 60-69 and 42% at the age over 70 (10). In France the distribution of patients from 30-39 was <5, 6% at each sex and at the age of 60-64 it was 17,5% (11). The growing industrialization in the world is connected to the larger percentage of obesity of population. In 2000 a metabolic syndrome was diagnosed at 47 million people in the USA which means that it is present at 40% from the adult population (12).

During the last years the interest from the consequences by grown deposing of iron towards people health grows more and more (13). Although, the mechanisms for the potential effect of the iron towards the risk by metabolic syndrome are unclear there are two basic hypotheses.

According to the first hypothesis the increased iron, which is due to overdosed deposing (born or gained) can lead to damage of the liver, heart and other organs. Pancreas beta cells are also important target of toxical iron which causes resistance of glycosis and diabetis. When iron concentration grows in the organism the liver and peripherial resistance towards insluine increases and pancreas secretion of inslulin is decreased (14). Subplus of iron is dangerous as it initiates atherosclerosis carciongenesis diabetis and other diseases connected to the way of life (15).

The second hypothesis for the influence of the iron to the appearance of metabolic syndrome is connected to the capability of the iron to form reactive oxygen radicals and it is considered that the increased oxidative stress is key mechanism on the basis of iron induced resistance although there are still no clear evidences for this hypotehsis (16). Oxidative stress influences to the metabolism of glucoses and iron and causes resistance to insulin with decreased entrance of insulin in the cells and increased synthesis of feritin (17).

...e capacity of iron to transform into two stabile oxidative forms ... potential for creation of reactive oxygen and amino type... hydroxical radicals with Fenton и Haber-Weiss reac... The oxidative stress can cause death of beta cells of pancreas and leads to diabetes and chronically oxidative stress of the liver muscles and mass tissues causing inflammatory reaction and resistance to insulin in these organs (18).

Stores of irons expressed through concentration of feritin in serum are suggested to be inseparable part of the metabolic syndrome. Feritin is clinic indicator for the level of iron in the organism. The potential reason for increase of feirtin in β- cells of pancreas is especially sensitive to the effects of the oxygen radicals (19). The level of feritin correlates with several components of metabolic syndrome: increased triglycerides reduced HDL-cholesterol obesity. These discoveries refer to the fact that the concentration of feritin can be used as biomarker for metabolic syndrome (13,20,21).

In our research it was confirmed that at patients with metabolic syndrome there are increased values of feritin compared to the control group. Numerous examinations prove increased values of feritin at patients with metabolic syndrome (22-27). High concentration of serum feritin can be potentially used as screening biomarker for revealing of people who were exposed to risk from development of metabolic syndrome and they can be treated even in early stadiums of the diseases through preventative measures (28).

For the first time in 2012 Martinelli N et al. (29) published that the level of hepcidin grows progressively as a result of the increased level of feritin in the serum of patients with metabolic syndrome and it was noticed that people with metabolic syndrome have significantly higher values of feritin and hepcidin compared to people without metabolic syndrome which was confirmed in our research.

We discover that at men from control and examined group there is difference in the value of serum iron and hepcidin. In our research it was confirmed that at the patients with metabolic syndrome from male sex has increased values of serum iron and hepcidin compared to healthy men.

At women from control and examined group we discovered significant differences in the values of hepcidin. In the group of healthy women significantly lower values of hepcidin are registered compared to the women from the group with metabolic syndrome.

We discovered that the sex has significant influence towards the values of serum iron, feritin and hepcidin as a result of the significantly higher values at man compared to women which is due to the lost of iron with period at the women. Men with metabolic syndrome have significantly higher values of hepcidin compared to women in menopause and women in period after menopause. Menopause status influences the concentration of hepcidin with significantly lower values of hepcidin in the group of women with metabolic syndrome in the period of pre meno pause.

Dislipidemy is in the basis of the etiological factors for appearance of metabolic syndrome. The violation of lipoproteins at the metabolic syndrome leads to reaction of the concentration of HDL- cholesterol which as a consequence of the changes in the structure and metabolism of HDL- cholesterol.

In our research difference in the values of cholesterol statically was confirmed as significant which is due to the significantly higher values of cholesterol in the group with metabolic syndrome compared to the group of healthy people. These results are confirmation of the results from other research Nea KR et al (30).

