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Content

Reviews

Original articles

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Категориални срещу дименсионални модели на класификация. Континуум на психозите

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Categorical vs dimensional models of classification. Continuum of psychoses

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РЕЗЮМЕ:

Настояща практика за диагностика в психиатрията е използване на синдромно базирани системи на класификация на психиатричните разстройства като петото издание на ДСМ (diagnostic and statistical manual of mental disorders) и десетото издание на МКБ (международна класификация на болестите), в които на практика се ползват симптоми и прояви за описателно категоризиране на диагностични единици. Основна цел на класификацията в психиатрията е подобряване на лечението и профилактиката.

Използваната в днешни дни нозологична класификация в Европа и САЩ е категориална и е наследник на дихотомните класификации на Крепелин и Блойлер. Дихотомният подход между шизофрения и разстройства на настроението има редица ограничения. В противовес на категориалния подход, възниква дименсионален, който описва по-добре комплексността и хетерогенността на разстройствата. Симптоми, общи за различни разстрой-

ABSTRACT

Contemporary practices of diagnostics in Psychiatry use syndrome based systems of classification of the psychiatric disorders as DSM (diagnostic and statistical manual of mental disorders) 5th edition and ICD (international classification of diseases) 10th edition, which use symptoms and signs as a tool of descriptive categorization. The aim of such classifications is improvement of therapy and prevention.

These classification systems, used in Europe and the USA are categorical and succeed Kraepelin and Bleuler's dichotomous classifications. The dichotomous approach, separating schizophrenia and mood disorders, is subject to numerous restrictions. A dimensional approach is created versus the categorical, which is better in covering the complexity and heterogeneity of the disorders. Symptoms common to different disorders produce clinical dimensions.

In dimensional classifications the existence of a continuum from normal to abnormal is assumed. Such concept stands for the exis-

ства, дават клинични дименсии.

При дименсионалните класификационни системи се приема наличието на континуум от норма до абнормност. Смята са, че концепцията за съществуване на континуум/дименсионален подход, всъщност е в подкрепа на хипотезата за съществуване на едно заболяване. Концепцията за единната психоза първи предлага белгийският психиатър Joseph Guislain. През 20 век тези идеи възражда и Klaus Conrad.

Ключови думи: категориален/дименсионален подход, континуум на психозите

tence of a unitary disorder. The concept of the unitary psychosis first is proposed by the Belgian psychiatrist Joseph Guislain. In the 20the century this ideas are resumed by Klaus Conrad.

Key words: categorical vs dimensional approach, continuum, psychosis

INTRODUCTION

Contemporary practices of diagnostics in Psychiatry use syndrome based systems of classification of the psychiatric disorders as DSM (diagnostic and statistical manual of mental disorders) 5th edition and ICD (international classification of diseases) 10th edition, which use symptoms and signs as a tool of descriptive categorization.

Deciding on a specific diagnose has few tasks, the main of them being reaching an expectance of a predictable therapeutic effect and a prognosis of the course of the disorder, and even working towards better prevention.

Do we need a classification? Classification is a process of simplifying complex phenomena by grouping them in categories, according to predefined criteria. The aim of such classifications in psychiatry is improvement of therapy and prevention.

CLASSIFICATIONS IN PSYCHIATRY THROUGH THE AGES

The first review of psychiatric classifications since ancient times has been done by Carl Menninger and his associates. They state that the first description of a psychiatric disease has been given as early as 3000 BC - prince Ptahhotep suffering of senile dementia. In Sumer and Egyptian texts melancholic and hysteria syndromes have been described about 2600 BC. In Eber papyruses (1500 BC) senile dementia and alcoholism has been described. In Ayurveda, 1400 BC, in India a classification of psychiatric maladies has been included.

Introducing the concept of psychiatric disorders in medicine is usually attributed to Hippocrates (460–370 BC). He describes acute psychiatric states with or without fever, chronic psychiatric without fever (melancholy), hysteria and Skit's disease (probably transvestism).

Mental retardation and dementia has been described by the French renaissance healer Felix Platter (1536–1614).

Philippe Pinel (1745–1826), French physician, simplifies the preceding complex diagnostic system to: melancholia, mania (insanity), dementia, and idiotism. He states that all psychiatric disorders are functional (without fever, inflammation, hemorrhage and anatomical damage) and so is a form of neurosis.

CONCEPT OF THE UNITARY PSYCHOSIS

The concept of the unitary psychosis is first proposed by the Belgian psychiatrist Joseph Guislain (1797–1860). In his book „Traité Des Phrénopathies ou Doctrine Nouvelle des Maladies Mentales" in 1833 he describes a complex system of classification of psychiatric disorders [2]: there are four consecutive stages in the course of a psychiatric state: 1) mental activity exaltation, 2) brain structural changes, 3) brain structural damages, 4) mental exhaustion [3]. He claims that the basis of every psychiatric state is the „phrénalgie" or „mental suffering"[3]. Later the psychiatric disorder would develop in seven successive stages: mania, insanity, stupidity, epilepsy, hallucinations, confusion, and dementia [18].

Guislain's ideas have been adopted by the German psychiatrist Ernst Albrecht von Zeller (1804–1877). He states that different psychiatric disorders are in fact separate stages of a single morbid process [4]. Zeller followed the idea that soul and character exist as a spiritual unity, according to the contemporary believes in Germany, based on Naturphilosophie and anthropology, and it is the spiritual self that is affected [15], and both psychological and moral causes combine to produce the psychiatric disorder [4]. The developed psychiatric state is universal and goes through four consecutive states: melancholia, mania, paranoia, dementia [4].

In the 19th century all psychiatric disorders have been widely accepted as a sign of underlying somatic pathology, so according specific damages have been searched for. First Morel (1809–1873) used the course of the disease as a basis for a classification and describes dementia praecox.

One of Zeller associates, Wilhelm Griesinger (1817–1868), grasps the concept of the unitary psychosis, even though he did not follow Zeller's ideas of the spiritual entity. Griesenger is one of the somaticist and one of the founders of materialist psychiatry [3]. In his opinion, psychiatric state is developed when „psychic reflex action" is either diminished: melancholia or accelerated: mania [15]. As Zeller, he believes that the single form of psychic state is melancholia, which might pass to mania and finally dementia [4]. In his work „Mental Pathology and Therapeutics" in 1861, he proposes a simplified classification of psychiatric disorders: one with predominantly emotional disturbances and other with predominantly intellectual and volitional disturbances [32]. According to him these two categories are two forms but also two consecutive phases of a single morbid process [32]. He also states that „psychiatric disorders are disorders of the brain", it is just that at that time there were not enough data on brain damages so psychiatric disorders are labeled functional or disorders with still unknown somatic origin.

One of the most passionate supporters of the theory of the unitary psychosis is the 19th century German psychiatrist Heinrich Neumann (1814–88) [23]. In his „Lehrbuch der Psychiatrie" in 1859 he evaluates every classification attempt in psychiatry as artificial [3] and states that there is only one type of psychiatric disorder, which he names `„Irresein" and one should define stages, not forms as: insanity (Wahnsinn), confusion (Verwirrheit) and dementia (Blödsinn) [23]. Neumann goes further and states that not only psychiatric conditions exist as continuum degrees, but there is a continuum of health and illness and all conditions in-between [3].

One of Neumann's critics is his coeval Kahlbaum, who in 1863 publishes his book „Die Gruppierung der psychischen Krankheiten" (Classification of Psychiatric Disorders) [16]. Kahlbaum separates four types of psychiatric disorders (vesania): vesania acuta, vesania typica, vesania progressiva, vesania catatonica [5]. In his opinion the major issues with declining any classification of psychiatric disorders would be the future development of the therapeutic approaches and studying the psychopathology in general [15]. He introduces the idea of (1) transient symptom complex instead of an underlying disease, (2) differentiation between organic and functional mental disorder and (3) the importance of the age of the patient at the onset of the disorder.

In the end of the 19th century, Kraepelin (1856–1926) unites manic and depressive states as manic-depressive psychosis, which differs from the chronic dementia praecox, named schizophrenia by Bleuler, because there are periods of remission. He also separates paranoia and dementia praecox, delirium and dementia and introduces the idea of psychogenic neuroses and psychopathies. His approach is based on combining clinical features, unlike Bleuler (1857–1939) who takes psychopathological processes as a basis.

August Hoch (1868– 1919) is the first to criticize the consideration of schizophrenia as a disorder. He differentiates disorder, syndrome (symptom complex) and single symptom, and thinks that in fact dementia praecox is characterized by the chaotic symptoms and that precisely changes in the symptoms are longitudinally specific to psychotic states [21], which are supported by other research in the field in Europe [9].

Freud (1856–1939) introduces the concept

of neurosis/ psychoneurosis (anxiety, anxious-hysteric (phobia), obsessive compulsive, hysteria).

In 20th century the idea of the unitary psychosis has been revived numerous times, predominantly in attempt to decline Kraepelin's dichotomy [4].

Klaus Conrad (1905–1961), German neuropsychiatrist, is convinced that there is only one endogenic psychosis, upon his research on the manifestation of schizophrenia in the offspring of patients with affective states. [3]. Based on his clinical practice, he postulates that such symptoms as hallucinations, delusions, catatonic state which are usually attributed to schizophrenia, might appear in patients with mania and depression as well and eventually lead to stabile personality changes [4]. His ideas are close to 19th century concept of the unitary psychosis [3; 4].

THE DICHOTOMOUS APPROACH

Although significant research takes place in the last hundred years, we still use basic categories, proposed by Kraepelin and Bleuler (organic disorders, affective disorders, schizophrenia) and Freud (neurosis and personality disorders).

Kraepelin is the first to propose the dichotomous approach, defining manic-depressive disorder and dementia praecox, based on the course of the disorder [24].

Bleuler proposes the name Schizophrenia, aiming to emphasize that patients with the chronic course of the disorder are not „demented", and it is not necessary for it to have an early onset [6]. He considers „schizophrenias" as a spectrum of psychoses possessing specific symptoms, with a schism in behavior and emotions.

According to Bleuler affective disorders are not that specific, so recognizing them should follow exclusion of possible schizophrenic state.

Later Bonhoeffer's works shows that the same disorder can present with different psychopathologic symptoms, where the conflict between etiologic and syndrome psychiatric classification origins.

Another theory, that schizophrenia leads to dementia and in manic-depressive disorder there is possible remission, is disclaimed as well. As early as 1909, one of Kraepelin's associates makes a research among 468 dementia praecox cases in Kraepelin's hospital in Munich. In 29.8% there was remission, which supposedly was due to misdiagnosis. This early data is confirmed by contemporary research on course and development of schizophrenia in Huber et al. [19], Ciompi and Müller [9], Bleuler [7], Marneros et al. [25], Möller et al. [29] works.

It is expected that in the course of the affective psychoses there is remission, but Bumke objects the opposite [8]: above 15% takes chronic course and in lot of the cases there are residual affective signs between episodes.

Now across Europe and the USA a nosological classification is used which appears to be e successor of Kraepelin's and Bleuler's dichotomy [1].

Separate disorders appear to be different signs and symptoms combinations. That is how we differentiate Bipolar disorder and Schizophrenia. Such approaches have certain advantages – high reliability and deficiencies – low validity.

The dichotomous approach has a number of drawbacks. In reality lot of the patients suffer from states that cover as affective symptoms and schizophrenic symptoms as well. This naturally leads to the introducing of a separate category – Schizoaffective disorder.

As counterbalance of the categorical approach comes the dimensional approach [10; 27; 28; 33], which surpasses the categorical in describing complexness and heterogeneity of the disorders. Symptoms common to different disorders produce separate dimensions...

For instance psychotic, anxious symptoms, impaired impulse control are observed in a number of disorders and in terms of the dimensional approach, new understanding and overcoming of the disorders are possible. In fact human biology in general is complex and heterogenic, so dimensional approach is much more fit [22].

CONTEMPORARY CLASSIFICATION SYSTEMS

The first predecessor of the DSM is published by APA (American Psychiatric Association) in 1844, as a statistical classification of institutionalized psychiatric inpatients. After World War Two, up to now there are five editions published and some Text revisions. The first edition of DSM is published in 1952.

International Classification of Diseases (ICD) is the main alternative of DSM and is the official nosological system used in Europe and the USA as well, because it considers not only psychiatric conditions. DSM is widely used in the USA as for psychiatric disorders and ICD in Europe and the rest of the world.

The last two editions of the DSM, DSM-IV-TR и DSM-V postulate that diagnoses have to be based on presence or absence of specific signs and symptoms, caused by different biological factors. Diagnoses are categorical, and defined.

Ever since 1974, the use of the term disorder in DSM-IV-TR (diagnostic and statistical manual of mental disorders IV edition - text revision), instead of syndrome or disease, supposes that these conditions are too complex to be defined as syndromes, and there is yet not enough data on etiology and pathogenesis, to be described as diseases. This comes on J. Wakefields idea of psychiatric disorders as impairing dysfunctions. His concept is based on quantitative data on manifestation of number of specific criteria out of the maximal, which are signs and symptoms, evaluated by structured clinical interview [35].

In DSM V there is an attempt of introducing dimensional approach but only as an alternative of the widely accepted categorical one, addressing personality disorders.

DSM is a categorical classification system. In such classifications there might be more than one diagnostic units, or comorbidity. Normal and abnormal are defined specifically.

In dimensional classifications there is continuum of normal to abnormal and different degree of manifestation of the same symptom in patients with the same diagnosis. Accordingly there are less nosological units, and lesser comorbidity.

Dimensional models suppose predisposition. Every patient should be evaluated with a specific score on different dimensions (as personality model of Eisenk and the dimensions: psychoticism, neuroticism, introvert/extrovert) [17].

Unfortunately the dimensional approach is not fit for the everyday clinical practice, because it does not ease the decision on a specific therapeutic plan [17].

NEW APPROACHES

In the last decades researchers work towards proposing alternative classification models as the prototype model – the nosological units are more general compared to the typical DSM and ICD categories, based on exclusionary criteria [36]; cluster model – grouping categories based on common signs and symptoms [36].

„Research Domain Criteria (RDoC)" project is an initiative of the American Mental Health Institute, which opts for a biologically validated classification of psychiatric disorders, based on new research methods in genetics, neurosciences, behavioral sciences. It is established in 2008, with predominantly research goals and is based on the dimensional approach concerning behavioral and neurobiological parameters. In order to clarify underlying causes of the psychiatric disorders, defining and connecting the major biological and behavioral models in normal and abnormal state is needed. This would lead to the definition of valid and reliable phenotypes, with measurable characteristics.

Contemporary work on the ideas in „Research Domain Criteria (RDoC)" model, is the Project on Translational Validity, which is based on the strong belief that all data concerning psychopathology, usually derived by structured clinical interviews, need to be validated by comparing it to neuroimaging results. Juxtaposition of clinical psychopathology data, from rating scales, and functional neuroimaging results in real time is needed [14].

CONCLUSIONS

Nowadays the existence of Schizoaffective Disorder as a diagnostic unit in opposition of Kraepelin's dichotomy supports the concept of the unitary psychosis, which even if it is out of

date is updated and allows the existence of the different psychoses as degrees of a continuum [30]. A list of authors as Crow [11, 12] and Taylor [34] state that psychotic disorders as Schizophrenia (Sch), Schizoaffective disorder (SchA) and Bipolar disorder (BPD), are in fact a continuum based on severity. Or they are continuum of severity, not a continuum of diagnoses. Psychotic features are just symptoms, not a diagnosis. It is accepted that the concept of a continuum/ dimensional approach, comes to support the hypothesis of the existence of a single disorder, with wide spectrum of severity of symptomatology.

Lake and associates [25] emphasize on the problem: does it really matter if the diagnosis is Sch/ SchA/ Psychotic BPD and if the exclusion of „neuroses" from DSM - III, is similar to eventually excluding well known diagnoses as Schizophrenia and Schizoaffective disorder, and finally uniting them with affective disorders in a spectrum of psychotic disorders, when there is not enough units. Psychotic features are criterial for Schizophrenia according to Bleuler, Schneider and the DSM, but only come to show the extent of severity of BPD, which is scientifically validated diagnostic unit.

Introducing the diagnostic unit Schizoaffective disorder creates the so important connection between Schizophrenia and Affective disorders in time when those two categories were mutually excluding which allows researchers to concentrate on comparing those categories.

The problem is that there is a continuum of the psychopathology from affective to schizophrenic symptoms. Mundt [31] in his review, says that Schneiders first rank symptoms, Huber's basic symptoms, negative symptoms of Andreasen and Olsen, delusions of Chapman and psychophysiological reactions of Hyman are non-specific. He concludes that if the symptoms are non-specific, then one cannot consider single disorder but a spectrum of the idiopathic psychoses. In 1995, Crow says: „Perhaps we should grasp the nettle and admit that the conditions we describe are in fact a continuum. There are no defining signs, needed for the existence of a specific diagnostic units...", „and if there are no diagnostic units, we cannot separate a part of the psychopathological spectrum, we need to consider the whole range." [13].