Results from our research show that triglycerides ApoA and ApoB significantly differ between men and women from control group and the group with metabolic syndrome except at LDL-cholesterol difference is insignificant at men a... wom... We discovered that men and women from the co... group have higher values of ApoA compared to the examin... while from the other side ApoB is significantly higher a... with metabolic syndrome compared to men from control group. We discovered higher values ApoB at women with metabolic syndrome and these results are similar to the examination of Lim Y et al (31).

Hypertriglyceridemy is an excellent marker for the state of resistance to insulin and important diagnostic marker for metabolic sydnrome (6). In the research we confirmed these observations and we proved that triglycerides are significantly higher at women and men with metabolic syndrome compared to the control group. These results are similar with the examination of Hea KP et al (30).

HDL-cholesterol is lower at patients with metabolic syndrome compared to control group and that is confirmed in the research of Kasapoglu B et al (32).

The sex significantly influence to the values of LDL-cholesterol triglycerides and ApoB at patients with metabolic syndrome. We confirmed that values LDL-cholesterol triglycerides and ApoB in the group of men are significantly higher compared to women. Kawamoto R et al. (33) in their research of patients with metabolic syndrome discovered higher values of triglycerides at men compared to women which was confirmed in our research.

Values of ApoA are higher in the group of women. Our results are not in correlation with Kawamoto R et al (33) who discover higher concentrations of cholesterol, HDL–cholesterol LDL-cholesterol at women compared to men.

Several examinations discover that the liver enzymes can be connected to the metabolic syndrome through many metabolic disturbances as obesity, dislipedemy, diabetes and hypertension while the insulin resistance is considered as basic reason. Marker for liver steatosis was proved as independent risk factor of metabolic syndrome, diabetes, heart diseases. Increased values of ALT have positive correlation to diseases connected to metabolic syndrome for example diabetes type 2 and heart diseases. Average values of ALT, AST и GGT are statistically significantly higher at patients with metabolic syndrome. GGT is basic in glutnative homeostasis and it is important protector of the cell. GGT plays important role in the protective antioxygen system. Increased levels of GGT can be a marker for oxigent stress and subclinical inflammation. Although the relation between GGT and metabolic syndrome is not clearly understood, some mechanisms including the presence of oxigent stress can explain the connection and GGT can play a role in early diagnosis of metabolic syndrome with high prognostic value for metabolic syndrome and heart diseases (32).

We discover that the tests for liver function are higher at women with metabolic syndrome compared to the control group of women. These results are confirmed in literature data of Hea KP et al (30), who found higher concentrations of AST and ALT at women with metabolic syndrome compared to control group. Analyses of the enzyme status at men from control group and from group with metabolic syndrome proved that both groups of men have insignificant different values of ALT and AST. At healthy and sick men significant difference in values of GGT has been registered with lower values at control group of men compared to examined group. These results are confirmation of the literature data of Kasapoglu B et al (32). The sex is with significant influence to the enzymes ALT, AST and GGT and it confirmed that men have higher serum concentrations compared to women.

CONCLUSION

Ten years in a row metabolic syndrome was connected generally to insulin reisistance.The fact that the increased stores of iron increases the risk of metabolic syndrome led to discovery of new views to this disease. The fact that worries is that

crease incidence of sick with metabolic syndrome is registered this tendency is defined as pandemics. Data have been collected for a numerous diseases as heart disease, diabetes and many others, whose progression could be connected to increased stores of iron measured through concentration of feritin in serum.

Conflict of interest

The authors state that there is no conflict of interest. The authors have not received any funding or benefits from industry to conduct this study.

Acknowledgements

I would like to express my special thanks to Pharmachem for ELISA kits that sponsored me and for paying my publication fees.

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International Journal of Medical and Health Research
ISSN: 2454-9142
Impact Factor: RJIF 5.54
www.medicalsciencejournal.com
Volume 3; Issue 9; September 2017; Page No. 56-60

Incidence of pregnancy chromosomal abnormalities detected by screening which include: fetal nuchal translucency thickness, maternal serum free beta human chorionic gonadotrophin hormone, and pregnancy associated plasma protein: A, in Bitola

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Abstract

Introduction: The most effective method of screening chromosomal abnormalities is by a combination of fetal nuchal translucency thickness and maternal serum free beta human chorionic gonadotrophin hormone and pregnancy associated plasma protein-A at the first 10–14 weeks of pregnancy gestation.

Methods: The serum of 526 pregnant women was separated and pregnancy associated plasma protein-A, and free beta human chorionic gonadotrophin hormone were measured. The ultrasound scan included a full structural survey, and nuchal translucency. Risks for chromosomal abnormalities were calculated using the software Prisca - mathematical model which gives individual risks for trisomy 21, 18 and 13.

Results: Over a 2 – year period of time, screening was carried out in 526 pregnancies. Median maternal age was 29,3 years old (range: 13, 4 to 43 years old), and 64 (12, 2%) of women who were 35 years old or older at the time of this assessment. In this prospective study, in among of the 526 pregnant women overall, 48 (9,1 %) fetuses had an estimated risk for trisomy 21 and trisomy 13/18. In the rest of 478 (90, 9 %) cases, chromosomal abnormality was not found.

Discussion: The first trimester screen has been available in Macedonia for several years, but only recently have been determined effective means of early chromosomal abnormality screening. In cases with chromosomal abnormalities we found a significant correlation between free beta human chorionic gonadotrophin hormone and nuchal translucency.

Conclusion: The screening of chromosomal abnormalities in pregnancy and the assessing risk of Down syndrome, Edward syndrome and Patay are of utmost importance for all pregnant women and the society as well.With this screening we are going to prevent their occurrence and we will reduce the psychological and physical suffering of parents and society, especially in today's modern society, where the technology is most advanced in the industry, and prevention is really possible!

Keywords: chromosomal anomalies, first-trimester, screening, pregnancy associated plasma protein-A

Introduction

Chromosomal anomalies are a leading cause of perinatal mortality and developmental abnormality. Consequently, the principal goal of prenatal testing is to screen for chromosomal anomalies and to provide genetic counseling for parents [1]. Trisomies 21, 18 and 13 and sex chromosome aberrations are the most frequently occurring chromosomal abnormalities.

First-trimester risk assessment of common chromosomal aneuploidy is based on a combination of maternal age, maternal serum free beta human chorionic gonadotrophin hormone, pregnancy associated plasma protein-A, and fetal nuchal translucency thickness [2].

The association between advancing maternal age and increased risk of trisomy 21 is well known, and pregnant women older than 35 years at delivery are routinely offered invasive prenatal diagnostic testing. The most commonly used test for genetic diagnosis is amniocentesis, but the rate of spontaneous fetal loss related to amniocentesis averages about one in every 200 procedures. Because of this risk, serum analytic testing has become an important, noninvasive first step in detecting patients at risk for congenital abnormalities [3].

First trimester screening is performed between first 10 and 14 weeks of the gestation. The markers used for the risk calculation are 2 serum markers: pregnancy associated plasma protein-Aand free beta human chorionic gonadotrophin hormone.

In 1974, pregnancy associated plasma protein-A was the one out of four proteins identified in the plasma of pregnant women [4]. Pregnancy associated plasma protein-A is produced in great amounts during pregnancy by the syncytiotrophoblast [5] and can be detected in placental tissue, decidua, maternal serum, amniotic and caulomic fluids [6, 7].

Maternal serum levels of pregnancy associated plasma protein-A, in the first trimester of pregnancy, are decreased in pregnancies with fetal trisomies 21, 18 or 13, dysgenictriploid,

monosomy X and those with impaired placentation resulting in pre-eclampsia and delivery of small for gestational age neonate.

In normal pregnancy, serum pregnancy associated plasma protein-A concentration is affected by gestational age and maternal characteristics, including: weight, racial origin, cigarette smoking, diabetes mellitus and method of conception [8].

Increased levels of free beta human chorionic gonadotrophin hormone are associated with an increased risk of Down syndrome.

The third marker is the fetal nuchal translucency which is performed by ultrasound. The nuchal translucency measurement needs to be performed by experienced sonographers and should be obtained between the first 10 and 13 weeks and 6 days of the gestation. The majority of fetuses with Down syndrome have an increase nuchal translucency measurement when compared to normal fetuses of the same gestational age [3]. Nuchal translucency is the sonographic appearance of a collection of fluid under the skin behind the fetal neck during the first trimester of pregnancy. An increased nuchal translucency is not only a marker for chromosomal anomalies, but also a nonspecific indicator of abnormal development, common to several pathologic pathways, including an increased risk of miscarriage or fetal death, from 1.6% in those with nuchal translucency between the 95th and 99th percentiles to approximately 20% for values above the 99th percentile, and a 15-fold increased likelihood of lethal or serious malformation [9].