REFERENCES

1. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th ed. Washington: American Psychiatric Association; 1994
2. Angst, Jules (2002). "Historical aspects of the dichotomy between manic–depressive disorders and schizophrenia". Schizophrenia Research. 57 (1): 5–13
3. Beer, M.D. (1996). "Psychosis: A history of the concept". Comprehensive Psychiatry. 37 (4): 273–91
4. Berrios, German E.; Beer, M.D. (1994). "The notion of unitary psychosis: a conceptual history". Journal of the History of Psychiatry. 5 (17): p 14-32
5. Berrios, German E.; Beer, M.D. (1995). "Unitary psychosis concept: clinical section". In German E. Berrios and Roy Porter. A History of Clinical Psychiatry: The Origin and History of Psychiatric Disorders. London: Athlone. p 322
6. Bleuler E. Dementia praecox oder gruppe der schizophrenien [Dementia praecox or the group of schizophrenias]. In: Aschaffenburg G, editor. Handbuch der Psychiatrie Spezieller Teil, 4. Abteilung, 1. HaË lfte, Leipzig: Franz Deuticke; 1911
7. Bleuler, M., 1972. Die schizophrenen Geistesstörungen im Lichte langjähriger Kranken-und Familiengeschichten. Thieme, Stuttgart
8. Bumke, O., 1909. Uber die Umgrenzung manisch-depressiven Irreseins. Zentralbl. Nervenheilkd. Psychiatr, N.F. 20, 381–403
9. Ciompi, L., Müller, C., 1976; Lebensweg und Alter der Schizophrenen. Eine Katamnestische Langzeitstudie bis ins Senium. (Monographien aus dem Gesamtgebiete der Psychiatrie 12). Springer, Berlin, pp. 1–242
10. Crow TJ. The continuum of psychosis and its implication for the structure of the gene. British Journal of Psychiatry 1986;149:419–29
11. Crow, T.J., 1990. The continuum of psychoses and its genetic origins. The sixty-fifth Maudsley lecture. British Journal of Psychiatry 156, 788 – 797
12. Crow TJ. Nature of the genetic contribution to psychotic illness: a continuum viewpoint. Acta Psychiatry Scand. 1990a;81:401-408
13. Crow, T.J., 1995. Psychotic continuum or disease entities? The critical impact of nosology on the problem of aetiology. In: Marneros, A., Andreasen, N.C., Tsuang, M.T. (Eds.), Psychotic Continuum. Springer, Berlin, pp. 151–163

14. Drozdstoj St. Stoyanov, Stefan J. Borgwardt and Somogy Varga, Translational validity across neuroscience and psychiatry, Alternative perspectives on psychiatric validation,Published on behalf of Oxford University Press, in print November 2014 Series: International Perspectives in Philosophy & Psychiatry
15. Engstrom, Eric J. (2003). Clinical Psychiatry in Imperial Germany: a History of Psychiatric Practice. New York: Cornell University Press.p 28-59
16. Engstrom, Eric J. (2003). Clinical Psychiatry in Imperial Germany: a History of Psychiatric Practice. New York: Cornell University Press: 263; Pillmann, F.; Marneros, A. (2003). "Brief and acute psychoses: the development of concepts". History of Psychiatry. 14 (2): 163
17. Gelder M, Harrison P, Cowen P. Classification and diagnosis. In: Shorter oxford textbook of psychiatry. 5 th ed. Oxford: Oxford University Press; 2006. p. 21-34
18. Guislain, Joseph (1835). Traité sur les phrénopathies, ou doctrine nouvelle des maladies mentales (2 ed.). Brussels: Etablissement encyюclographique
19. Huber, G., Gross, G., Schüttler, R., 1979. Schizophrene Verlaufsund Sozial-Psychiatrische Langzeituntersuchungen an den 1945–1959 in Bonn Hospitalisierten Schizophrenen Kranken. Springer, Berlin
20. Hoff, Paul (2009). "Historical roots of the concept of mental illness". In I.M. Salloum and J.E. Mezzich. Psychiatric Diagnosis: Challenges and Prospects. Chichester: John Wiley and Sons., p 11
21. Janzarik, W., 1968. Schizophrene Verlaüfe. Eine Strukturdynamische Interpretation. Springer, Berlin
22. Ketter TA, Wang PW, Becker OV, et al: Psychotic bipolar disorders: dimensionally similar to or categorically different from schizophrenia? Journal of Psychiatric Research 38 (2004) 47–61
23. Kraam, Abdullah (2004). "On the Origin of the Clinical Standpoint in Psychiatry : By Dr Ewald Hecker in Görlitz". History of Psychiatry. 15 (3): 348-51
24. Kraepelin E. Psychiatrie. Ein Lehrbuch fü r Studirende und Aerzte. 6th ed. Leipzig: J.A. Barth; 1899
25. Lake CR, Hurwitz N: Schizoaffective disorders are psychotic mood disorders; there are no schizoaffective disorders. Psychiatry Research 143 (2006) 255–287
26. Marneros, A., Deister, A., Rohde, A., 1991. Affektive, Schizoaffek-tive und Schizophrene Psychosen. Eine Vergleichende Langzeit-studie. Springer, Berlin
27. Meltzer HY. Schizoaffective disorder: is the news of its nonexistence premature? Editor's introduction. Schizophrenia Bulletin 1984;10: 11–13
28. Moldin SO, Gottesman II, Erlenmeyer-Kimling L. Psychometric validation of psychiatric diagnoses in the New York High-Risk Study. Psychiatry Research 1987; 22:159–77
29. Möller, H.J., von Zerssen, D., Werner-Eilert, K., Wüschner-Stockheim, M., 1982. Outcome in schizophrenic and similar paranoid psychoses. Schizophr. Bull. 8, 99–108
30. Möller, Hans-Jürgen (2008). "Systematic of psychiatric approaches between categorical and dimensional approaches: Kraepelin's dichotomy and beyond". European Archives of Psychiatry and Clinical Neuroscience. 258 (Suppl. 2): p 60
31. Mundt, C., 1995. Psychotic continuum or distinct entities: perspec¬tives from psychopathology. In: Marneros, A., Andreasen, N.C., Tsuang, M.D. (Eds.), Psychotic Continuum. Springer, Berlin, pp. 7–15
32. Noll, Richard (2007). The Encyclopedia of Schizophrenia and Other Psychotic Disorders (3rd ed.). New York: Infobase Publishing. ISBN 0-8160-6405-9, p 145
33. Strik WK, La Malfa G, Cabras P. A bidimensional model for diagnosis and classification of functional psychoses. Comprehensive Psychiatry 1989;30:313–19
34. Taylor, M.A., 1992. Are schizophrenia and affective disorder related? A selective review. American Journal of Psychiatry 149, 22–32
35. Wakefield JC. Disorder as harmful dysfunction: A conceptual critique of DSM-III-R's definition of mental disorder. Psychol Rev 1992;99:232-47
36. Стоянов Д., 2016 Учебник по психиатрия за студенти по медицина под редакцията на проф. д-р В.Акабалиев, д.м.н., IV издание, 17. Психиатрична нозология: Класификация и Номенклатура, 81-84

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Кростолк между различни сигнални пътища и витамин Д и витамин $Д_3$ рецептор - ВДР в процесът на онкогенеза в кожа

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Crosstalk between different signal pathways and Vitamin D and Vitamin D_3 Receptor - VDR in the process of oncogenesis in skin.

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РЕЗЮМЕ:

ВДР (Витамин Д Рецетор, VDR) протеинът се експресира в почти всички нормални човешки клетъчни типове и тъкани, и също в ракови клетъчни линии и тумори. Увеличената ВДР експресия се асоциира с по-високи нива на диференциация, липса на лимфни метастази, и добра прогноза в дебелочревен рак, с по-ниска туморна степен, късно развитие на на метастази в лимфните възли, и по-дълга преживяемост без рецидиви при рак на млечната жлеза, и с подобрена обща преживяемост при простатен, немалък белодробен рак и меланом. Беше доказано че, метаболитите на Витамиин Д инхибират пролиферацията и индуцират диференциация на меланомните клетки експресиращи ВДР. Редуцираните нива или липсата на ВДР се асоциират с меланомната прогресия (меланогенезата може да супресира експресията на рецепторът (лош прогностичен маркер), водещо до намалено оцеляване на меланомните пациенти. Витамиин Д супресира ключов туморен път в развитието на БЦК (BCCs) - Hedgehog сигналният път.

ABSTRACT

VDR protein is expressed in almost all normal human cell types and tissues, and also in cancer cell lines and tumors of several origins. Remarkably, elevated VDR expression is associated with high tumor differentiation, absence of node involvement, and good prognosis in colon cancer, with lower tumor grade, late development of lymph node metastases, and longer disease-free survival in breast cancer, and with improved overall survival in prostate and non-small cell lung cancer and melanoma. It was shown that the me¬tabolites of vitamin D inhibit proliferation and induce differentiation of melanoma cells expressing VDR. Reduced level or absence of VDR is associated with melanoma pro¬gression (melanogenesis can suppress the expression of the receptor (bad prognostic marker), resulting in deteriorated survival of melanoma patients. vitamin D suppresses a key tumour pathway in BCCs development - Hedgehog signalling pathway. 1α,25(OH)2D3 also inhibits the growth of SCCs *in vivo* as well as *in vitro*, inhibiting Wnt/β-catenin and interferes in ERK1/2 signaling, connected with

1α,25(OH)2D_3 инхибира също растежът на СЦК (SCC) *in vivo* както и *in vitro,* инхибирайки Wnt/β-катенин и повлиявайки ERK1/2 сигналът, свързан с развитието на меланом. Въпреки че изследванията, *in vitro* и в животински модели, предполагат че vitamin D може да предотврати развитието на БЦК и СЦК, допълнителни изследвания са необходими за да се оцени ефектът от топикалното или системно (орално) използване на Витамин D_3 в профилактиката на ракът в лицево-челюстната област (кожен рак) при хора. Използването на 1,25(OH)2D3 при ракови пациенти се ограничава от хиперкалцемичният ефект на витаминът в терапевтични дози, довело до развитието на няколко аналози, притежаващи антитуморна активност, но проявяващи по-слабо калцемично действие, включително и при пациенти с панкреатичен рак, орган смятан за нетаргетен за действието на витаминът.

Ключови думи: 1α,25(OH)2D_3, ВДР, БЦК, СЦК, меланом, Кожен рак

development of melanoma. Although the in vitro and animal studies suggested that vitamin D may prevent development of BCCs and SCCs, additional studies on humans are needed to assess the suitability of topical or oral vitamin D_3 supplementation in chemoprevention of head and neck skin cancers. Administration of 1,25(OH)2D3 to cancer patients is restricted by its hypercalcemic effects at the therapeutic doses, enforcing the development of several analogs that maintain the antitumoral properties but have less calcemic actions, including pancreatic cancer patients, organ which is thought not to be target for vitamin D action.

Keywords: 1α,25(OH)2D_3, VDR, BCC, SCC, melanoma, Skin cancer

MELANOMA

Nowadays the most effective treatment for melanoma is surgical removal of the neoplastic lesions. Therefore, early detection of melanoma in patient increases the chances for successful curing of this disease. The significant mortality is caused by the ineffectiveness of standard treatment methods, particularly dur¬ing the stage of metastasis. Notable advance in the treatment of this type of cancer was the introduction of targeted therapy with inhibitors for tyrosine kinase receptor KIT. Recent developments, including BRAF-inhibitors, as well as drugs targeting the MAPK pathway, bring new perspectives to the field, especially when used as a combined immunotherapy [4].

The RAS/RAF/MEK/ERK pathway has been reported to be activated in over 80% of all cutaneous melanomas, making it the focus of many scientific studies in the melanoma field. Discoveries of mutations and aberrant expression of components in this cascade, in particular, BRAF and NRAS render a deeper understanding of the mechanisms responsible for oncogenesis and provide new therapeutic strategies for this deadly disease. Mutations in B-raf are found in 67% of melanomas [16], 50 % according Ascierto PA et al., 2012, activating mutations in the TERT promoter were recently identified in up to 71% of cutaneus melanoma [7].

In fact, approximately 50% of melanomas harbor activating BRAF mutations. Among the BRAF mutations observed in melanoma, over 90% are at codon 600, and among these, over 90% are a single nucleotide mutation resulting in substitution of glutamic acid for valine (BRAFV600E: nucleotide 1799 T>A; codon GTG>GAG). The second most common mutation is BRAFV600K substituting lysine for valine, that represents 5-6 % (GTG>AAG), followed by BRAFV600R (GTG >AGG), an infrequent two-nucleotide variation of the predominant mutation, BRAF V600’ E2’ (GTG>GAA), and BRAF V600D (GTG>GAT). The prevalence of BRAFV600K has been reported as higher in some populations. In melanoma, BRAF mutation is most common in patients whose tumors

arise on skin without chronic sun-induced damage, whereas BRAF mutations are rare in melanomas arising from mucosal and acral sites [1].

Several RAF mutations have been implicated in the induction of genomic instability, driving the proliferation of cancer cells with the highest frequency in melanoma. For instance, mutated BRAF signals as a monomer, independent of upstream growth stimuli. The most frequent BRAF mutation, BRAFV600E, causes constitutive activation of the kinase as well as insensitivity to negative feedback mechanisms. BRAFV600E has been implicated in different mechanisms of melanoma progression, and principally the activation of the downstream MEK/ERK pathway, evasion of senescence and apoptosis, unchecked replicative potential, angiogenesis (through MEK-dependent activation of HIF-1α and VEGF), tissue invasion and metastasis (via upregulation of several proteins involved in migration, integrin signaling, cell contractility, tumor - and microenvironment-derived interleukin-8), as well as the evasion of immune response [1].

In melanoma, BRAF mutation is most common in patients whose tumors arise on skin without chronic sun-induced damage, whereas BRAF mutations are rare in melanomas arising from mucosal and acral sites [1].

No clear differences in prognosis (time from primary diagnosis to distant metastasis) were noted between BRAF-mutated versus wild-type melanomas. Features of the antecedent primary melanoma significantly associated with a BRAF mutation ($P<0.05$) were the superficial spreading and nodular histopathological subtypes, the presence of mitoses, the presence of a single or occult primary melanoma, a truncal location and age at diagnosis of the primary tumor (≤50 years) [1].

Mechanistically, mutated BRAF and NRAS exert most of the oncogenic effects through the activation of the MAPK pathway, which both drive the uncontrolled growth of melanoma cells and regulate the cell survival. In a subsequent section, clinical efficacy of targeted small-molecule inhibitors is highlighted. BRAF-targeted therapies (e.g., vemurafenib, dabrafenib) have showed impressive results in systemic therapy for melanoma harboring activating BRAF V600E mutations (most frequent BRAF mutation [1]. MEK inhibitors show limited activity in phase I trials, and inhibitors directly targeting mutated NRAS, to date, have not been realized. Furthermore, the emerging mechanisms underlying both intrinsic and acquired drug resistance as well as approaches to prevent or abrogate the onset of therapeutic escape are addressed [25]. Nevertheless, seeking for new drugs is still required, since during the treatment many tumours acquire resistance to the therapies [20].

Studies conducted in the 70's showed, that vitamin D can stimulate the activity of tyrosinase, the principal enzyme involved in melanin synthesis in cultured mel¬anoma cell line. Following studies confirmed the presence of the vitamin D receptor (VDR) in the primary melanoma tissue. Having in mind its antiproliferative properties, vitamin D and its analogues are potential candidates for melanoma treatment. In fact, it was shown that the metabolites of vitamin D inhibit proliferation and induce differentiation of melanoma cells expressing VDR. Interestingly, it seems that not transformed melanocytes and keratinocytes are protected by 1,25(OH)2D3 and its analogues. There is also evidence, that 1α,25$(OH)_2D_3$ promotes survival of the cells and inhibits the tumour invasion and angiogenesis. In the Gupta's study, keratinocytes after the treatment with 1α,25$(OH)_2D_3$ and exposure to UV radiation were characterized by the increased survival and reduced amount of DNA damage. Similar protective effect was observed in melanoma cells. Furthermore, VDR was shown to take part in activation of DNA repair mechanism. Thus, it seems that vitamin D is an important, physiologically relevant factor preventing UV-induced carcinogenesis. It is not surprising, that a number of recent studies pointed out that the development of melanoma can be linked with vitamin D deficiency or defects in vitamin D signalling pathway. In humans, VDR gene has multiple splicing variants, which are likely to affect protein expression and activity of the VDR. In addition, over 1000 polymorphic sites have been detected in VDR gene, some of which have been correlated with increased risk of melanoma occurrence, its aggressiveness and prognosis, as well as with other pathologies [20].

Reduced level or absence of VDR is associa-

ted with melanoma progression (melanogenesis can suppress the expression of the receptor), resulting in deteriorated survival of melanoma patients. Recent studies also provided evidence that vitamin D and its analogues may be effective in melanoma treatment, especially recently tested low-calcemic analogues such as 20-OH D_3 or analogues with pregnenolone-like side chain (21-hydroxypregnacalciferol) [20].

ERK1/2 SIGNALING PATHWAY

Study on breast carcinoma showed that calcitriol inhibits Src tyrosine kinase in Ras/ Raf kinase cascade, thereby leading to subsequent decrease in activity of ERK 1/ 2 and MAP kinase, this in turn reduces the formation of cyclin/ cdk proteins, thereby leading to inhibition of cell proliferation and alteration of differentiation in G1 and other phases of the cell cycle (MAP kinase plays an important role in cell proliferation and differentiation). Bernardi et al. have also investigated the action of calcitriol on VEGF-induced TDEC (tumor-derived endothelial cells) proliferation and found the vitamin to reduce phospho-ERK 1/ 2 and phospho-Akt levels in them. Such observation shows that calcitriol interferes with both the proliferation - transduction pathways induced by RTKs, thereby inhibiting angiogenesis [3].

The prognostic role of VDR expression or its relationship with PIK3CA or KRAS mutation is uncertain. Among 619 colorectal cancers in two prospective cohort studies, 233 (38%) tumors showed VDR overexpression by immunohistochemistry, which significantly associated with KRAS mutation (odds ratio, 1.55; 95% confidence interval, 1.11-2.16) and PIK3CA mutation (odds ratio, 2.17; 95% confidence interval, 1.36-3.47). These data support potential interactions between the VDR, RAS-MAPK and PI3K-AKT pathways, and possible influence by KRAS or PIK3CA mutation on therapy or chemoprevention targeting VDR [13].

Gilad LA et al. [6] previously demonstrated that 17β-estradiol (E2) regulates the transcription and expression of the vitamin D receptor (VDR) in rat colonocytes and duodenocytes in vivo. E2 has been shown to increase the number of VDR in the osteoblastlike cell line ROS 17/2·8, an increase associated with enhanced responsiveness of the cells to $1,25(OH)2D_3$. Increased VDR expression following E2 treatment has also been noted in other tissues and cell types, such as the uterus, liver, and human breast cancer cells. They compared E2-associated signaling activity in HT29 colon cancer cells, a non-classical E2-target, with that in MCF-7 breast cancer cells, the natural targets of the hormone. E2 did not affect proliferation of HT29 cells, but enhanced proliferation of MCF-7 cells. Vitamin D inhibited proliferation of both cell lines and the combined treatment induced potentiation of vitamin D activity. E2 upregulated VDR transcription and protein expression concomitantly with ERK 1/2 phosphorylation in both cell lines. PD98059, a specific mitogen-activated protein kinase (MAPK) inhibitor, prevented E2-mediated activation of ERK 1/2, with concomitant inhibition of VDR expression. Rapid activation of mitogen-activated protein kinase (MAPK) by E2 in ROS 17/2·8 cells has provided the first evidence of MAPK activation by E2 through phosphorylation, indicating the involvement of putative plasma membrane receptors. Rapid effects exerted by E2 on growth-factor related signaling pathways have also been demonstrated in neuronal cells, suggesting a potential mechanism by which E2 might affect the expression of genes with promoters that do not contain strictly estrogen-responsive elements but are responsive to factors acting through other response elements, such as activation protein-1 (AP-1) and serum response elements [6].

ICI182780 (ER-specific inhibitor) inhibited VDR expression in HT29 and MCF-7 cell lines. A conjugate of E2 and bovine serum albumin upregulated phosphorylation of ERK 1/2 and concomitantly enhanced VDR expression in a similar fashion as the nonconjugated hormone. Expression of ERα and ERβ was detected in MCF-7 and HT29 cell lines respectively, which localized to the nuclei, cytosol and caveolar membrane rather than non-caveolar membrane. Disruption of lipid rafts/caveolae by depleting cellular cholesterol with the cholesterol-binding reagent β-methylcyclodextrin blocked ERK 1/2 phosphorylation concomitantly with VDR upregulation. The tyrosine phosphorylation inhibitor suramin and src kinase inhibitor PP2 inhibited both ERK 1/2 phosphorylation

and VDR expression. E2 induced phosphorylation of Raf and Jun in a time-dependent manner. The Ras/Raf dependent inhibitor of transactivation sulindac sulfide also blocked E2 effects. The specific c-Jun phosphorylation inhibitor SP600125 dose dependently inhibited c-Jun phosphorylation and VDR expression. The MAPK/ERK kinase inhibitor PD 98059 downregulated both c-Jun phosphorylation and VDR expression indicating that upstream and downstream events in the signaling cascade are all related to the control of VDR expression, through AP-1 binding site on the VDR promotor. Taken together, the authors' data suggest that E2 binds to receptors (ERα or ERβ with different tissue distribution) compartmentalized to membranal caveolar domains in HT29 and MCF-7 cells, tyrosine phosphorylation activity triggers the Ras-ERK pathway, which ultimately can activate transcription factors such as c-Jun or c-Fos to bind specific sequences within the VDR gene (such as the AP-1 binding site) and finally induce upregulation of transcription and expression of the VDR gene [6].

For better understanding the functional interactions between VDR and the ERK signaling pathway, Narayanan R *et al.* [17] sought to determine whether 1,25-D activates ERK in the osteoblastic cell lines, MG-63 (human osteosarcoma) and MC3T3-E1 (osteoblastic cell line from a C57BL/6 mouse calvaria with high alkaline phosphatase (ALP) activity in the resting state), and to evaluate the effects of ERK on VDR activity. The authors found that 1,25-D rapidly induced ERK activity and that this activation persisted at 24 h in both cell lines. Surprisingly, the effects of ERK activation on VDR activity in the two cell lines were very different. Overexpression of Raf-1 (an upstream activator of ERK) reduced VDR activity in MC3T3-E1 cells, but stimulated activity in MG-63 cells. Similarly, inhibition of ERK by the MEK inhibitor U0126 stimulated VDR activity in MC3T3-E1 cells. However, it inhibited VDR activity in MG-63 cells as well as in HeLa cells, a cervical carcinoma cell line commonly utilized to study the functions of nuclear receptors. Despite this difference, U0126 treatment enhanced expression of VDR in both cell lines. Although previous studies in keratinocytes suggested a potential for differential coactivator usage as a function of differentiation, and coactivators are phosphoproteins, more highly differentiated MC3T3-E1 cells responded similarly to the undifferentiated cells. Supplementation with either SRC-1 or DRIP205, two VDR coactivators, had no effect on the response to the ERK pathway [17].

The primary effect of U0126 in MC3T3-E1 cells appears to be enhancement of nuclear localization and DNA binding. The increase in DNA binding in MC3T3-E1 cells may, in part, be due to increased nuclear levels of VDR, but the binding is not strictly proportional to VDR expression as 1,25-D treatment alone was less effective than U0126 in increasing DNA binding despite the higher levels of VDR. The lack of correlation between nuclear VDR levels and DNA binding suggested that the VDR heterodimer partner, RXR, might be limiting. An analysis of RXR isoform expression levels revealed that MC3T3-E1 cells had lower levels of RXRβ and RXRγ than did HeLa or MG-63 cells, both of which are cancer cell lines, suggesting that in MC3T3-E1 cells the VDR may be more dependent upon RXRα.Previous studies have shown that RXRα is a substrate for ERK and there is evidence that phosphorylation of this site reduces the effectiveness of RXRα as a VDR partner. Ras-transformed keratinocytes are resistant to 1,25-D-mediated differentiation, and this resistance is caused by the phosphorylation of Ser260 of RXR. Mutation of this serine to alanine eliminated the resistance. Interestingly, the response of TR, which also forms heterodimers with RXR, was not differentially affected by ERK signaling in the two cell lines. Consistent with the correlation of the levels of RXR isoforms, elevated expression of RXRγ eliminated the U0126 stimulation of VDR activity. Thus, although coactivator function may be modified by ERK signaling, the dominant determinant of the effect of ERK signaling on VDR function is its RXR partner. In cells in which RXRα predominates, the elevation of ERK signaling by 1,25-D likely blunts the VDR transcriptional responses whereas in cells containing higher levels of the other isoforms, the enhanced ERK signaling stimulates VDR activity. The RXR isoform-dependent response indicates that depending upon the cellular milieu, treatment with 1,25-D will activate ERK with little activation of the transcriptional activity of VDR (RXRα dominant cells) whereas in other

cells (RXRβ or RXRγ dominant), 1,25-D potentiates VDR activity both through its action as a ligand as well as through activation of ERK. ERK activity is often associated with cell growth, whereas 1,25-D acting through VDR often inhibits growth and induces differentiation, although 1,25-D stimulates proliferation in some cell types. Thus, the relative abundance of RXR may play a role in determining the extent to which 1,25-D alters proliferation. in cells in which RXRα is the dominant VDR partner, activation of ERK by 1,25-D reduces the activity of VDR, whereas in cells utilizing RXRβ or RXRγ, the activation of ERK enhances the activity of VDR [17].

IN SKIN

Inflammation, elicited in the skin following tissue damage or pathogen invasion, may become chronic with deleterious consequences. Tumor necrosis factor (TNF) is a key mediator of cutaneous inflammation and the keratinocyte an important protagonist of skin immunity. Calcitriol (v.D_3, 1,25$(OH)_2D_3$) and its analogs attenuate epidermal inflammation and inhibit the hyperproliferation of keratinocytes associated with the inflammatory disorder, psoriasis. Since activation of extracellular signal-regulated kinase (ERK) promotes keratinocyte proliferation and mediates epidermal inflammation, the effect of calcitriol on ERK activation in HaCaT keratinocytes exposed to the ubiquitous inflammatory cytokine TNF was studied. Ziv E. et al.established that TNF activated ERK in an EGFR and Src dependent and an EGFR and Src independent modes. EGFR dependent activation resulted in the upregulation of the transcription factor, c-Fos, while the EGFR independent activation mode was of a shorter duration, did not affect c-Fos expression but induced IL-8 mRNA expression. Calcitriol, enhanced TNF-induced EGFR-Src dependent ERK activation and tyrosine phosphorylation of the EGFR, but abolished the EGFR-Src independent ERK activation. These effects were mirrored by enhancement of c-Fos and inhibition of IL-8 induction by TNF. Treatment with calcitriol increased the rate of the de-phosphorylation of activated ERK, accounting for the inhibition of EGFR-Src independent ERK activation by TNF. It is possible that effects on the ERK cascade contribute to the effects of calcitriol and its synthetic analogs on cutaneous inflammation and keratinocyte proliferation [30].

Cystatin A, a cysteine proteinase inhibitor, belongs to the cystatin superfamily, is a cornified cell envelope constituent and a differentiation marker of keratinocytes. In the epidermis cystatin A is expressed in the upper spinous to granular cell layers. 1,25(OH)2D3 suppressed NHK proliferation in a dose-dependent manner with the maximal effect at 1×10^{-7} M. It also stimulated cystatin A promoter activity and its expression with similar dose effects. The increased cystatin A was detected by 24 h and the effect was accompanied by the suppression of ERK activity. The increased cystatin A was detected by 24 h and the effect was accompanied by the suppression of ERK activity. Cystatin A promoter activity was not affected by cotransfection of vitamin D_3 receptor or retinoid X receptor. Further analyses disclosed that the 12-*o*-tetradecanoylphorbol-13-acetate (TPA)-responsive element (TRE), T2 (−272 to −278), in cystatin A promoter is critical for the regulation by 1,25(OH)2D3. Transfection of the dominant-negative form of ERK adenovirus (Ad-dnERK) increased cystatin A promoter activity and its expression, which was markedly augmented by 1,25$(OH)_2D_3$ treatment. Transfection of the dominant-active form of Raf-1 (Ad-daRaf-1) or MEK1 (Ad-daMEK1) inhibited 1,25(OH)2D3-dependent cystatin A promoter activity and its expression. Consistent with these results, the MEK1 inhibitor, PD98059, further augmented 1,25$(OH)_2D_3$-induced cystatin A promoter activity and its expression. This study demonstrated that the 1,25(OH)2D3-responsive element in the cystatin A gene is identical to the TRE, T2 (−272 to −278), and that the suppression of Raf-1/MEK1/ERK1,2 signaling pathway increases cystatin A expression of NHK [11]. Additionally, cystatin A is positively regulated via the Ras/MEKK1/MKK7/JNK signal transduction pathway. In contrast, transfection of dominant negative forms of MKK3, MKK4, or p38 did not affect cystatin A promoter activity [22]. The AP-1 proteins, c-Jun and c-Fos, or by c-Jun and JunD, regulate cystatin A promoter activity probably via PKCα activation [21]. Loss-of-function mutations in the gene for pro-

tease inhibitor cystatin A (CSTA) result in skin fragility due to impaired cell-cell adhesion: autosomal-recessive exfoliative ichthyosis or acral peeling skin syndrome [8] and is the underlying genetic cause of exfoliative ichthyosis [15]. Electron microscopy of skin biopsies from affected individuals revealed that the level of detachment occurs in the basal and lower suprabasal layers. Immunostaining against keratin 14 and E-cadherin showed a widening of intercellular spaces and major disorganization of keratinocytes in the lower suprabasal and basal layers [2]. siRNA knockdown of CSTA resulted in cytoplasmic localization of Dsg2 (desmoglein 2), perturbed cytokeratin 14 staining and reduced levels of desmoplakin in response to mechanical stretching. CSTA is deregulated in several skin cancers, including squamous cell carcinomas (SCC) and loss of function mutations lead to recessive skin fragility disorders [8].

We could not find data concerning Cystatin D expression in human epidermis (keratinocytes), influencing E-cadherin expression and EMT. Cystatin M/E (CST6) is another member of cystatin family, a nonredundant, epithelium-specific protease inhibitor with a presumed role in epidermal differentiation and tumor suppression. Zeeuwen PL et al., have previously reported that cystatin M/E deficiency in Cst6(-/-) mice causes neonatal lethality because of excessive transepidermal water loss. The absence of cystatin M/E in adult animals caused scarring alopecia. Biochemical evidence suggests that cystatin M/E controls the activity of legumain, cathepsin L, cathepsin V, and transglutaminase-3. The authors concluded that a tightly regulated balance between cathepsin L and cystatin M/E is essential for tissue integrity in epidermis, hair follicles, and corneal epithelium [28].

Furthermore, Verdier-Sevrain S *et al.* have demonstrated that keratinocytes express both estrogen receptor ER-α and ER-β. At physiological concentrations, estradiol up-regulates the level of ER-α receptors in keratinocytes and induces proliferation in neonatal keratinocyte [24]. E2 increased protein and mRNA levels of cyclin D2, and resultantly enhanced assembly and kinase activities of cyclin D2-cyclin-dependent kinases 4 or 6 complexesin human keratinocytes. 17β-estradiol (E2) enhanced cyclin *D2* promoter activity, via CREB phosphorylation by protein kinase A, dependent on cAMP. These effects of E2 may be mediated via cell surface GPR30 (G-protein-coupled receptor) [11]. It is known that in human keratinocytes ERK1/2 induces keratinocytes proliferation, and increases expression of cyclin B1, but not that of cyclin D [5].

The ER-α agonist, TGF β1 dependently increases fibroblast migration and keratinocyte proliferation. By contrast, the ER β agonist does not affect cell migration and increases keratinocyte proliferation in a TGF β1 independent manner. Accordingly, in vivo experiments have shown that targeting ER β, but not ER α leads to accelerated re epithelialization in mice and rats. Furthermore, ER α has been proven to be responsible for impaired wound healing in male mice. Perzelova V *et al.* demonstrated that the pharmacological activation of ER β, but not that of ER α, in HaCaT keratinocytes, expressed both ER α and −β, led to an increase in cell proliferation and keratin 19 expression, characteristic which exemplifies the stem cell like character of keratinocytes. In relation to markers, their previous research has demonstrated that the ER β agonist does not induce Sox 2 expression, a character¬istic of stem like properties, in keratin 19 positive cells [19].

Typical of the type II nuclear receptors, $1,25(OH)_2D_3$-induced VDR activation inhibited proliferation and promoted differentiation of keratinocytes, though VDR/RXRα heterodimers, which can transactivate keratinocytic genes independent of $1,25(OH)_2D_3$ binding [10].

RXRα, the primary isoform in skin and hair follicles, has stronger expression levels compared to RARs, and RXRα/RARγ heterodimer appears to be the major retinoid transducing element in epidermal biology. RXRα/RARγ heterodimerization is also critical in the development and formation of epidermal lamellar granules by repression of target genes, as RARγ agonists promote lamellar granule defects in murine skin. Similarly, RXRα/PPARβ/δ heterodimers are equally important to stratum corneum homeostasis through activation of gene transcription [10].

Mice with an epidermal-specific ablation of RXRα ($RXR\alpha^{ep-/-}$) presented epidermal hyperplasia, keratinocyte hyperproliferation

and aberrant terminal differentiation, alopecia, dermal cysts and a cutaneous inflammatory response [10,15]. Likewise, mice lacking both RXRα and RXRβ in keratinocytes (RXRαβ$^{ep-/-}$) developed a chronic dermatitis similar to human AD (Atopic dermatitis) patients, elevated serum IgE/IgG and cytokine production associated with Th2-type response. Importantly, thymic stromal lymphopoietin (TSLP) was strongly upregulated from the basal keratinocytes, potentially influencing the systemic AD phenotype in these mice. To further support the hypothesis that loss of RXRα contributed to a derepressive mechanism on gene expression leading to inflammatory responses, expression of RXRα has been reported to decrease in human psoriatic lesions, with levels in progressive disease further reduced compared to stable stages [10].

The transcription factor KLF4 is required for proper EPB formation in mice and dysregulated KLF4 activity is shown to be oncogenic. KLF4 regulates expression of RXRα and KLF4-induced malignant transformation is sensitive to retinoids *in vitro.* Importantly, rexinoid application drastically prevented formation of SCC in a KLF4-activated transgenic mouse line. These results suggest existence of a crosstalk between RXR and KLF4 signaling, where KLF4-mediated expression of RXR contributes to tumor suppressor activity within the epidermis. RXRα$^{ep-/-}$ mice, selectively lacking RXRα in epidermal keratinocytes, subjected to a 7,12-dimethylbenz(a)anthracene (DMBA)/12-*O*-tetradecanolyphoral-13-acetate (TPA) induced two-step chemical carcinogenic protocol developed higher numbers of epidermal tumors compared to control mice. The RXRα$^{ep-/-}$ papillomas progressed towards SCC in a murine model, further validating the role of RXRα as a cutaneous tumor suppressor [10].

Type II NR-mediated signaling via RXR dimerization is essential to melanocyte biology. RXRα and β expression has been detected in B16 and S91 murine melanoma cells, with RXRβ being the predominant isoform. Interestingly, loss of melanocytic RXRα expression was seen in human primary and metastatic melanoma compared to benign nevi, indicating the importance of this signaling pathway to the differentiation of these cell types. RARβ expression was also downregulated in melanoma and its RA-induced expression was linked to growth inhibition and differentiation in these cells. Sequential occupation of the RA response element located on the *Rarβ* promoter by RXR/RXR and RXR/RAR combinations is believed to be a molecular switch responsible for *Rarβ* transcriptional activation in these cells. Since ligand activation of RXR heterodimeric partners regulates transcriptional activity of target genes involved with differentiation and growth arrest, it is possible that combinatorial activation of both dimer partners may assist in melanoma therapy [10].

Interestingly, in a two-step carcinogenesis model, RXRα$^{ep-/-}$ mice developed a higher number of dermal melanocytic growths (nevi) compared to control mice, implicating contribution of keratinocyte-derived factors in melanomagenesis. Only nevi from RXRα mutant mice progressed to melanoma-like tumors, suggesting that RXRα-mediated distinct non-cell autonomous actions suppressed nevi formation and melanoma progression in mice [10].

Similarly, VDR$^{-/-}$ mice undergoing identical treatments also developed higher numbers of melanocytic lesions, indicating RXRα/VDR heterodimerization may be the causative factors, at least in part, in these non-cell autonomous events. Finally, the loss of keratinocytic RXRα alongside an activated-CDK4 mutation enhanced the metastatic transformation of cutaneous melanoma after chemical carcinogenesis. Loss of epidermal RXRα was also seen in human melanoma progression and could potentially be utilized as a therapeutic biomarker [10].

NON-MELANOMA SKIN CANCERS

Similarly as in case of melanomas, there are number of studies concerning the role of vitamin D in basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). Just like keratinocytes, BCC cells also express VDR, and furthermore, peripheral cells forming BCC tumours show even higher expression of VDR than neighbouring, un¬affected epidermal cells. It was demonstrated that vitamin D sup¬presses a key tumour pathway in BCCs development - Hedgehog signalling pathway. It was shown

that VDR-knockout mice, after the exposure to a carcinogen, were more prone to BCCs skin tumours development than the wild type animals. Another studies on mice showed that topical application of vitamin D_3 reduces BCC cell proliferation and also inhibits the Hedgehog signalling pathway, both in vitro and in vivo. 1α,25$(OH)_2D_3$ also inhibits the growth of SCCs *in vivo* as well as *in vitro.* Similar studies on animals showed that mice lacking VDR and exposed to high and prolonged doses of UVB are predisposed to SCC tumour formation. In addition, as in BCCs, topically applied 1α,25(OH)2D3 inhibits formation of chemically induced tumour in a dose-dependent manner [20].

What is more, 20(OH)D3, 20,22$(OH)D_3$ and 20,23$(OH)D_3$, novel vitamin $D3_3$ analogues produced by P450scc, show pro-differentiation, anti-proliferative and anticancer properties. Although the in vitro and animal studies suggested that vitamin D may prevent development of BCCs and SCCs, additional studies on humans are needed to assess the suitability of topical or oral vitamin D3 supplementa¬tion in chemoprevention of non-melanoma skin cancers [20].

HEDGEHOG SIGNALING PATHWAY

In humans, the hedgehog (Hh) genes were found to be an essential developmental signaling pathway in maintaining tissue polarity and stem cell population. Inactivation or hyperactivation of this pathway can cause serious health problems, including developmental defects such as holoprosencephaly, and different forms of cancer including Basal Cell Carcinomas (BCCs), medulloblastomas, leukemia, gastrointestinal, prostate, ovarian, breast and lung cancers. The receptor for Hhs is a transmembrane protein called Patched (PTC, Ptch). In the absence of Hh ligands, PTC is bound to another transmembrane protein, smoothened (SMO), and functions as an inhibitor of SMO. The binding of Hh ligands to PTC releases SMO from the inhibitory effect of PTC and allows SMO to transduce signals leading to the activation of transcription factor, called glioma associated (Gli), and the expression of genes involved in regulating embryonic and postnatal development, and the transformation of cancer- and metastasis-initiating cells [4].

In the absence of Hh ligands (A), Ptch1 receptor at the base of the primary cilium inhibits the function of SMO by preventing its entry into the cilium. Gli3 and, to a lesser extent Gli2, are converted to C-terminally truncated repressor forms (Gli-R) and translocate into the nucleus, where they inhibit the transcription of Hh target genes. Formation of Gli-R is promoted by sequential phosphorylation of full length Gli by a kinase cascade that includes PKA (Protein Kinase A), GSK-3β (Glycogen Synthase Kinase 3β), and CK1 (Casein Kinase 1), which creates binding sites for the adapter protein β-TrCP (β-transducin repeat-containing protein). The Gli/β-TrCP complex is ubiquitinated by Cul1-based E3 ligase, which results in partial Gli degradation by the proteasome and formation of Gli-R. In addition to partial degradation, full-length Gli may be completely degraded by the proteasome through SPOP (Speckle-type POZ Protein)-mediated Cul3-based E3 ligase ubiquitination. Upon Hh ligand binding (B), Ptch is displaced from the cilium, becomes internalized in endosomes, and degraded. SMO relocates from intracellular vesicles to the cilium. Active SMO promotes a signaling cascade that leads to translocation of activated forms of Gli (Gli-A) into the nucleus, where they induce the transcription of Hh target genes, such as Gli1, Ptch1, and HHIP (Hh-interacting protein). HHIP competes with binding of the Hh ligands, while the GPI-linked Gas1 (Growth Arrest-Specific protein 1) and the Ig/Fn repeat-containing surface proteins Cdo and Boc (brother of Cdo) act as coreceptors of Hh [12,18].