The crown – rump length was obtained by measuring the fetal length from the tip of the cephalic pole to the tip of the caudal pole in the midsagittal plane [1].

Edward syndrome (trisomy [18] is the second most common form of chromosomal aneuploidy. The first trimester screening of trisomy 18 is based on the ultra-sonographic finding of nuchal translucency and decreases in maternal serum pregnancy associated plasma protein-A and free beta human chorionic gonadotrophin hormone. This test can detect 86% to 89% of cases with a 0.5% to 1.0% false-positive rate [1].

In Macedonia there is none published data on the association between serum biochemical and ultra-sonographic markers and adverse pregnancy outcomes. Consequently, the purpose of this study was to examine screening for chromosomal abnormalities in first trimester of pregnancy using ultrasound and maternal serum markers.

Methods

This prospective interventional study was performed between April 2015 and March 2017 in the Clinical Hospital "Dr. Trifun Panovski" in Bitola, Macedonia. A total number of 526 pregnant women were screened during the first trimester.

Maternal weight was measured using a digital weight scale eighth a precision of 0.1 kg. The serum was separated and pregnancy associated plasma protein-A and free beta human chorionic gonadotrophin hormone were measured using solid phase, enzyme labeled chemiluminescent immunometric assay (Siemens Healthcare Diagnostics, Inc., Llanberis, UK).

Transabdominal and transvaginal ultrasound examination was performed by certified maternal fetal medicine specialists. The ultrasound scan included a full structural survey, and nuchal translucency was measured according to established guidelines. Ultrasound examinations were performed with high resolution equipment (Voluson E Expert 2008, General Electric, Austria or Siemens G50 Ultrasound, Siemens Medical Solutions USA, Inc.)

Risks for chromosomal abnormalities were calculated using the software Prisca - mathematical model which gives individual risks for trisomy 21, 18 and 13. This mathematical model takes into consideration the maternal age, the serum levels of various biochemical markers and the fetus ultrasound measurements. In addition, a number of factors play an important role in the calculation of the risk as they will affect the values of the maternal serum biochemical analyzes. This includes: gestational age, weight, race, smoking, diabetic status of the individual, the number of fetuses present, and whether in vitro fertilization treatment was used for conceiving.

A calculated risk ≥1:250 were defined as high-risk for Down syndrome and ≥1:300 was defined as high-risk for Edward syndrome.

Results

Over a 2 - year period of time, screening was carried out in 526 pregnancies. Median maternal age was 29, 3 years old (range: 13, 4 to 43 years old), and 64 (12, 2%) of women who were 35 years old or older at the time of the assessment.

Table 1: Demographic characteristics of studied population (526 women, 535 fetuses)

Variable	Median (range) (%)	Total subjects
Maternal age (years)	29.3 (13.4-43)	462
≥ 35 years		64
Maternal weight (kg)	66,9(40 -208)	422
Not reported	19.7%	104
Mode of conception		526
spontaneous	96.7%	509
In vitro fertilization	1.5%	8
Not reported	1.7%	9
Smoking status		526
Smoker	9.5%	50
Non – smoker	88.8%	467
Not reported	1.7%	9
Racial origin		526

Caucasian	100%	526
Diabetes mellitus		526
Mother with diabetes mellitus	0.7%	4
Mother without diabetes mellitus	97.3%	512
Not reported	1.9%	10
Number of fetuses		526
singleton	98.3%	517
twins	1.7%	9
Gestational age at screening (days)	86.6 (70-97)	526
Crown – rump length (mm)	60.7(6.6 – 83.3)	526
Pregnancy associated plasma protein-A (mIU/ml)	3.9 (0.26- 19.4)	526
Free beta human chorionic gonadotrophin hormone (ng/ml)	44.6 (6.2- 170)	526
Nuchal translucency (mm)	0.89(0.14-4.73)	526

The median gestational age at screening was 86, 6 days or 12+2 gestational weeks (range: 70 to 97 days or 10 to 13+6 gestational weeks). The median crown – rump length was 60, 77 mm (range: 6, 6 to 83,3mm). The median maternal weight was 66, 94 kg (range: 40 to 108 kg).

Our study shows that smoker mother are 50, non-smokers are 467 and 9 were not reported. About the mode of conception, 502 was spontaneous, assisted with in vitro fertilization were eight, and nine were not reported.

Among the 526 pregnant women, four mothers were with diabetes mellitus, non-diabetes were 51, 2, and ten were not reported.