Recent studies indicate a cross-talk between vitamin D_3 and Hh signaling mediated by at least two mechanisms. First, PTC has been shown to stimulate the secretion of a vitamin D_3-related compound, which is likely responsible for the inhibitory action of PTC on SMO. Second, 1α,25$(OH)_2D_3$ can down regulate the expression of some members of the Hh pathway genes, including PTC, SMO and Gli in an epidermal explants culture system, suggesting a direct regulation by 1α,25(OH)2D3. These results are in agreement with the increased expression of Shh in the keratinocytes of the

VDR-null animal and hyperactivation of the Hh pathway, predisposing the skin to the development of both malignant and benign epidermal neoplasms. More interestingly, Uhmann *et al.* demonstrated that $1\alpha,25(OH)_2D_3$ was capable of inhibiting Hh signaling at the level of SMO in the absence of VDR. Similar conclusion was obtained by Tang *et al.* who studied murine basal cell carcinomas (BCC) *in vitro* and *in vivo,* and found that the effect of $1\alpha,25(OH)_2D_3$ induced Gli expression is likely independent of VDR. The results provide strong evidence of the non-genomic and rapid non-VDR action of $1\alpha,25(OH)_2D_3$ on cell growth and differentiation mediated by Hh/Gli signaling pathway [4].

In the absence of the Hh ligands, Hh receptor (PTC) binds SMO, the key Hh signal transducer, *via* a vitamin D_3-related compound. Under this condition, Gli molecules are processed into repressor forms (Gli R), which turn off the expression of Hh-inducible genes. In the presence of Hh, Hh binds to PTC. The binding causes the release of SMO from T. After SMO is freed from the PTC inhibition, it undergoes conformational changes to activate Gli to form active Gli (Gli A) in the cytosol. Cytosolic Gli A is then transported into the nuclei to act as a transcription factor to induce gene expression. Vitamin D may affect Hh signaling pathway via two potential mechanisms. PTC can stimulate the secretion of a vitamin D_3-related compound, which is responsible for the inhibitory action of PTC on SMO in the absence of Hh. This results in a down-regulation of Hh signaling and increased expression of p21 and p27. Right panel depicts that the active form of vitamin D_3, $1\alpha,25(OH)_2D_3$, can down-regulate the expression of PTC, SMO and Gli proteins, that in turn decreases Hh signaling, leading to enhanced p21 and p27 expression and other actions, including rapid nongenomic responses [4].

WNT/B-CATENIN SIGNALING PATHWAY

Expression of E-cadherin and β-catenin (proteins forming intracellular junctions) are also decreased in skin malignant tumours, including basal cell carcinoma, squamous cell carcinoma and melanoma . One of the studies revealed, that 4-day incubation of human keratinocytes with $1\alpha,25(OH)_2D_3$ caused the assembly of adherens junctions, upregulating E-cadherin expression, but not of desmosomes. The same study demonstrated that $1\alpha,25(OH)_2D_3$ may induce formation of intracellular junctions by protein kinase C (PKC) activation. Thus, it is speculated that $1\alpha,25(OH)_2D_3$-induction of cell–cell junctions formation may be a novel, promising mecha¬nism of the anti-neoplastic and anti-proliferative cancer treatment [20].

Moreover, protein kinase C inhibitors block E-cadherin, P-cadherin, α-catenin, and vinculin translocation to cell-cell contacts and the assembly of adherens junctions promoted by $1,25(OH)_2D_3$ in cultured human keratinocytes [24].

Many epidermal genes induced by WNT/β-catenin contain VDR response elements and were activated independently of TCF/LEF, implying that it is part of a TCF/LEF-independent aspect of WNT signaling. Likewise, depletion of follicular keratinocyte populations in VDR-null mice was linked to aberration of the canonical WNT pathway [9].

Wnt/β-catenin is an evolutionarily conserved signaling pathway that plays an essential role in a diverse array of biologic processes, including organogenesis, tissue homeostasis and, in some instances, pathogenesis of diseases, including cancers. Earlier studies indicate that $1\alpha,25(OH)_2D_3$ and its analogs are able to promote the differentiation of colon cancer cells by inhibiting Wnt/β-catenin signaling pathway mediated by VDR competing with transcription factor TCF-4 for β-catenin binding. The molecular mechanism of the induction by $1\alpha,25(OH)_2D_3$ was further studied in LS180 colon cancer cells using chromatin immunoprecipitation-seq and gene expression analyses. It was found that VDR and RXR co-localized to VDRE sites in a ligand-dependent manner near a set of genes that included c-FOS and c-MYC. The expression of both c-FOS and c-MYC was modulated by 1α,25(OH)2D3. At the c-FOS gene, both VDR/RXR and TCF4/β-catenin bound to a single distal enhancer located 24kb upstream of the transcriptional start site. At the c-MYC locus, binding was at a cluster of sites between - 139 and -165 kb

and at a site located -335 kb upstream (down-regulation), where both VDR and β-catenin activation was interlinked to basal and 1α,25$(OH)_2D_3$-inducible activities. In addition, 1α,25$(OH)_2D_3$ is known to regulate two genes encoding two extracellular Wnt inhibitors, DICKKOPF-1(DKK-1) and DICKKOPF-4 (DKK-4), in opposite directions; 1α,25$(OH)_2D_3$ up-regulates DKK-1 which acts as a tumor suppressor in human colon cancer cells, whereas 1α,25(OH)2D3 down-regulates DKK-4, an oncogenic protein and a target of the Wnt/β-catenin pathway. Taken together, these data reveal complex modes of action in the regulation of target genes 1α,25(OH)2D3 [4].

NON-GENOMIC ACTION OF VITAMIN D_3

The genomic actions of 1α,25$(OH)_2D_3$ are mediated through its binding to vitamin D receptor (VDR), a member of the nuclear receptor superfamily, to modulate the expression of various genes. The liganded VDR forms a heterodimer with retinoid X receptor (RXR) and binds to vitamin D response element (VDRE) to modulate the gene expression. Since VDR is expressed in almost all tissues, 1α,25$(OH)_2D_3$ has been found to exert various anti-cancer actions, including anti-proliferation, anti-inflammation, pro-differentiation, pro-apoptosis and antiangiogenesis in a tissue- and cell-specific manner. Recent evidence indicates that 25$(OH)D_3$ is a natural ligand for VDR, and is capable for causing biologicaleffects without converting to 1α,25$(OH)_2D_3$ [4].

So-called, non-genomic mechanism of rapid vitamin D response has been described recently. This mechanism does not di¬rectly affect gene expression or require additional protein synthesis. Rapid vitamin D response was shown to mod¬ulate intracellular calcium levels, affects activity of sev¬eral intracellular signalling pathways, through activation of selected phosphate kinases and phosphatases. These activities take minutes and occur in the cytoplasm of the cell rather than in the nucleus. Potential mechanism of non-genomic response involves interaction of vitamin D to 1α,25$(OH)_2D_3$ membrane–associated rapid response steroid-binding protein (1,25 D-MARRSBP), also known as the protein-disulfideisomerase-associated 3 (PDIA3) or endoplasmic reticulum stress protein 57 (ERp57). PDIA3 activates phospholipase C in a G protein-coupled process and results in production of inositol trisphosphate (IP3) and diacylglycerol. These two cellular messengers mediate the rapid release of calcium from the cellular stores [26].

Furthermore, an alternative ligand-binding pocket in the VDR has been identified by molecular docking using a receptor conformational ensemble model generated from x-ray crystal structure of the VDR ligand binding domain.This structural flexibility has been proposed as a base of rapid and nongenomic biological responses induced by 1α,25$(OH)_2D_3$ and some of its analogs. The non-genomic rapid (diect) response has been shown in several biological systems, that may be mediated through a functional VDR in some systems or may be independent of VDR in other systems. To support the involvement of VDR in the rapid non-genomic actions of vitamin D, it has been reported that VDR is present in caveolae-enriched plasma membranes and binds 1α,25$(OH)_2D_3$ with high affinity in vivo and in vitro [4].

The existence of non-classical membrane VDR has been found to be related to the rapid actions, including activation of protein kinase C and protein phosphatase PPIc [4].

The actions have been shown to result in subsequent ion channel activity modulation. Furthermore, it has been known for some time that 1α,25$(OH)_2D_3$ reduces UV induced DNA damage in the form of cyclobutane pyrimidine dimers (CPD) in human keratinocytes in culture, and in mouse and human skin. The photoprotection by 1α,25$(OH)_2D_3$ against oxidative insults is thought to be mediated by a non-genomic signaling mechanism, because 1α,25$(OH)_3$ lumisterol 3, which has almost no transactivating activity, reduces UV-induced DNA damage, apoptosis and immunosuppression with similar potency as 1α,25$(OH)_2D_3$[4].

In the earlier section under Hedgehog Hh/Gli signaling pathway, it was mentioned the work by Uhmann et al. who demonstrated that 1α,25$(OH)_2D_3$ was capable of inhibiting Hh signaling at the level of SMO in the absence of

VDR, and by Tang et al. who also found that 1α,25$(OH)_2D_3$-induced Gli expression in murine BCC cells was independent of VDR. Thus, in addition to the report by Wali et al, these two studies provide strong evidence of the non-genomic and rapid non-VDR action of 1α,25$(OH)_2D_3$ on cell growth and differentiation [4].

Tang *et al.* who studied murine basal cell carcinomas (BCC) in vitro and in vivo, and found that the effect of 1α,25$(OH)_2D_3$ on Gli expression is likely independent of VDR. The results provide strong evidence of the non-genomic action of 1α,25$(OH)_2D_3$ on cell growth and differentiation mediated by Hh/Gli signaling pathway [4].

Rapid activation of extracellular signal-regulated kinases, ERK1/ERK2 in NB4 promyelocytic leukemia cells can be induced not only by 1,25-D, but also by analogs that are unable to activate VDR, suggesting the possibility of a separate receptor. Antibodies to a membrane protein block the ability of 1,25-D to induce rapid calcium uptake and activation of PKC in cartilage cells. VDR-/- osteoblasts take up calcium and activate PKC similar to the wild-type osteoblasts, implicating proteins other than VDR in these actions. In contrast, Gniadecki has described activation of ERK through 1,25-D-induced activation of Raf as a result of interactions between VDR and the adaptor protein Shc. VDR-null osteoblasts do not exhibit ion channel responses in response to 1,25-D and Erben et al. have reported that deletion of the VDR DNA binding domain also eliminates non-genomic responses. Thus some of the rapid actions of 1,25-D may be dependent upon VDR, whereas others are not [17].

SUMMARY AND FUTURE DIRECTIONS

The reversal of EMT through the use of VDR has been found to play critical roles in the control of tumor invasion, metastasis, and drug resistance in PDAC (pancreatic ductal adenocarcinoma). Downregulation of VDR could trigger EMT by several factors, including cytokines and cellular signaling pathways such as β-catenin, FoxM1, and CSCs. More importantly, specific natural compounds could partially reverse the EMT phenotype to mesenchymal-to-epithelial transition (MET), resulting in the reversal of drug resistance. Therefore, targeting the VDR pathway with nontoxic natural agents could be a novel potential therapeutic strategy for the treatment of metastatic PDAC. However, the molecular mechanisms of EMT are very complicated and are not yet fully elucidated. Therefore, further investigation is necessary to explore the mechanisms underlying EMT progression in PDAC. Future research will surely focus on uncovering the molecular similarities and differences among the EMT programs. EMT research in the next few years promises to be exciting, as new mouse models and molecular probes are identified to address the important, still-unanswered questions [29].

Cell fate and phenotype are strictly regulated by extracellular signals. 1,25$(OH)_2D_3$ and EMT-TFs have opposite effects on epithelial cell phenotype and they antagonize each other (Figure 3 - part I). 1,25$(OH)_2D_3$ induces epithelial differentiation while it inhibits the expression of several EMT inducers. Conversely, expression of keyEMT-TFs in epithelial cells promotes the acquisition of a mesenchymal phenotype, which in the case of SNAIL1 and SNAIL2 is associated with *VDR* gene repression and the blockade of 1,25$(OH)_2D_3$ action on epithelial differentiation. Thus, a double negative feedback loop operates between 1,25$(OH)_2D_3$ and EMT inducers that may contribute to the complete acquisition of the phenotype dictated by the extracellular cues.The loop may first amplify the signal and later stabilize cell fate once the process is completed. Hence, the balance between 1,25$(OH)_2D_3$/VDR and SNAIL family of transcription factors determines cell fate, and its imbalance may explain the reversibility of the EMT process. Of note, the transition between epithelial and mesenchymal phenotypes is also governed by similar double negative feedback loops among EMT-TFs and certain microRNAs, such as the ZEB/*miR-200* and the SNAIL1/*miR-34* regulatory circuits [14].

The implication of EMT in cancer progression and organ fibrosis and the inhibitory effect of 1,25$(OH)_2D_3$ on EMT have opened the possibility of a therapeutic use of VDR agonists against these diseases. However, the downregulation of VDR expression found in several

types of cancer, frequently associated with advanced stages of the disease, limits the applicability of vitamin D compounds to prevention in highrisk populations and treatment in patients at early stages of tumor progression [14]. In addition, EMT is a transient event during tumorigenesis, and it has been proposed that the reverse process (MET) in the metastatic sites was postulated to be part of the process of metastatic tumor formation [27,14]. These lines of evidence have led to controversy about anticancer therapeutic strategies designed to inhibit EMT, as they may favor the formation of metastases, and suggest that these therapies may be limited to patients diagnosed at early stages of the disease to prevent invasion and dissemination. Nevertheless, vitamin D compounds as inhibitors of EMT may be interesting therapeutic agents for fibrosis-associated pathologies, in which the EMT process is not reverted and the mesenchymal phenotype is maintained during disease progression [14].

$1\alpha,25(OH)_2D_3$ is a potent anti-cancer agent, affecting cancer cells in cultures and tumor progression in animal models by a variety of mechanisms, including but not limited to anti-proliferation, anti-angiogenesis, pro-apoptosis, pro-differentiation and anti-inflammation (Figure 2-part I). The major hurdle is the hypercalcemic side effect induced by administering a high dose of $1\alpha,25(OH)_2D_3$, that is necessary to exert the anti-tumor effects of vitamin D in humans. The potential of using less calcemic analogs of vitamin D with much higher potency than $1\alpha,25(OH)_2D_3$ for treating cancers still exists (Seocalcitol was with positive opinion from EMA for treatment of hepatocellular carcinoma and recommended from the producer also for treatment of pancreatic cancer , showen cardiotoxic activities), especially in combination with other anti-cancer agents or immunomodulatory drugs. The association between decreased sun exposure/vitamin D deficiency and the risk of chronic diseases, including many types of cancer, indicates that maintaining adequate vitamin D nutrition should be a paramount priority for men and women of all ages [4].

REFERENCES:

1. Ascierto PA, JM Kirkwood , JJ Grob, E Simeone, AM Grimaldi, M Maio, G Palmieri, A Testori, FM Marincola, N Mozzillo. The role of BRAF V600 mutation in melanoma. J Translational Medicine. 2012, 10: 85.
2. Blaydon DC, D Nitoiu, KM Eckl, RM Cabral, Ph Bland, I Hausser, DA van Heel, Sh Rajpopat, J Fischer, V Oji, A Zvulunov, H Traupe, H Christian Hennies, and DP Kelsell. Mutations in CSTA, Encoding Cystatin A, Underlie Exfoliative Ichthyosis and Reveal a Role for This Protease Inhibitor in Cell-Cell Adhesion. Am J Hum Genet. 2011, 89(4): 564–571.
3. Chakraborti CK. Vitamin D as a promising anticancer agent. Indian J Pharmacol. 2011, 43(2): 113–120.
4. Chiang KC and TC Chen. The Anti-cancer Actions of Vitamin D. Anti-Cancer Agents in Medicinal Chemistry. 2013, 13: 126-139.
5. Dumesic PA, FA Scholl, DI Barragan, and PA Khavari. Erk1/2 MAP kinases are required for epidermal G2/M progression. J. Cell Biol. 2009, 185(3): 409–422.
6. Gilad LA, T Bresler, J Gnainsky , P Smirnoff and B Schwartz. Regulation of vitamin D receptor expression via estrogen-induced activation of the ERK 1/2 signaling pathway in colon and breast cancer cells. J Endocrinol. 2005, 185: 577–592.
7. Griewank K, R Murali, B Schilling, T Schimming, I Moller, I Moll, M Schwamborn, A Sucker, L Zimmer, D Schadendorf, U Hillen. TERT promoter mutations are frequent in cutaneous Basal Cell Carcinoma and Squamous Cell Carcinama. PLOS One. 2013, 8: 11: e80354.
8. Gupta A, D Nitoiu, D Brennan-Crispi, S Addya, NA Riobo, DP Kelsell, MG Mahoney. Cell cycle- and cancer-associated gene networks activated by Dsg2: evidence of cystatin A deregulation and a potential role in cell-cell adhesion. PLoS One. 2015, 10(3): e0120091. 14.
9. Hyter S and AK Indra. Nuclear hormone receptor functions in keratinocyte and melanocyte homeostasis, epidermal carcinogenesis and melanomagenesis. FEBS Lett. 2013, 587(6): 529–541.
10. Hyter S, AK Indra. Nuclear hormone receptor functions in keratinocyte and melanocyte homeostasis, epidermal carcinogenesis and melanomagenesis. FEBS letters. 2013, 587(6): 529-541.
11. Kanda N, S Watanabe. 17β-Estradiol Stimulates the Growth of Human Keratinocytes by Inducing Cyclin D2 Expression. J Investigative Dermatol. 2004, 123(2): 319-328.
12. Kasper M, V Jaks, D Hohl, R Toftgard. Basal cell carcinoma — molecular biology and potential new therapies. J Clin Invest. 2012, 122(2): 455-63.

13. Kure S, K Nosho, Y Baba, N Irahara, K Shima, K Ng, JA Meyerhardt, EL Giovannucci, CS Fuchs, S Ogino. Vitamin D receptor expression is associated with PIK3CA and KRAS mutations in colorectal cancer. Cancer Epidemiol Biomarkers Prev. 2009, 18(10):2765-72.
14. Larriba MJ, AG Herreros, A Muñoz. Vitamin D and the Epithelial to Mesenchymal Transition. Stem Cells International, 2016, (2016): Article ID 6213872, 11 p.
15. Li M, H Chiba, X Warot, N Messaddeq, C Gerard, P Chambon, D Metzger. RXR-alpha ablation in skin keratinocytes results in alopecia and epidermal alterations. Development. 2001, 128: 675-688.
16. Murphy LO and J Blenis. MAPK signal specificity: the right place at the right time. Trends Biochem Sci. 2006, 31(5):268-75.
17. Narayanan R, VAT Sepulveda, M Falzon, NL Weigel. The Functional Consequences of Crosstalk between the Vitamin D Receptor and ERK Signaling Pathways Are Cell-specific. J Biol Chemistry. 2004, 279(45): 47298 –47310.
18. Pandolfi S, B Stecca. Cooperative integration between HEDGEHOG-GLI signalling and other oncogenic pathways: implications for cancer therapy. Expert Rev Mol Med. 2015, 9;17:e5.
19. Perzelova V, F Sabol, T Vasilenko, M Novotny, I Kovac, M Slezak, J Ďurkac, M Holly, M Pilatova, P Szabo, L Varinska, Z Čriepokova, T Kucera, H Kaltner, S Andre, HJ Gabius, P Mucaji, K Smatana Jr and P Gal. Pharmacological activation of estrogen receptors-α and -β differentially modulates keratinocyte differentiation with functional impact on wound healing. Int J Mol Med 2016, 37: 21-28.
20. Piotrowska A, J Wierzbicka and MA Żmijewski. Vitamin D in the skin physiology and pathology. ACTA Biochimica Polonica. 2016, 63: 1-13.
21. Takahashi H, K Asano, M Kinouchi, A Ishida-Yamamoto, KD Wuepper and H Iizuka. Structure and Transcriptional Regulation of the Human Cystatin A Gene. The 12-O-tetradecanoylphorbol-13-acetate (TPA) responsive element-2 site (−272 TO −278) on cystatin A gene is critical for TPA-dependent regulation. JBC. 1998, 273, 17375-17380.
22. Takahashi H, M Honma, A Ishida-Yamamoto, K Namikawa, H Kiyama, H Iizuka. Expression of human cystatin A by keratinocytes is positively regulated via the Ras/MEKK1/MKK7/JNK signal transduction pathway but negatively regulated via the Ras/Raf-1/MEK1/ERK pathway. J Biol Chem. 2001, 276(39): 36632-8. .
23. Takahashi H, M Ibe, M Honma, A Ishida-Yamamoto, Y Hashimoto, H Iizuka. 1,25-Dihydroxyvitamin D3 increases human cystatin A expression by inhibiting the Raf-1/MEK1/ERK signaling pathway of keratinocytes. Arch Dermatol Research 2003, 295(2): 80–87.
24. Verdier-Sevrain S, M Yaar, J Cantatore, A Traish, BA Gilchrest. Estradiol induces proliferation of keratinocytes via a receptor mediated mechanism. FASEB J. 2004,18(11): 1252-4.
25. Wang AX, XY Qi. Targeting RAS/RAF/MEK/ERK Signaling in Metastatic Melanoma. IUBMB Life. 2013, 65(9): 748-758..
26. Wierzbicka J, A Piotrowska and MA Żmijewski. The renaissance of vitamin D. ACTA Biochimica Polonica. 2014, 61(4), 679–686.
27. Yao D, C Dai, S Peng. Mechanism of the Mesenchymal–Epithelial Transition and Its Relationship with Metastatic Tumor Formation. Mol Cancer Res. 2011, 9(12): 1608-1620.
28. Zeeuwen PL, IM van Vlijmen-Willems, T Cheng, D Rodijk-Olthuis, K Hitomi, I Hara-Nishimura, S John, N Smyth, T Reinheckel, WJ Hendriks, J Schalkwijk. The cystatin M/E-cathepsin L balance is essential for tissue homeostasis in epidermis, hair follicles, and cornea. FASEB J. 2010, 24(10): 3744-55.
29. Zhiwei L, G Junli, X Keping, and Zh Shaojiang. Vitamin D Receptor Signaling and Pancreatic Cancer Cell EMT. Curr Pharm Des. 2015, 21(10): 1262–1267.
30. Ziv E, C Rotem, M Miodovnik, A Ravid, R Koren. Two modes of ERK activation by TNF in keratinocytes: Different cellular outcomes and bidirectional modulation by vitamin D. J. Cell. Biochem. 104, 606–619: 2008.

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Сравнително изследване на телесни маси, някои телесни обиколки и нивата на триглицериди и холестерол при различни групи пациенти, включени в НИРДИАБО проекта от Пловдивска област

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Comparison of the fat mass, some body circumferences (waist, hip and thigh) and the levels of cholesterol and triglycerides in different groups of patients included in NIRDIABO project from Plovdiv

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РЕЗЮМЕ:

Въведение: Цел на изследването е да се сравнят някои телесни обиколки (талия, ханш и бедро), телесните маси и нивата на триглицериди и холестерол при различ-

ABSTRACT

Introduction: The aim of the study was to compare some body circumference measures, fat mass and triglycerides and cholesterol levels in different age groups in NIRDIA-

ни групи пациенти, включени в НИРДИАБО проекта от пловдивска област. **Метод:** В изследването са включени доброволци с BMI над 25, разделени на 5 възрастови групи: а) 20-29 г.; б) 30-39 г.; в) 40-49 г.; г) 50-59 г.; д) 60-69 г. (n=5-9). Вземането на кръвни проби е единствената слабо болезнена процедура. Първите 2 месеца пациентите са на ниско калорична диета и прием на пробиотик. Определени са нивата на триглицериди и общ холестерол, както и на HDL and LDL холестерола при първата визита. **Резултати:** Измерените телесни обиколки показват завишени стойности в средната възрастова група, телесните масти са също по-високи в тази група. Липидният профил показва високи нива на триглицериди, холестерол (HDL и LDL) в същата група. **Заключение:** Клиничното проучване показва важността на измерването на тези телесни обиколки, сравняването им с телесните масти и нивата на триглицериди и холестерол, особено на HDL и LDL холестерола в различните възрастови групи. Основна рискова група са хората на средна възраст – 40-49 години. Използването на пробиотик и подходяща нискокалорична диета може да помогне за понижаването им, да подобри стила на живот и да го удължи.

Ключови думи: телесни масти, телесни обиколки, липиден профил

BO project from Plovdiv region. **Method:** Volunteers with BMI over 25 were included (male and female), divided in 5 age groups: a) 20-29 years; b) 30-39 years; c) 40-49 years; d) 50-59 years; e) 60-69 years (n=5-9). There was one mild invasive procedure – taking blood samples from patients. The levels of triglycerides, cholesterol (incl. total fraction, HDL and LDL) were measured at first visit. **Results:** The body circumferences were increased in the middle age group, the fat mass also was higher in the same group. The lipid profile showed higher levels of triglycerides, cholesterol (HDL and LDL) in the same group. **Conclusion:** The present study reveals the importance of measuring some body circumferences that have to be compared with the fat mass levels, the levels of triglycerides and cholesterol, with focus on HDL and LDL, in different age groups. The main group at risk with higher parameters and levels is the middle aged patients – 40-49 years. The use of probiotics with an appropriate low caloric diet may help them to decrease it and to improve the life style.

Key words: fat mass, body circumferences, lipid profile

INTRODUCTION

The tendency to develop diabetes mellitus type 2 is mainly due to the rise of overweight and obesity in population [10]. The obesity has increased globally over the last few decades and its association with insulin resistance has affected the ability to reduce population morbidity and mortality. Traditionally, adipose tissue in the visceral fat depot has been considered one of the major culprits in the development of insulin resistance. However, there is growing evidence supporting the role of subcutaneous abdominal adipose tissue in the development of insulin resistance. There are significant differences in the functional characteristics of subcutaneous abdominal vs. intra-abdominal vs. gluteofemoral fat depot [11].

Therapeutic lifestyle changes continue to be the most important intervention in clinical practice to improve adipose tissue function and to avoid development of insulin resistance.

The ratio between the body circumference at the waist and the hips (WHR) is unique to humans. The waist-hip ratio is an accurate anthropometric parameter for predicting hypertension in overweight and obese patients [6]. High WHR doubles the risk of vascular diseases. BMI and fat mass are not the only and strongest body predictors of „idiopathic" diseases, but also WHR should be considered as such [9]. The WHR measures also the distribution of body fat and it has

been a focus of extensive research. The distribution of body fat provides information about age, health and fertility [2]. Usually fat mass is the marker for some physiological and pathological conditions, like fat infiltration of cells [13].

Cholesterol plays a vital role in cell biology. Cholesterol is the precursor for all steroid hormones, including gluco- and mineral- corticoids, sex hormones, vitamin D. All these regulate carbohydrate, sodium, reproductive and bone homeostasis, respectively [4]. Diet-increased serum cholesterol levels do not increase the low-density lipoprotein/high-density lipoprotein (LDL/HDL cholesterol ratio). Increased levels of LDL cholesterol reduce HDL cholesterol levels and are considered independent risk factors for arterial diseases. [8].

The aim of our study was to compare some body circumference measures as waist, hip and thigh, the fat mass and the levels of cholesterol and triglycerides in different age groups of volunteers that took part in the project „NIRDIABO". All volunteers were from the Plovdiv region and they participated in the first year of the project.

METHODS

The project aimed to examine the quality of life and the effects of some non-pharmacological interventions on volunteers with obesity and high BMI. Both male and female volunteers with BMI over 25 were included in this study, and were divided in the following groups: a) 20-29 years of age; b) 30-39 years of age; c) 40-49 years of age; d) 50-59 years of age; e) 60-69 years of age. The number of patients in each age group was relatively small (n=5-9).

All requirements to perform human study were done, as it was described in our previous paper [5]. There weren't any harmful procedures on the patients excluding the weak discomfort when blood samples were taken.

The including as well as excluding factors and criteria and the non-pharmacological intervention of the study was also performed as it was cited in previous paper [5].

We used „TANITA C 300 BC-420 MA body composition analyzer" apparatus (USA) to make the following measurements: age, sex, body weight, BMI and fat mass. The circumference measures (waist, hip and tight) were done using simple linear measure in centimeters.

The levels of cholesterol, triglyceride, HDL and LDL were done at Clinical Laboratory Department of the Medical University – Sofia. The estimations were done using the widely accepted method on the system Roche - Hitachi, Cobas 6000 (Japan), according to the instructions of the producer.

Statistics: all observed values were calculated and the mean for each group was estimated. Because of the small number of patients in each age group the statistical significance was not determined.

RESULTS

The average body measures of circumferences of the waist, hip and thigh in centimeters (cm) are shown on **Fig. 1.** There is a tendency, not statistically significant, for all measures to increase over the years in the first 3 age groups, but in the two last groups there is a tendency of decreasing in all circumferences measured.

The average fat mass was measures bioelectrically on the first and the second visits. There is interesting tendency in the first 2 group, more pronounced in the younger group, to decrease the fat mass after 2 months low caloric diet, performed by the patients. The third group do not show any changes between first and second visit. The older 2 groups even show slight increasing of the fat mass **(Fig. 2).**

The basal levels of the cholesterol and the triglycerides were estimated on the first visit. The 1st and the 2nd group of patients were with normal range levels of cholesterol and triglycerides. The 3rd, 4th and 5th groups of patients were with higher levels of cholesterol **(Fig. 3).**

The levels of HDL and LDL in all groups studied were in the normal range, but the levels of LDL wwere higher in the last 3 groups. Evidently the ration HDL/LDL is moved towards up due to increased levels of LDL **(Fig. 4).**

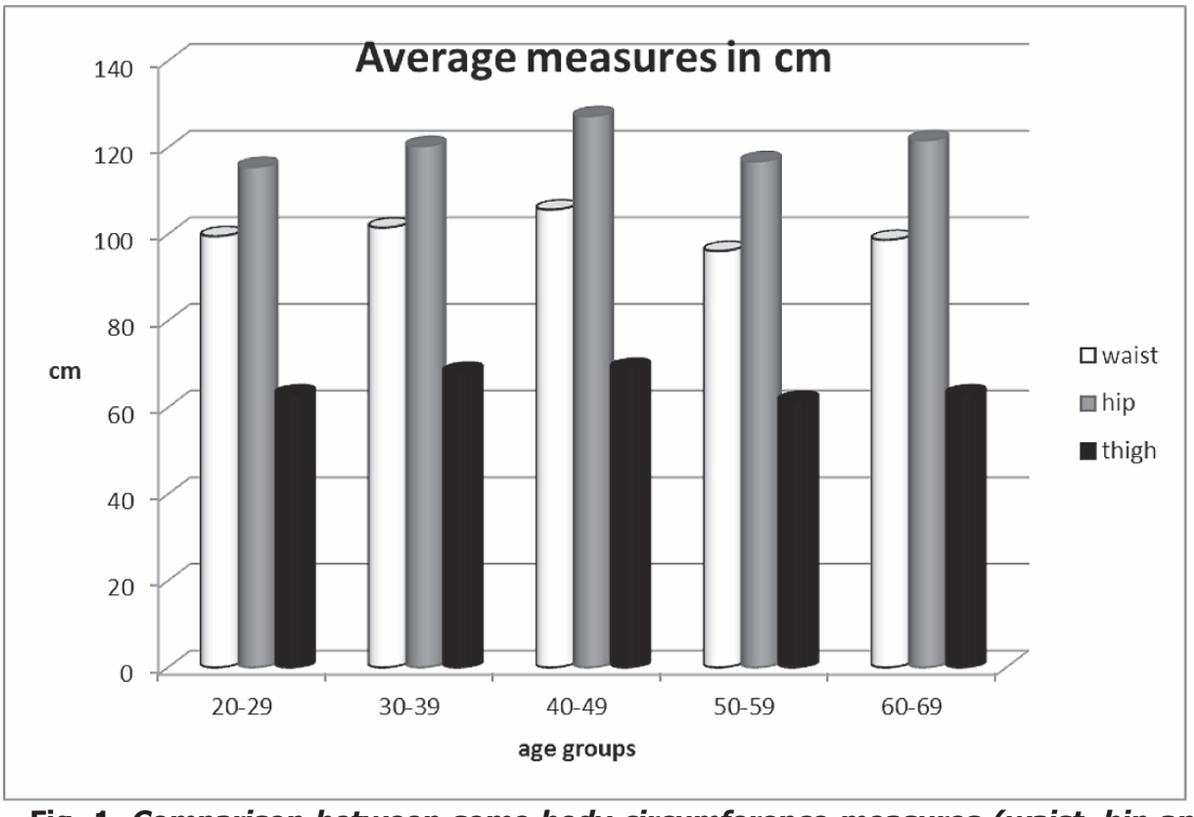

Fig. 1. ***Comparison between some body circumference measures (waist, hip and thigh) in the examined age groups.***

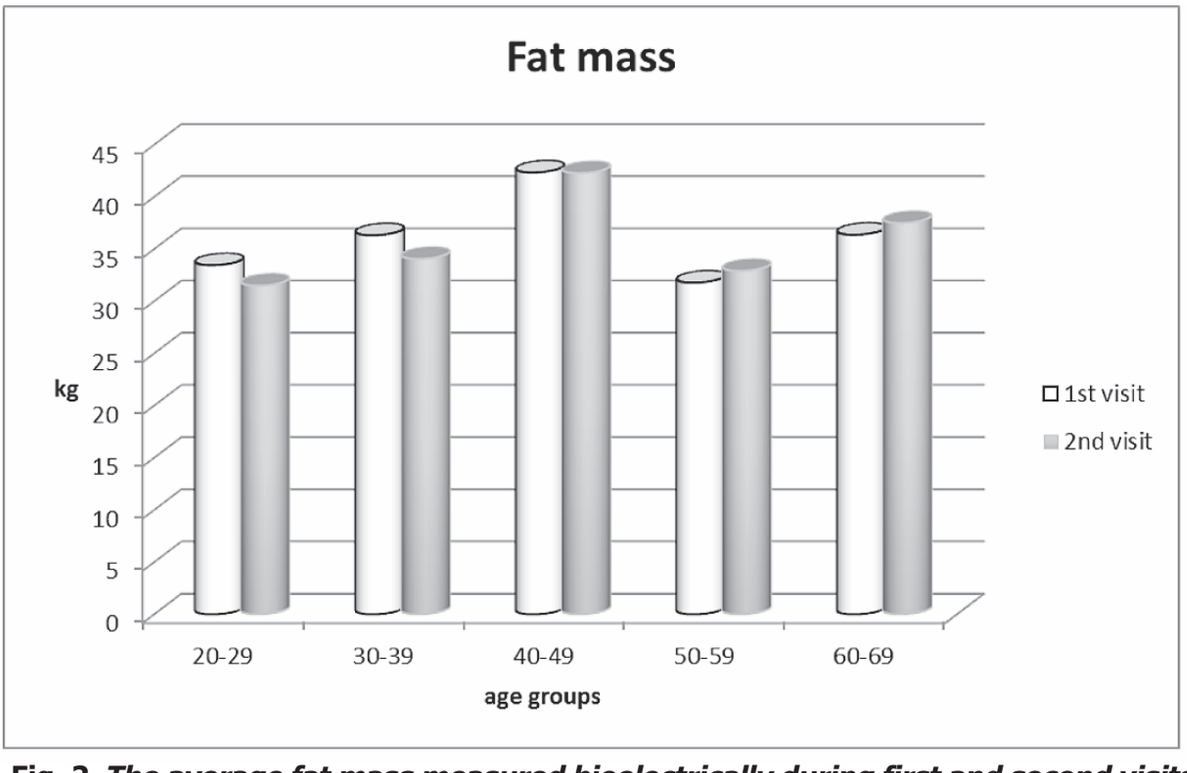

Fig. 2. ***The average fat mass measured bioelectrically during first and second visits in all age groups of patients.***

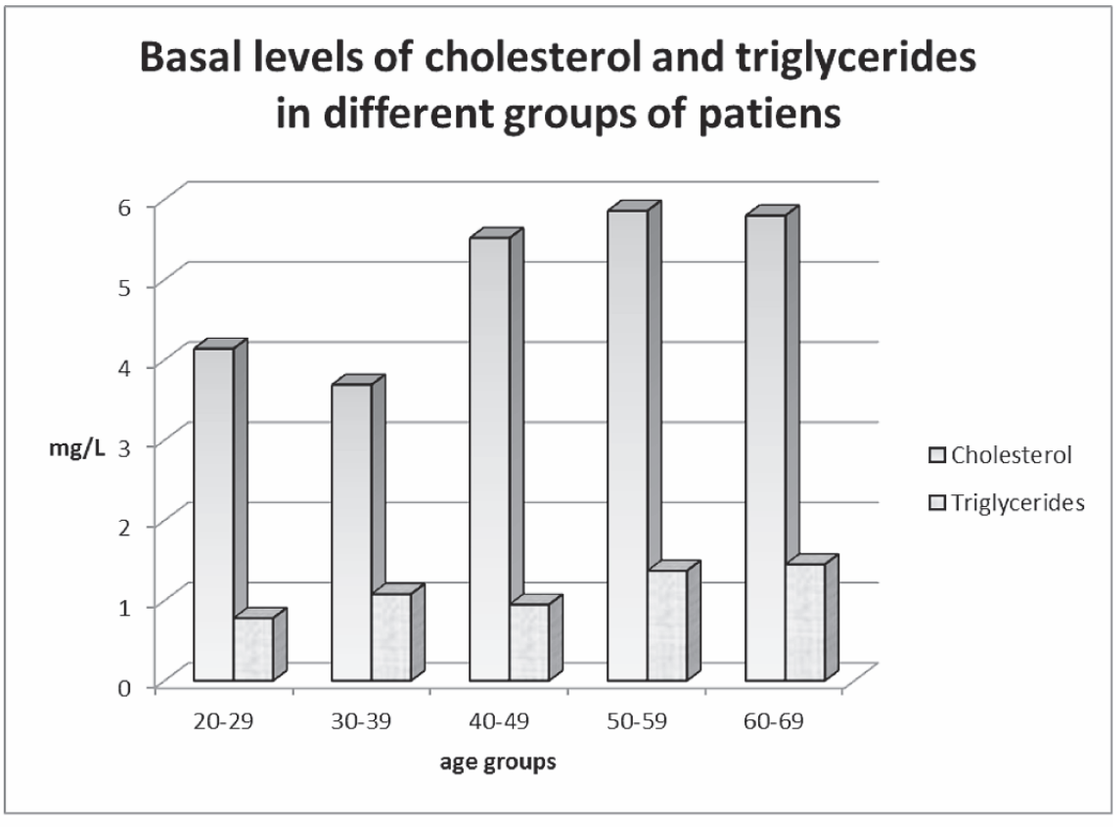

Fig. 3. ***Basal levels of cholesterol and triglycerides in the different groups of patients.***