About the number of fetuses present, singleton pregnancies were 517 and nine were twins.

Table 2: Demographic characteristics of 48 cases with chromosomal abnormalities and 478 cases with a low risk of trisomy 21, 13, 18.

Variable	**Median (range)(%) in unaffected pregnancies**	**Median (range)(%) in cases with chromosomal abnormalities**
Maternal age (years)	29.08 (13.4-43)	32,2 (15,1 – 42,9)
Maternal weight (kg)	67.07 (41 - 108	65,6 (40 -90)
Not reported		
Mode of conception		
spontaneous	96.3%	97,9%
In vitro fertilization	1.7%	0
Not reported	1.7%	2,1%
Smoking status		
Smoker	9%	14,6%
Non – smoker	89.3%	83,3%
Not reported	1.7%	2,1%
Racial origin		
Caucasian	100%	100%
Diabetes mellitus		
Mother with diabetes mellitus	0.9%	0
Mother without diabetes mellitus	99.1%	97,9%
Not reported	0.9%	2,1%
Number of fetuses		
singleton	98.3%	97,9%
twins	1.7%	2,1%
Gestational age at screening (days)	86.6 (69-97)	86,4 (76 -95)
Crown – rump length (mm)	60.8 (6.6 – 83.3)	59,7 (39,9 – 77,9)
Pregnancy associated plasma protein-A (mIU/ml)	4 (0.37- 19.4)	2,41 (0,26 – 9,37)
Free beta human chorionic gonadotrophin hormone (ng/ml)	40.6 (6.2- 157)	95 (11,2 – 558)
Nuchal translucency (mm)	0.86 (0.14-2.02)	1,2 (0,26 – 4,73)

Among the 526 pregnant women overall, 48 (9, 1 %) fetuses had an estimated risk for trisomy 21 and trisomy 13/18. Out of the 526 women, 478 (90, 9 %) were cases with a low risk of trisomy 21, 13, 18, where chromosomal abnormality was not found.

In this interventional study we identified 48 cases of chromosomal abnormality: thirty eight of trisomy 21, nine of trisomy13/18. We identified 37 fetuses with biochemical T21 risk: six are with risk > 1:50; eight with risk 1: 50 –1:100; eight with risk 1: 100 –1:150; nine with risk 1: 150 –1:200 and 6 with risk 1:200 –1:250. Also, we identified 16 fetuses with combined trisomy 21 risk: eleven of them are with risk > 1:50; one with risk 1: 50 –1:100; three with risk 1: 100 –1:150 and one with risk 1: 150 –1:200.

Among the 526 pregnant women, nine (1, 7%) women had high risk for trisomy 13 or trisomy 18 using a cutoff value of 1:300. Three of them are with risk > 1:50; one with risk 1: 50 –1:100; two with risk 1: 100 –1:150; one with risk 1: 150 –1:200 and two with risk 1:200 –1:250.

Seven fetuses had biochemical trisomy 21 risk and combined

trisomy 21 risk. Thirty fetuses had just biochemical trisomy 21 risk. Nine fetuses had just combined trisomy 21 risk. Six are with combined trisomy 21 risk and trisomy 13/18 risk. One was with biochemical trisomy 21 risk, combined trisomy 21 risk and trisomy 13/18 risk. Two are just with trisomy 13/18 risk.

Table 3: Chromosomal abnormality frequencies

Chromosomal abnormality	n
Normal	478
Trisomy 21 and trisomy 13/18	48
Biochemical trisomy 21 rick	39
Biochemical trisomy 21 rick > 1:50	6
Biochemical trisomy 21 rick 1: 50 –1:100	8
Biochemical trisomy 21 rick 1: 100 –1:150;	8
Biochemical trisomy 21 rick 1: 150 –1:200	9
Biochemical trisomy 21 risk 1:200 –1:250	6
Combined trisomy 21 risk	16
Combined trisomy 21 risk > 1:50	11
Combined trisomy 21 risk1:50 –1:100	1
Combined trisomy 21 risk1:100 –1:150	3
Combined trisomy 21 risk1:150 –1:200	1
Trisomy 13 or trisomy 18	9
Trisomy 13 or trisomy 18 risk > 1:50	3
Trisomy 13 or trisomy 18 risk 1: 50 –1:100	1
Trisomy 13 or trisomy 18 risk 1: 100 –1:150	2
Trisomy 13 or trisomy 18 risk 1: 150 –1:200	1
Trisomy 13 or trisomy 18 risk 1:200 –1:250	2
Biochemical trisomy 21 risk and combined trisomy 21 risk	7
Just biochemical trisomy 21 risk	30
Just combined trisomy 21 risk	9
Combined trisomy 21 risk and trisomy 13/18 risk	6
Biochemical trisomy 21 risk, combined trisomy 21 risk and trisomy 13/18 risk	1
Just with trisomy 13/18 risk.	2