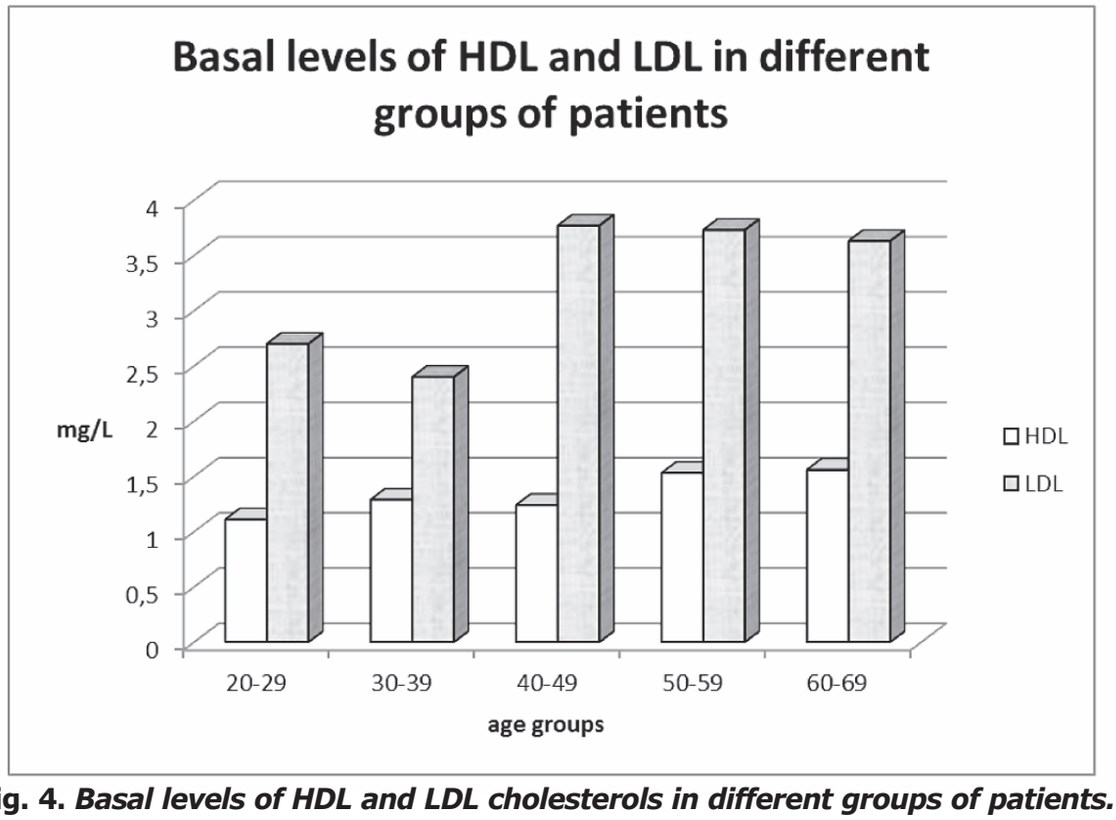

Fig. 4. ***Basal levels of HDL and LDL cholesterols in different groups of patients.***

DISCUSSION

Recent research suggests that distribution of body fat is the most important indicator for cardiovascular risk [3]. In particular, increased visceral fat has been shown by them as an important risk factor for diabetes type 2 and other metabolic disorders. An indicator of visceral fat is not only fat mass, but also the waist circumference is valid marker as more sensitive to the distribution of body fat than other markers, as BMI. Other authors [1] showed that some other indices of abdominal obesity should be considered – hip and thigh circumferences, which gave better information about metabolic risk factors. They believe that ethic and racial variation among population from different regions might need different cut-off points or use of different anthropometric measurements to diagnose obesity and metabolic disorders.

In our study all circumferences measured – waist, hip and thigh showed increasing values in cm with age (from 20 to 49 years), but they decreased after 50 – 69 years of age. Evidently our findings are in accordance with those in the literature and the main risk group is in the 3rd one – 40-49 years, probably due to hormonal changes in human body during this period of life. We assume that our data is valid for the Eastern European population and showed the European tendency for increasing the body fat due to more caloric diet traditionally used.

It has been established that abdominal obesity assessed by waist circumference predicts obesity-related health risk [7]. It has been shown that waist circumference and hip or thigh circumferences have independent and opposite effects on metabolic health risk. They suggest that waist circumference is positively associated with health risk, but hip and thigh circumferences are negatively associated with health. Our data do not support such view, because in our study all circumferences measured have linear relationship.

Other believe, that a large hip or thigh circumferences, or both, which are due probably to a greater lean mass, have a protective effect on health [1]. Our data shows that fat mass measured bioelectrical is slightly decreased after 2 months of low caloric diet performance by patients only in 20-39 years of age, do not change in older age groups. Probably those measures have no significant influence on metabolic changes associated with obesity or diabetes type 2.

It is well known that the levels of triglycerides and cholesterol, as well as its HDL and LDL forms play a significant role in obese patients or diabetic type 2 patients. In our study triglycerides and cholesterol increased after 40 years of age and the levels of HDL and LDL cholesterol also.

Clinical and epidemiologic studies have constantly shown that there is an inverse relationship between the HDL cholesterol concentration and vascular risk [12]. HDL cholesterol has been proposed to have several anti-atherosclerotic properties, including the ability to mediate macrophage cholesterol efflux, antioxidant capacity, anti-inflammatory properties, nitric-oxide promoting activity and an ability to transport proteins with their own intrinsic biological activity. HDL particles are critical acceptors of cholesterol from lipid macrophages and thereby play an important role in the maintenance of cholesterol balance in the arterial wall. On the other hand LDL cholesterol is an important target in clinical studies and decreased levels below1.8 moll/L is of great interest. High LDL cholesterol is prerequisite for formation of atherosclerotic plaque. In our study LDL cholesterol levels are higher in aged groups – 50-69 years of age, i.e. the bad cholesterol shifts the ration HDL/LDL towards itself.

CONCLUSION

The present study reveal the importance of measure some body circumferences, compare it with fat mass and the levels of triglycerides and cholesterol, especially HDL and LDL cholesterols, in different age groups. The main group at risk with higher measures and levels is the middle aged patients – 40-49 years. The use of probiotics with appropriate low caloric diet may help them to decrease it and to improve life style and prolong life time.

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REFERENCES:

1. Bener A, Yousafzai MT, Darwish S, Al-Hamaq AOAA, Nesralia EA, Abdul-Ghani M. Obesity index that better predict metabolic syndrome: bosy mass index, waist circumference, waist hip ratio or waist thigh ratio. J. Obes, 2013, 13: 269-78.
2. Bovel J, Raymonds M. Preferred women's waist-to hip ration variation over the last 2 500 years. Plus One, 2016, 10(4): e0123284.
3. Bozeman SR, Hoaglin DC, Burton TM, Pashos CL, Ben-Joseph RH, Hollenbeak CS. Predicting waist circumference from body mass index. BMC Med Res Methodol, 2012, 12: 115-123.
4. Cortes VA, Busso D, Maiz A, Arteaga A, Nervi F, Rigotti A. Physiological and pathological implications of cholesterol. Bront. Biosci, 2014, 19: 416-28.
5. Getova D, Dimitrova D, Topolov M. Comparison of body weight, BMI, glucose level, blood pressure and heart rate in different groups of patients included in NIRDIABO project from Plovdiv. Bulgarian Medicine, 2017, 7(1): 23-29.
6. Gonzalez-Jimenez E, Montero-Alonso MA, Schmidt-RioValle J. Waist-hip ration as a predictor of arterial hypertension risk in chidren and adolescents. Nutr. Hosp, 2013: 28(6): 1993-6. Doi: 10:3305/nutr hosp.v28in06.6653.
7. Janssen I, Katzmarzyk PT, Ross R. Waist circumference and not body mass index explains obesity related health risk. The American Journal Clinical Nutrition, 2004, 79: 379-84.
8. Kapourchali FR, Surendiran G, Goulet A, Moghadasian MH. The role of dietary cholesterol in lipoprotein metabolism and related metabolic abnormalities: a mini review. Crit. Rev. Food Sci, Nutr., 2016, 56(14): 2408-15.
9. Mondelli M, Aretini A, Ginanneschi F, Greco G, Mattioli S. Waist circumference and waist-to-hip ration in carpal tunnel syndrome: a case-control study. J. Neurol. Sci, 2014, 338(1-2): 207-13.
10. Nicolls SJ, Brown A. Targeting obesity, diabetes and the metabolic syndrome: are we trying to close the barn door after the horse bolted? Exprt. Rev. Cardiovasc. Ther., 2014, 12(3): 279-90. Doi: 10.1586/1479072.2014.894362.
11. Patel P, Abate N. Body fat distribution and insulin resistance. Nutrients, 2013, 5(6): 2019-27.
12. Ono K. Current concept of reverse cholesterol transport and novel strategy for atheroprotection. Journal of Cariodlogy, 2012, 60: 339-343.
13. Vetrovska R, Vilikus Z, Klaschka J, Stranska Z, Svacine S, Svobodova S, Matoulek M. Does impedance measure a functional state of the body fat? Physiol. Res, 2014, suppl. 2: S309-320.

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Анализ на експресията на серумните нива на тумор некрозисфактора алфа и TNF при пациенти с остър коронарен синдром във връзка с наличието или отсъствието на възпалително ставно заболяване, тип заболяване на ставите и активност на заболяването

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Analysis of the expression of the Tumor Necrosis Factor alpha and TNF serum levels in patients with acute coronary syndrome in relation to the presence or absence of inflammatory joint disease, type of joint disease and disease activity

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РЕЗЮМЕ:

Повишени нива на Туморния Некрозен Фактор алфа (TNF-a) и TNFa серум се асоциират с наличието на възпалителни ставни и сърдечни заболявания. Повечето от съществуващите научни проучвания са изследвали тази връзка поотделно, в рам-

ABSTRACT

Elevated levels of the Tumour Necrosis Factor alpha (TNF-α) and TNFα serum have a significant association with inflammatory joint disease and cardiac disease. However, most of the existing research has examined this relationship separately, within inflamma-

ките на възпалителни ставни заболявания и в рамките на сърдечни заболявания. Настоящото проучване има за цел да обедини тези две области като проследява нивата на експресия на TNFa и TFNa серум при пациенти с остър коронарен синдром (ACS), разделени на две групи: със и без възпалително ставно заболяване. Също се изледва потенциална асоциация с тип ставно заболяване (RA срещу PsA) и ниво на болестна активнот, измерено чрез DAS28 при пациенти с RA и ASDAS при пациенти с PsA.

Изледвана е извадка от 95 пациентите с остър коронарен синдром (ОКС). От тях, 46 са с възпалително ставно заболяване (ВСЗ) и 49 без ВСЗ. TFN-a експресия е измерена чрез имунохистохимичен анализ на материал, съдържащ атероматозна плака от операция на сърдечен байпас или друга манипулация без риск за пациента. TNFa серумните нива се измерват с протокол ELISA Kit за тумор некрозис фактор ELISA (TNF) AA 77-233, човешки, сандвич ELISA, чувствителност 1.0 pg / mL ABIN411361- BOSTER.

Резултатите показват значително повисоки нива на експресия на TNFa и TNFa серум (48 часа) при ОКС пациенти с ВСЗ в сравнение с ОКС групата без ВСЗ. Не се установява статистически значима разлика при TNFa серум на 24 часа. В групата без ВСЗ има значителен спад в серумното ниво на TNFa на 48 часа; докато в групата с ВСЗ не се наблюдава значително понижение. Установява се, че TNF-a експресията и серумното ниво на TNFa не са свързани с вида на възпалителното ставно заболяване (RA спрямо PsA), нито с нивото на активност на заболяването.

Ключови думи: възпалително ставно заболяване, остър коронарен синдром, TNF-α, TNFα серум, ревматоиден, артрит, псориатичен артрит

tory joint disease and within cardiac disease. The present study aimed to put together both areas by comparing the levels of TNF-α expression and TFNα serum in patients with acute coronary syndrome (ACS), divided into two groups: with and without inflammatory joint disease. Associations with type of joint disease (RA vs. PsA) and inflammatory joint disease activity (as measured by DAS28 in patients with RA and ASDAS in patients with PsA) were also explored.

The sample included 95 ninety-five patients with ACS. Among then, 46 had inflammatory joint disease and 49 did not. TFNα was measured through immunohistochemical analysis of material containing atheromatous plaque from cardiac bypass surgery or other manipulation without a risk for the patient. TNFa serum levels were measured with the following protocol Tumor Necrosis Factor ELISA Kit (TNF) AA 77-233 Human, Sandwich ELISA, sensitivity 1.0 pg / mL ABIN411361- BOSTER.

TNFα expression and TNFα serum levels (48hrs) were significantly higher in ACS patients with inflammatory joint disease than in those without inflammatory join disease. There was no significant difference regarding TNFα serum levels (24hrs). In the group without inflammatory joint disease, there was a significant drop in TNFα serum level at 48 hrs; whereas in the group with inflammatory joint disease, no significant decrease was observed. It is observed that TNFα expression and TNFα serum levels were not associated with type of inflammatory joint disease (RA vs. PsA), nor with level of disease activity.

Key words: inflammatory joint disease, acute coronary syndrome, TNFα, TNFα serum, rheumatoid arthritis, psoriatic arthritis

INTRODUCTION

Rheumatoid (RA) and psoriatic arthritis (PsR) are chronic inflammatory autoimmune diseases that are associated with higher cardiovascular risk and accelerated atherosclerosis. They influence both traditional risk factors as well as specific factors such as comorbidity, inflammation level, treatment of the disease and inflammatory activity. TNF-α is an important cytokine with a central role in inflamma-

tory joint disease, atherogenesis and acute coronary syndromes. Its production may be related to the level of activity and the extent of systemic and local inflammation. The normal heart does not express and does not produce TNF-α, but in heart failure or other cardiac diseases, the heart produces extreme levels of TNF-α [1, 2].

Atherosclerosis is characterized by hyperplasia, neointimal and local inflammatory response. TNFα mediates proinflammatory, proliferative, cytostatic and cytotoxic effects in various cell types, including endothelial cells and vascular smooth muscle cells (VSMCs) [3]. In addition, the myocardium may synthesize TNF-α de novo which causes TNF-α serum level to rise extremely in response [4-7]. Scientific research about these processes is beneficial to therapeutic approaches utilizing cytokine inhibition and can help achieve control and risk reduction in acute events.

However, most of the existing research has examined this relationship separately, within inflammatory joint disease and within cardiac disease. The present study aimed to put together both by comparing the levels of TNFα expression and TFNα serum in patients with acute coronary syndrome (ACS), divided into two groups: with and without inflammatory joint disease. Associations with type of joint disease (RA vs. PsA) and inflammatory joint disease activity (as measured by DAS28 in patients with RA and ASDAS in patients with PsA) were also explored.

MATERIAL AND METHODS

Patients

The sample was drawn from cardiac surgical patients at a Bulgarian medical institution. Ninety five patients with acute coronary syndrome (ACS) (mean age 69.59 ± 7.22), of whom 46 (mean age 68.74 ± 7) with inflammatory joint disease and 49 (mean age 70.39 ± 7.40) without inflammatory joint disease were included in the study. Among the 46 patients with inflammatory joint disease, 23 had rheumatoid arthritis (RA - mean age 67.39 ± 7.42) and 23 were with psoriatic arthritis (PsA - mean age 70.09 ± 6.37). Table 1 contains stratified demographic information. There was no significant difference between the groups in terms of average age, $p > .05$ for all comparisons. The samples were predominantly male (77.5% without joint disease; 80% with joint disease; 65% RA; 95.6% PsA). There was no significant sex difference between the groups with joint disease and without joint disease, $p = .730$; however, there was a significant sex difference between the RA and PsA groups as the male sex was predominant in the PsA group, $p = .009$. Table 1 contains stratified demographic information.

The procedures were conducted in accordance with the WMA Declaration of Helsinki. All patients provided written informed consent prior to the investigation.

Immunohistochemical analysis was performed on material containing atheromatous plaque from cardiac bypass surgery or other manipulation without a risk for the patient. The immunohistochemical analysis was performed according to the IHH protocol using the following kits to detect the presence of TNF alpha: *anti-Tumor Necrosis Factor antibody (TNF) - (AA 180-230) -tnf-alpha-Rabbit, Polyclonal, IgG, 1: 100 Biosystems and Polymer Visualization System - CRF™ Anti-Polyvalent HRP Polymer (DAB) Stain Kit.* For the color intensity, the following numeric codes were used: 0 - absence; 1 - weak; 2 - moderate; 3 - strong. Strong intensity is counted as this of a positive control, and as absent based on a negative control. For a more accurate assessment of the color reaction, all materials were prepared with a negative control.

TNFα serum levels were tested at 24 and 48 hours after onset of ACS with protocol *Tumor Necrosis Factor ELISA Kit (TNF) AA 77-233 Human, Sandwich ELISA, sensitivity 1.0 pg / mL ABIN411361- BOSTER.* Their relationship to the presence of absence of inflammatory joint disease was sought.

Inflammatory joint disease activity in patients with RA was established with DAS28, and in the PsA patients with ASDAS. DAS28 values were used to determine categories of disease activity as follows: remission – DAS28 ≤ 2.6; low disease activity – 2.6 < DAS28 ≤ 3.2; moderate disease activity – 3.2 < DAS28 ≤ 5.1; high disease activity – DAS28 > 5.1. Accordingly, ASDAS levels were defined as:

remission – ASDAS < 1.3; low disease activity – 1.3 ≤ ASDAS ≤ 2.1; medium disease activity – 2.1 ≤ ASDAS ≤ 3.4; high disease activity – ASDAS > 3.5.

Analysis

SPSS software package version 24 (SPSS, Chicago, Illinois, USA) was used for statistical analysis. Normally distributed continuous variables are described in mean and standard deviation (SD), dichotomous variables are expressed in number and percentage, and for ordinal variables, the median and range are provided. The dependent variables of interest to this investigation (TNFα, TNFα serum 24hrs; and TNFα serum 48hrs) were examined for normality through Kolmogorov-Smirnov goodness-of-fit test. The results revealed that all three of them were not normally distributed, $p < .001$ for all three variables. This determined the use of non-parametric tests, including Mann-Whitney U test for between group comparisons and Spearman Rho correlation for exploring associations. Additionally, chi-square test was used for nominal variables. All statistical results are interpreted at level of significance, alpha (α) = .05.

RESULTS

First are presented the results of the between-groups comparison of ACS patients with and without inflammatory joint disease in relation to the values of TFNα and TFNα serum (24 and 48 hrs). The descriptive statistics for these variables are given in **Table 1.** As mentioned earlier, all three of them were not normally distributed and for this reason the comparison was performed with Mann-Whitney U test for two independent samples. The results showed statistically significant differences on two of the three parameters, TNFα and TNFα serum (48hrs). For TNFα, the difference was highly significant, $p < .001$. The group without inflammatory joint disease had a lower median value of 1 (range 0-2), whereas the group with inflammatory joint disease had a median value of 2 (range 0-3). A similar trend was found for TNFα serum (48 hrs), where the difference was highly significant, $p < .001$. The group without inflammatory joint disease had a significantly lower mean value of 5.01 (± 3.31) as compared to the group with inflammatory joint disease, mean of 9.98 (± 4.57). No significant difference was found between the two groups regarding TNFα serum at 24 hours, p =.690.

Since the between-group comparisons revealed lack of significant differences regarding TFN α serum (24 hrs) and a highly significant difference for TFN α serum (48 hrs), it was considered important to follow it up with within-group comparisons between the two serum levels. Wilcoxon Signed Ranks test was used to explore potential internal differences in TFN α serum levels between 24 and 48 hrs. The results revealed significant decrease in TFN α serum levels for the ACS group without inflammatory joint disease, p < .001. The mean value dropped from 9.48 to 5.01. However, for the ACS group with inflammatory joint disease no significant reduction in TFN α serum levels was found, p = . 288, with an even slight increase in mean value, from 9.19 to 9.62. The within-group trends of TFN α serum levels between 24 and 48 hrs are illustrated on **Figure 1.**

Additionally, the internal relationship between TNFα and TNFα serum (24 and 48 hrs) was also explored through correlation analysis employing Spearman Rho test. Two correlations were examined within each ACS patient group: 1) between TNFα and TNFα serum (24hrs) and 2) between TNFα and TNFα serum (48hrs), amounting to a total of four correlation analyses. In both groups, no significant association between TNFα expression and TNFα serum were found; $p > .05$ in all four correlations as shown in **Table 2.**

At the next level, a comparative analysis was performed within the group of ACS patients with inflammatory joint disease involving the two subgroups of RA and PsA patients. The Mann-Whitney U test was used to explore potential differences associated with the type of joint disease. None of the three comparisons showed statistical significance. The median value for TNFα was 2 in both RA and PsA patients. Although the range was different (0-2 for RA) and (0-3 for PsA), only one patient in the PsA group had level 3 of TNFα expression. The Mann-Whitney U test showed lack of significant difference, p = .616. The mean values of TNFα serum (24hrs) were

Table 1: ***Demographic features of the participants in the study and dependent variables (TNFα and TNFα serum at 24 and 48 hours***

Variables		Inflammatory Joint Disease			Absence of Inflammatory Joint Disease
		Rheumatoid Arthritis N = 23	Psoriatic Arthritis N = 23	Total N = 46	N = 49
Age: mean (SD)		67.39 (± 7.42)	70.09 (± 6.37)	68.74 (± 7)	70.39 (±7.40)
Sex: N (%)	Male	15 (65%)	22 (95.6%)	37 (80%)	38 (77.5%)
	Female	8 (35%)	1 (4.4%)	9 (20%)	11 (32.5%
TNF α (0-3): Median, (Range)		2 (0 – 2)	2 (0-3)	2 (0-3)	1 (0- 2)
TFN α serum (24 hrs): ng/ml mean, (SD)		9.19 (± 4.75)	10.35 (± 7.20)	9.77 (± 6.06)	9.48 (±7.86)
TFN α serum (48 hrs): ng/ml mean, (SD)		9.62 (±3.64)	10.34 (±5.40)	9.98 (±4.57)	5.01 (±3.31)

TNFα = Tumor Necrosis Factor measured on an ordinal scale of 0 to 3, where 0 = absence and 3 = high expression; TNFα serum is measured in ng/ml and is a continuous variable.

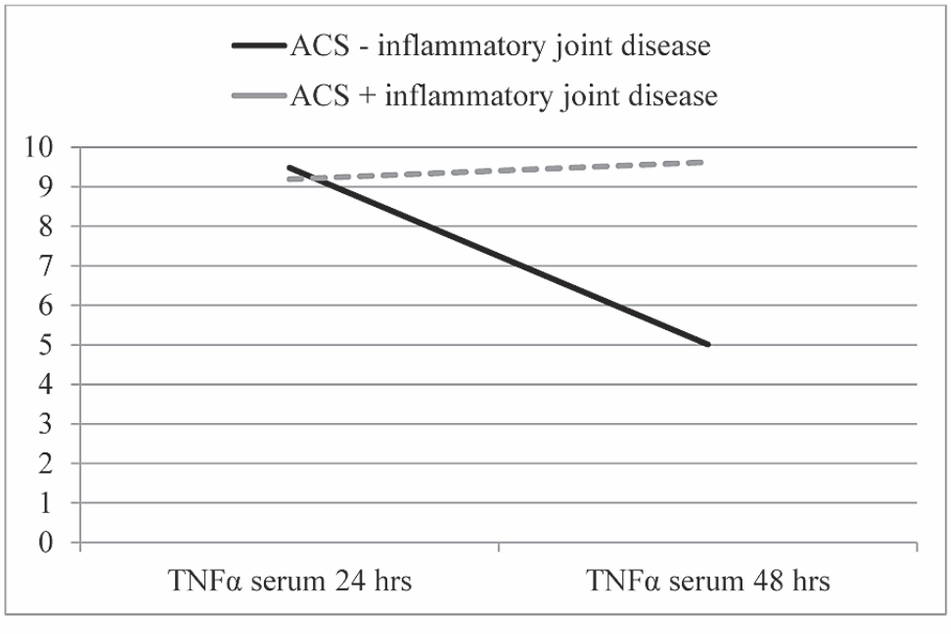


Figure 1: ***Line plot of group means for TFN a serum levels between 24 and 48 hrs***

Table 2: ***Spearman correlation results for TNFα and TNFα serum (24 and 48 hrs) within both groups***

Spearman Rho			TNF serum 24hrs	TFN serum 48hrs
ACS group - inflammatory joint disease	TNFα	Correlation Coefficient	-.139	.076
		Sig. (2-tailed)	.358	.614
		N	49	49
ACS group + inflammatory joint disease	TNFα	Correlation Coefficient	-.086	.092
		Sig. (2-tailed)	.569	.554
		N	46	46

Note: None of the correlations are significant, $p < .05$.

slightly higher in the PsA group (10.35 vs. 9.19), but the difference was not statistically significant, $p = .974$. A similar trend was observed regarding TNFα serum (48hrs), where the PsA group had a slightly higher mean of 10.34 vs. 9.62 of the RA group, with no statistical significance, $p = .953$.

Within the RA and PsA groups of patients, a potential relationship between TNFα and TNFα serum (24 and 48 hrs) and disease activity level was explored through Spearman Rho correlation. Disease activity within the RA subgroup was established on the basis of DAS28, following the standard values and ranges for each level. There were no patients in remission (DAS28 ≤ 2.6) and no patients with low disease activity (2.6 < DAS28 ≤ 3.2). Nine patients (42.9%) had moderate disease activity (3.2 < DAS28 ≤ 5.1) and 12 (51.1%) had high disease activity (DAS28 > 5.1).

For the PsA patients, disease activity was determined on the basis of ASDAS, again in accordance with the established reference values. There were no patients in remission (ASDAS < 1.3) and no patients with low disease activity (ASDAS 1.3 – 2.1). Five patients showed moderate disease activity (ASDAS 2.1 -3.4) and 16 patients had high disease activity (ASDAS > 3.5). The descriptive statistics for TNF-α TNF-α serum levels at 24 and 48 of the stratified samples are shown in **Table 3.**

Correlation analysis was also performed within the RA and PsA groups between disease activity level (DAS28 and ASDAS), TNFα expression and TNFα serum (24 and 48 hrs). All correlation coefficients were low with level of significance exceeding .05 **(Table 4).**

CONCLUSIONS

The study provides information about differences and similarities in TNF-α expression and TNFα serum levels (24hrs and 48hrs) in patients with ACS in relation to presence and absence of inflammatory joint disease, type of inflammatory joint disease (RA vs. PsA) and levels of disease activity. The results show significantly elevated levels of TNF-α expression in patients with ACS and inflammatory joint disease as compared to patients with ACS, but without inflammatory joint disease. Extrapolating from this finding, it can be concluded that the presence of inflammatory joint disease in patients with ACS adds up to the production of TNF-α and further elevates its expression. However, when patients of two different types of inflammatory joint disease were compared, RA vs. PsA, the expression of TNF-α did not reveal significant differences, which leads to the conclusion that the type of joint disease does not have association with the levels of TNF-α expression.

Table 3: ***Stratification of RA and PsA groups according to disease activity***

Variables	Rheumatoid Arthritis Moderate DAS28 3.2 - 5.1	Rheumatoid Arthritis High DAS28 > 5.1	Psoriatic Arthritis Moderate ASDAS 2.1 - 3.4	Psoriatic Arthritis High ASDAS > 3.5
Number, %	9 (42.9%)	12 (57.1%)	5 (23.80%)	16 (76.20%)
TNF α (0-3): Median, (Range)	2 (1 – 2)	1.50 (0-2)	2 (1- 2)	3 (0-3)
TFN α serum (24 hrs): ng/ml mean, (SD)	8.80 (± 4.81)	8.84 (± 5.20)	8.99 (±5.98)	11.59 (±7.62)
TFN α serum (48 hrs): ng/ml mean, (SD)	7.96 (±4.53)	8.68 (±2.84)	9.21 (±4.82)	11.61 (±5.70)

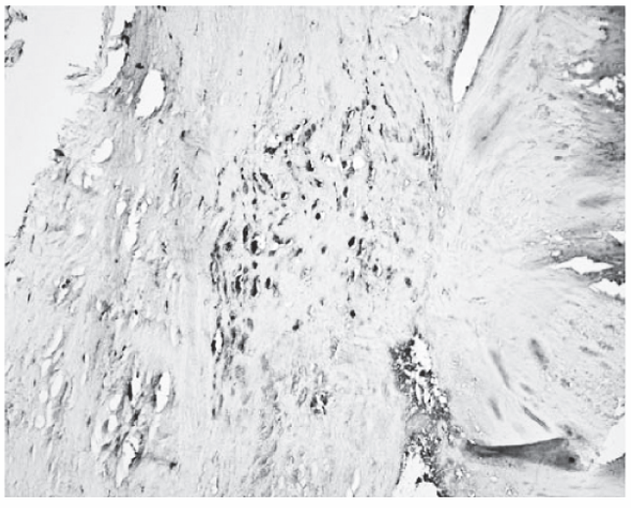
Note: DAS28 used for determining disease activity in RA patients; ASDAS in PsA patients

Table 4: ***Correlation analysis between disease level TNFα expression and TNFα serum***

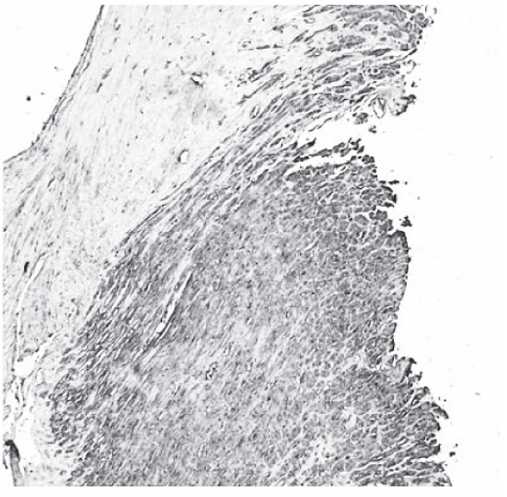
Spearman Rho			TNFα	TNFα Serum 24 hrs	TFNα Serum 48 hrs
RA patients	DAS28 level	Correlation Coefficient	-.311	.086	.218
		Sig. (2-tailed)	.170	.712	.343
		N	21	21	21
PsA patients	ASDAS level	Correlation Coefficient	.020	.098	.176
		Sig. (2-tailed)	.932	.673	.445
		N	21	21	21

Note: None of the correlations are significant, $p < .05$.

Regarding serum levels at 24hrs after the onset of the coronary syndrome, the study found no significant differences between patients with ACS with and without inflammatory joint disease. However, a significant difference was observed in relation to the TNFα serum levels at 48hrs as the group without joint disease showed significantly lower levels. The post hoc analysis revealed a significant drop in TNFα serum level at 48 hrs as compared to the level at 24hrs within the group of patients with ACS, but no inflammatory joint disease. This suggests that the presence of a systematic inflammatory joint disease leads to induction of cytokine synthesis after 48 hours. This conclusion is valid for both the RA and PsA patients based on the lack of significant differences between them regarding TNFα serum level at 24 and 48 hrs. Inflammatory joint disease leads to induction of cytokine synthe-



TNF X PLAQUE



TNF X CIKATRIX INFARKT

sis regardless of the type of joint disease.

The study also sought to explore possible associations between TNF-α expression and TNF-α serum levels at 24 and 48 hours within the groups of ACS patients with and without inflammatory joint disease. No significant relationship was found suggesting that TNF-α expression and TNF-α serum levels serve as independent measures.

In relation to disease activity, the patients with RA and PsA fell into two categories of moderate and high disease activity as measured by DAS28 and ASDAS, respectively. No significant relationship was found between disease activity level, TNF-α expression and TNF-α serum levels. This maybe due to the limited variation in disease activity parameters as the patients in both groups were between moderate and high levels.

REFERENCES

1. Kleinbongard P, Heusch G, Schulz R TNFα in atherosclerosis, myocardial ischemia/reperfusion and heart failure j.pharmthera.2010.05.002
2. Avina-Zubieta JA, Choi HK, Sadatsafavi M, Etminan M, Esdaile JM, Lacaille D. Risk of cardiovascular mortality in patients with rheumatoid arthritis: a meta-analysis of observational studies. Arthritis Rheum. 2008;59(12):1690-1697
3. Ridker PM, Rifai N, Pfeffer M, Sacks F, Lepage S, Braunwald E.Elevation of tumor necrosis factor-alpha and increased risk of recurrent coronary events after myocardial infarction. Circulation 2000; 101: 2149–53
4. Diwan A, Tran T, Misra A, Mann DL. Inflammatory mediators and the failing heart: a translational approach. Curr Mol Med. 2003;3(2):161-182
5. Doherty T.M., Uzui H., Fitzpatrick L.A., et al., "Rationale for the role of osteoclast-like cells in arterial calcification," The FASEB Journal, vol. 16, no. 6, pp. 577–582, 2002
6. EULAR recommendations for cardiovascular disease risk management in patients with rheumatoid arthritis and other forms of inflammatory IJD 2016
7. Faure, E.,Thomas L., Xu H., Medvedev A. E. , Equil O. and Arditi M., "Bacterial lipopolysaccharide and IFN-γ induce toll-like receptor 2 and toll-like receptor 4 expression in human endothelial cells: role of NF-κB activation," Journal of Immunology, vol. 166, no. 3, pp. 2018–2024, 2001
8. Kapadia S, Lee J, Torre-Amione G, Birdsall HH, Ma TS, Mann DL. Tumor necrosis factor-alpha gene and protein expression in adult feline myocardium after endotoxin administration. J Clin Invest. 1995;96(2):1042-1052
9. Bassuk Sh., N. Rifai, P. Ridker. High sensitive C-reactive proteinb: clinical importance. Curr. Probl. Cardiol., 2004; 29: 439-493.
10. Andus T, Geiger T, Hirano T, Northoff H, Ganter U, et al. 1987. Recombinant human B cell stimulatory factor 2 (BSF-2/IFN-β2) regulates β-fibrinogen and albumin mRNA levels in Fao-9

cells. FEBS Lett. 221:18–22
11. Avina-Zubieta JA, Choi HK, Sadatsafavi M, Etminan M, Esdaile JM, Lacaille D. Risk of cardiovascular mortality in patients with rheumatoid arthritis: a meta-analysis of observational studies. Arthritis Rheum. 2008;59(12):1690-1697
12. Kleinbongard P, Heusch G, Schulz R TNFα in atherosclerosis, myocardial ischemia/reperfusion and heart failure j.pharmthera.2010.05.002
13. Lampugnani MG, Resnati M, Raiteri M, Pigott R, Pisacane A, Houen G, Ruco LP, Dejana E. A novel endothelial-specific membrane protein is a marker of cell-cell contacts. J Cell Biol. 1992;118(6):1511–22
14. Libby P. Molecular bases of the acute coronary syndromes. Circulation. 1995;91:2844 –2850
15. McKellar GE, McCarey DW, Sattar N, McInnes IB. Role for TNF in atherosclerosis? Lessons from autoimmune disease. Nat Rev Cardiol. 2009;6:410–417
16. Morrison L. and Bogan I.K., "Calcification of the vessels in diabetes," Journal of the AmericanMedical Association, vol. 92, pp. 1424–1426, 1929.
17. Ridker PM, Rifai N, Pfeffer M, Sacks F, Lepage S, Braunwald E.Elevation of tumor necrosis factor-alpha and increased risk of recurrent coronary events after myocardial infarction. Circulation 2000; 101: 2149–53

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Проучвания върху шума в околната среда и здравето в България (1970 – 2017)

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Environmental noise and health research in Bulgaria (1970 – 2017)

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РЕЗЮМЕ:

Шумът в околната среда е повсеместно разпространен замърсител и е свързан с редица здравни резултати, от лошо психично здраве до сърдечно-съдови заболявания. Това е свързано със значителни социално-икономически загуби. Шумът е проникнал във всички сфери на живота, от транспорта до дейностите през свободното време, а неблагоприятното социално-икономическо положение в България допълнително усложнява проблема. В настоящата работа авторът цели да направи кратък обзор на българската литература по темата, с намерението да установи съществуващите пропуски и да подчертае необходимостта от развитието на тази научна област. Обзорът е базиран на електронни и неавтоматизирани търсения, без времеви ограничения, на актуални проучвания, публикувани на български или английски. Включени бяха собствените публикации на автора заедно с такива на специалисти по комунална хигиена, които бяха помолени да прегледат архивите си. Резултатите показаха, че докато в по-заможните държави са натрупани значителни данни, тази област на търсене е все още в зародиш в България. Въпреки това, вдъхва увереност фактът, че проучванията върху шума в страната набират скорост и са все по-признати от международната научна общност.