In group of fetuses with chromosomal abnormalities we found this correlations: a significant correlation between mother age and mother weight (p= 0,023; p<0, 05) and between mother age and free beta human chorionic gonadotrophin hormone (p= 0, 22); a significant correlation was established between gestational age and pregnancy associated plasma protein-A (p= 0,000; p<0, 01) and between gestational age and crown – rump length (p= 0,000). Also we found a significant correlation between mother weight and crown – rump length (p= 0,047; p<0, 05); we found a significant correlation between pregnancy associated plasma protein-A, and crown – rump length (p=0,000; p<0, 01) and we found a significant correlation between free beta human chorionic gonadotrophin hormone and nuchal translucency (p= 0,036; p<0, 05).

Discussion

The first trimester screen has been available in Macedonia for several years, but only recently have been determined effective means of early chromosomal abnormality screening. This screening is the most accurate, non-invasive screening method available. In this prospective study of first-trimester screening for chromosomal abnormalities by a combination of maternal serum biochemical markers and ultrasound markers in among the 526 pregnant women overall, 48 (9, 1 %) fetuses had an estimated risk for trisomy 21 and trisomy 13/18. Out of the 526 women, 478 (90, 9 %) were cases where chromosomal abnormality was not found.

In cases with chromosomal abnormalities we found a significant correlation between mother's age and mother's weight and between mothers' age and free beta human chorionic gonadotrophin hormone. A significant correlation was found between gestational age and pregnancy associated plasma protein-A, and between gestational age and crown – rump length. Also we found a significant correlation between mother weight and crown – rump length; we found a significant correlation between pregnancy associated plasma protein-A, and crown – rump length, and between free beta human chorionic gonadotrophin hormone and nuchal translucency.

The results of this study demonstrate that an enlarged nuchal translucency (above the 95th percentile) and advanced maternal age are associated with adverse perinatal outcomes in a mixed population (low- and high-risk), which is consistent with the findings reported in other studies [10, 11].

Measurement of serum pregnancy associated plasma protein-A may be useful in screening for aneuploidies, neural tube defects and adverse pregnancy outcome. Effective use of serum pregnancy associated plasma protein-A in risk assessment and screening necessitates that variables from maternal characteristics and medical history which affect this measurement in normal pregnancy are taken into account. We found lower values of pregnancy associated plasma protein-A in cases with chromosomal abnormalities. About the values of free beta human chorionic gonadotrophin hormone we detect almost double higher values of free beta human chorionic gonadotrophin hormone in cases with high risk for chromosomal abnormalities compared between those with normal outcome. A positive correlation between high free beta

bi[illegible]n chorionic gonadotrophin hormone and increased risk for chromosomal abnormalities is reported in other studies as well [12, 13].

In this study, we have outlined our first 2 - year experience in screening pregnancies. The limitation of this study is a small sample size in comparison with larger studies. Additionally, because this study was conducted in only one center, the result cannot fully represent the screening performance in Macedonian population.

In summary, the purpose of this study was to provide information on screening performance of the first trimester combined test, in a medical center of Bitola. To our knowledge, this study is the first population study analyzing the result of the first trimester combined test performed in Macedonia.

Conclusion

The screening of chromosomal abnormalities in pregnancy and the assessing risk of Down syndrome, Edward syndrome and Patay are of utmost importance for all pregnant women and the society as well.With this screening we are going to prevent their occurrence and we will reduce the psychological and physical suffering of parents and society, especially in today's modern society, where the technology is most advanced in the industry, and prevention is really possible!

Competing Interests

Authors have declared that no competing interests exist.

Authors Contributions

Author Biljana Ilkovska designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors Bisera Kotevska Trifunova, Georgi Trifunov, Marina Trajkovska and Branimir Kanazirev managed the analyses of the study. Author Sandra Hristovska managed the literature searches. All authors read and approved the final manuscript.

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