Ключови думи: Раздразнение от шума; ССЗ; Психично здраве; Шумова експозиция; Транспортен шум

ABSTRACT

Environmental noise is a ubiquitous pollutant, and it is associated with a plethora of health outcomes, ranging from mental ill health to cardiovascular disease. This translates into considerable socioeconomic loses. Noise has penetrated all aspect of modern life, from transportation to leisure time activities, and the unfavorable socioeconomic situation in Bulgaria further compounds the problem. In the current paper, the author aims to briefly review the Bulgarian literature on the subject matter, with the intention to identify existing gaps and emphasize the necessity to advance the field. The review was based on electronic and manual searches, with no time restrictions, of relevant studies published in Bulgarian and English. Author's own publications were included along with those of specialists in environmental hygiene who were asked to search their archives. Results showed that while substantial data has been accumulated in more affluent countries, this field of inquiry is still in its infancy in Bulgaria. Still, it is reassuring that noise research in the country is gaining momentum and is increasingly acknowledged by the international scientific community.

Keywords: Noise annoyance; CVD; Mental health; Noise exposure; Traffic noise

INTRODUCTION

Environmental noise is a ubiquitous pollutant, with road traffic being the major noise source in urban settlements (15). In 2012, around 37.8% of Europeans living in major agglomerations were exposed to road traffic noise > 55 dB(A), while this proportion was more than double, 84.44%, in Bulgaria (19). Houthuijs et al. recently estimated that 46% of Europeans were expose to ≥ 55 dB(A) day-evening-night noise level, and 32%, to ≥ 50 dB(A) at night (18). Median daily and nighttime noise levels in the EU were 54.5 dB(A) and 46.5 dB(A), respectively (18).

In the report „Burden of disease from environmental noise", WHO experts calculated that at least one million healthy life years are lost in Europe every year due to road traffic noise; of those, 61 000 disability-adjusted life-years (DALYs) are due to ischemic heart disease, 45 000 DALYs – to cognitive impairment in children, 903 000 DALYs – to sleep disturbance, 22 000 DALYS – to tinnitus, and 587 000 – to noise annoyance (23). According to Swinburn et al., nationwide reduction of noise levels with 5 dB(A) would result in 1.4% and 1.8% lower prevalence of hypertension and ischemic heart disease, respectively, which would save around $ 3.9 billion in the US (20). In Europe, road traffic noise is culpable for 1.1 million cases of hypertension, 52 600 hospitalizations, and 12 200 cases of premature mortality from ischemic heart disease and stroke (18).

Evidently, environmental noise has a substantial impact on public health. Its control, however, remains challenging on a global scale, let alone in Bulgaria. Noise has penetrated all aspect of modern life, from transportation to leisure time activities, and the unfavorable socioeconomic situation in Bulgaria further compounds the problem. In the current paper, the author aims to briefly review the Bulgarian literature on the subject matter, with the intention to identify existing knowledge gaps and emphasize the necessity to advance the field. The review was based on electronic and manual searches, with no time restrictions, of relevant studies published in Bulgarian and English. Author's own publications were included along with those of specialists in environmental hygiene who were asked to search their archives.

MENTAL WELL-BEING AND PSYCHOSOCIAL REACTIONS TO NOISE

Evidence of the psychosocial effects of environmental noise in Bulgaria dates back to the 1970-ties. For instance, Radneva-Dimitrova conducted a socio-acoustic survey of the resident of 1 030 apartments in the capital Sofia and indicated that 94.6% of those living close to a highway construed traffic noise as a major nuisance (29). In a follow-up study, she investigated general noise annoyance comparing people exposed to 68 – 78 dB(A) and 51 – 60 dB(A) (30). Another study focused on noise penetrating residential buildings and its association with restoration and sleep (26).

Several studies were conducted in the city of Pazardzhik. In 2000, Gatseva et al. looked at the association between traffic noise exposure and self-reported health status of 100 adults and found some indication for higher noise annoyance, sleep disturbance and neurological complaints among participants living in noisier neighborhoods (16). In addition to adults, Gatseva and Turnovska sampled 285 children from Pazardzhik and found higher irritability, more complaints and difficulties concentrating in children living on streets with higher traffic intensity (17).

In 2011, Turnovska et al. sampled 400 residents of the town of Smolyan exposed to 67.4 – 70.6 dB(A); 60 – 70% reported high, and 25%, very high noise annoyance (21). The National Centre for Public Health and Analyses reported results of a pilot study conducted in 2013 in the capital Sofia and also focused on citizens living in a noisy area (≈ 73 dB(A)) (25). Noise was measured objectively and it was linked to participants' perception of the acoustic environment in the neighborhood. High traffic intensity and noise exposure were negatively associated with participants' residential satisfaction; 32% reported high noise annoyance, and more than half reported sleep disturbance due to transportation noise.

Two multi-item questionnaires for the assessment of noise sensitivity were validated in Bulgarian to herald the use of this psychometric construct in future noise research (8, 11). It was confirmed that noise sensitivity was an important correlate of annoyance,

predicting it above and beyond noise exposure (8, 11). Another study indicated that perceived traffic intensity on the residential street was a better predictor of noise annoyance than the actual number of motor vehicles and measured noise level (14).

Dzhambov and Dimitrova examined the exposure-response relationship between road traffic noise exposure and noise annoyance in Plovdiv (2). They extracted noise data from the strategic noise map of the city for 513 participants who also filled a socio-acoustic questionnaire. The empirically derived relationship in this sample resembled the polynomial recommended by the WHO for calculation of the proportion of highly noise annoyed people in different noise bands. Burden of disease calculations following the WHO methodology showed an annual loss of 1 188 DALYs in Plovdiv, which translated into around € 14 million; on a national scale, these estimates were 6 400 DALYs and € 77 million (2). The socioeconomic burden of traffic noise-attributed sleep disturbance was even greater – 15 468 DALYs and € 185 million (4).

In the recent years, the interest in mental health as a potential outcome of environmental noise exposure was renewed. In 2013, Dzhambov and Dimitrova sampled 182 residents in one of the neighborhoods of Plovdiv where noise levels were around 72 dB(A) (1). The authors assessed participants' perceived acoustic environment at home and looked at the association between displaced aggression and noise. Hearing noises above the perceived normal threshold more often, exposure to continuous noise (vs intermittent), and higher noise sensitivity were independently associated with higher aggression (1).

More recently, the same authors investigated different pathways linking road traffic noise to general mental health in a sample of 399 youth (3). That study extended the inquiry to the social sphere and integrated environmental and social aspects of the noise – health relationship. Results showed that higher noise exposure was associated with worse mental health only indirectly through noise annoyance, which was associated with lower neighborhood restorative quality, in turn with less social cohesion and physical activity, and consequently with worse mental health (3).

CARDIOVASCULAR AND METABOLIC OUTCOMES

There is limited evidence linking traffic noise to cardiovascular and metabolic outcomes in Bulgaria. To name a few examples, Tzenova et al. surveyed 1 062 residents of 52 buildings in Sofia and measured their noise exposure and arterial blood pressure (31). The study indicated higher prevalence of hypertension in those exposed to > 60 dB(A) compared to < 60 dB(A) (36.97% vs 28.41%, $p < 0.01$). A similar trend was observed for ischemic heart disease (17.38% vs 10.56%, $p < 0.01$) (31). Differences in systolic and diastolic blood pressure, measured on a continuous scale in mmHg, between residents in quiet and noisy areas were also statistically significant and more pronounced in hypertensive individuals (27). These findings were confirmed in a sample of 84 normotensive medical students (28). In an ecological study of 115 819 participants living in the city of Burgas, Turnovska et al. found that those living near the airport and exposed to > 50 dB(A) had higher prevalence of circulatory diseases and myocardial infarction (22). Of note, the aforementioned studies were carried out some 10-15 years ago and suffered methodological shortcomings, such as exposure misclassification and lack of adequate control for confounding factors.

More recently, data were collected for 513 residents of Plovdiv to study the long-term relationship between road traffic noise and the prevalence of cardiovascular disease, diabetes, and obesity in healthy adults (24). In contrast to previous research, this study accounted for a wide range of confounding factors, e.g. sociodemographic variables, co-exposure to air pollutants, other contextual factors. Results for CVD were inconclusive, as there were indications that higher noise exposure was associated with higher odds for hypertension and ischemic heart disease, but the effect estimates were non-significant (5, 10). According to the authors, these null findings could be due to the smallish sample size. Still, burden of disease calculations for the year 2012 showed that 2.9% of all myocardial infarction cases in Bulgarian urban areas could be attributed to road traffic noise. Those addi-

tional cases were valued at around € 11.6 million per annum (6). As regards metabolic outcomes, participants exposed to > 70 dB(A) at home had higher odds for diabetes (7) and obesity (9).

In order to expand the scope of previous research, which was mostly concerned with the effects of noise in the general population, Dzhambov et al. investigated the impact of road traffic noise on several cardiovascular and metabolic indicators in patients with pre-existing cardiovascular disease (24). In a series of studies of patients from the Plovdiv Province, they observed higher blood pressure, lower glomerular filtration rate, and less favorable lipid profile in some patients exposed to higher residential noise (12, 13). The authors identified potentially vulnerable subgroups in which the effect was both substantive and statistically significant. These findings added to the limited body of evidence on the subject and suggested the clinical relevance of noise pollution.

CONCLUSION

Environmental noise is a conspicuous threat for human health and well-being. To persuade stakeholders to take action and mitigate noise exposure, the current body of evidence needs to be expanded further. While substantial data has been accumulated in more affluent countries, this field of inquiry is still in its infancy in Bulgaria. Still, it is reassuring that the current review found a positive trend in the recent years. Noise research in Bulgaria is gaining momentum and is increasingly acknowledged by the international scientific community.

REFERENCES

1. Dzhambov A, Dimitrova D. Neighborhood noise pollution as a determinant of displaced aggression: a pilot study. Noise Health. 2014;16(69):95-101
2. Dzhambov A, Dimitrova D. Road traffic noise and annoyance: exposure-response relationship and burden of disease calculations in Bulgaria. Scr Sci Med. 2015;47(2):22-30
3. Dzhambov A, Tilov B, Markevych I, Dimitrova D. Residential road traffic noise and general mental health in youth: The role of noise annoyance, neighborhood restorative quality, physical activity, and social cohesion as potential mediators. Env Int. 2017;109:1-9.
4. Dzhambov AM, Dimitrova DD, Mihaylova-Alakidi VK. Burden of Sleep Disturbance Due to Traffic Noise in Bulgaria. Fol Med (Plovdiv). 2015;57(3-4):264-9.
5. Dzhambov AM, Dimitrova DD. Association between Noise Pollution and Prevalent Ischemic Heart Disease. Fol Med (Plovdiv). 2016;58(4):273-281.
6. Dzhambov AM, Dimitrova DD. Evaluation of the social and economic burden of road traffic noise-attributed myocardial infarction in Bulgarian urban population. Arh Hig Rada Toksikol. 2015;66(1):15-21
7. Dzhambov AM, Dimitrova DD. Exposures to road traffic, noise, and air pollution as risk factors for type 2 diabetes: A feasibility study in Bulgaria. Noise Health. 2016;18(82):133-42.
8. Dzhambov AM, Dimitrova DD. Psychometric properties of the Bulgarian translation of Noise Sensitivity Scale Short Form (NSS-SF): Implementation in the field of noise control. Noise Health. 2014;16:361-7.
9. Dzhambov AM, Dimitrova DD. Road traffic noise exposure association with self-reported body mass index. Noise Contr Eng J. 2015;63(6):572-81.
10. Dzhambov AM, Dimitrova DD. Road traffic noise-related hypertension and ethnicity as an effect modifier. In: Łucjan C, Gérard D (Editors), Traffic Noise: Exposure, Health Effects and Mitigation. New York: Nova Science Publishers, 2017, pp. 41-58. ISBN: 978-1-53612-550-4.
11. Dzhambov AM, Dimitrova DD. Validating a short Bulgarian version of a psychometric instrument for multidimensional noise sensitivity assessment. Fol Med (Plovdiv). 2014;56(2):116-25
12. Dzhambov AM, Tokmakova M, Gatseva P, Zdravkov N, Gencheva D, Ivanova N, Karastanev K, Vladeva S, Donchev A, Dermendzhiev S. Community noise exposure and its effect on blood pressure and renal function in patients with hypertension and cardiovascular disease. Fol Med, in press.
13. Dzhambov AM, Tokmakova MP, Gatseva PD, Vladeva SV, Zdravkov NG, Vasileva EV, Gencheva DG, Ivanova NG, Karastanev KI, Donchev AT. Is community noise associated with metabolic control in patients with cardiovascular disease? Acoust Aust. 2017;45(1):61-75.
14. Dzhambov AM. Validity of self-reported traffic intensity as a proxy for road traffic counts and noise. Noise Contr Eng J. 2015;63(1):11-9.

15. European Community. Commission green paper on future noise policy. Brussels, 1996. Retrieved from http://aei.pitt.edu/1204/1/noise_gp_COM_96_540.pdf
16. Gatseva P, Aleksandrova A, Chardaklieva N. Assessment of the impact of urban noise on the residents in some areas in the town of Pazardzhik. In: Ecology and Health, October 26th, 2000. Plovdiv.
17. Gatseva P, Turnovska T. Influence of transport traffic on children's health in Bulgaria. Balkan Ecol. 2002;5(3):283-286
18. Houthuijs D, Swart W, van Beek A, van Kempen E, de Leeuw F. Health Impact Assessment for Noise in Europe: Expected consequences of the limitations of the available noise exposure data. ETC/ACM Technical Paper 2014/9 February. 2015. Available at: http://acm.eionet.europa.eu/reports/docs/ETCACM_TP_2014_9_HIA-noise_europe.pdf
19. NOISE (Noise Observation and Information Service for Europe). Copenhagen, European Environment Agency. 2017. Available at http://noise.eionet.europa.eu/
20. Swinburn TK, Hammer MS, Neitzel RL. Valuing Quiet: An Economic Assessment of U.S. Environmental Noise as a Cardiovascular Health Hazard. Am J Prev Med. 2015;49(3):345-53.
21. Turnovska T, Kubateva M, Stefanova S, Tosseva E. Impact of automobile transport pollution on the quality of life in the town of Smolyan. Arch Balkan Med Union 2013;48(1):85-90.
22. Turnovska T, Staykova J, Petkov T. Health assessment of populations living close to the airport of Bourgas, Bulgaria. Arh Hig Rada Toksikol. 2004;55(1):5-10.
23. WHO Regional Office for Europe. Burden of Disease from Environmental Noise. Quantification of Healthy Life Years Lost in Europe. Copenhagen: WHO Regional Office for Europe; 2011.
24. Джамбов АМ. Шумовото замърсяване от автомобилен трафик – рисков фактор за някои сърдечно-съдови и метаболитни заболявания сред пълнолетното население [дисертация]. Пловдив: Медицински университет – Пловдив; 2017.
25. Иванова П, Израел М, Петкова Хр, Димитрова М, Димитрова Д. Оценка на въздействието на шума върху населението в район с установена наднормена шумова експозиция. Юбилейна конференция с международно участие: Обществоздравна политика и практика. Плевен, 2015.
26. Радев Ат, Раднева-Димитрова Р, Радева-Александрова Н. Проникващи шумове в жилищните сгради и въздействието им върху почивката и съня на населението. В: СНМД - Дружество по Хигиена. Резюмета на докладите, изнесени на III Национален конгрес по хигиена. София, 13 - 15 X, 1977, стр. 34.
27. Раднева Р, Торбова С, Ценова Б, Цветков Д, Боев М. Акустични характеристики на жилищната среда и епидемиология на артериалната хипертония. Хигиена и здравеопазване. 2001(44);1:14-19.
28. Раднева Р, Ценова Б, Торбова С, Цветков Д, Боев М, Попова К. 24-часово мониториране на артериалното налягане и пулсовата честота при лица, живеещи в шумни градски зони. Хигиена и здравеопазване. 2001;44(1):19-22.
29. Раднева-Димитрова Р. Проучване на субективната оценка на въздействието на шума върху отдиха и възстановяването на човешкия организъм. В: СНМД - Дружество по Хигиена. Резюмета на докладите, изнесени на III Национален конгрес по хигиена. София, 13 - 15 X, 1977, стр. 34.
30. Раднева-Димитрова Р. Шумът в жилището и степента на общата обезпокоеност на обитателите. В: СНМД - Дружество по Хигиена. Резюмета на докладите, изнесени на III Национален конгрес по хигиена. София, 13 - 15 X, 1977, стр. 34.
31. Ценова Б, Раднева Р, Цветков Д, Вуков М. Характеристики на шумовото въздействие в съвременната жизнена среда и техните здравни аспекти. Хигиена и здравеопазване. 2000;5/6:29-33.

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Анализ на познанието за епидемиологията и клиниката на бисфосфонатно-асоциираната остеонекроза на челюстите

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Analysis of the knowledge of the epidemiology and clinic of bisphosphonate-related osteonecrosis of the jaws

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РЕЗЮМЕ:

Бисфосфонатно асоциираната остеонекроза на челюстите (БАОНЧ) е състояние, описано сред работещите във фабрики за кибритени клечки през 19 век под името „фосфорна челюст", но докладвано официално за първи път едва през 2003 година. БАОНЧ е страничен ефект от приложението на бисфосфонати (БФ) – група медикаменти, повлияващи костния метаболизъм. Развитието на състоянието е причина за сериозни увреждания и дискомфорт на част от увеличаващия се брой пациенти, лекувани с БФ, особено при интравенозна апликация. Съществуват множество дилеми и спорове на различни групи специалисти относно определението, епидемиологията и рисковите фактори,

ABSTRACT

Bisphosphonate related osteonecrosis of the jaws (BRONJ) is a condition, first described in the 19th Century among workers in match factories as "Phossy Jaw", but officially reported in 2003. BRONJ is a side effect from the use of bisphosphonates (BP), a group of drugs that affect bone metabolism. The development of the condition is the cause of serious impairment and discomfort of part of the increasing number of patients treated with BP, especially by intravenous application. There are several dilemmas and controversies among different groups of experts on the definition, epidemiology and risk factors, mechanisms of development, classification, clinical manifestations and approaches to treatment and prevention. That's why, in the literature,

механизма на възникване, класификацията, клиничните изяви и подходите за лечение и превенция. Това говори, че се касае за изключително сложен и многофакторен процес, изискващ внимателно мониториране и индивидуален подход към всеки пациент. Тъй като състоянието се счита за необратимо, усилията трябва бъдат насочени основно към неговата превенцията, както преди, така и след началото на БФ терапия. От ключово значение е увеличаването на информираността по проблема на дентални лекари, онколози и пациенти и готовността им да работят заедно като екип с цел свеждане до минимум риска от развитие на това усложнение.

Ключови думи: Бисфосфонати, Бисфосфонатно асоциираната остеонекроза на челюстите, епидемиология, рискови фактори, превенция

BRONJ is described as a complicated process requiring careful monitoring and individual approach to each patient. As the condition is considered to be irreversible, efforts should be directed primarily to its prevention, both before and after the onset of BP therapy. It is crucial to increase the awareness of dental practitioners, oncologists and patients and their willingness to work together as a team to minimize the risk of developing this complication.

Key words: Bisphosphonates, Bisphosphonate Related Osteonecrosis of the Jaws, Epidemiology, Risk Factors, Prevention

INTRODUCTION

Bisphosphonates (BP) are a group of drugs, synthesized in the late 1960s, that affect the cycle of bone metabolism by suppressing the processes of absorption. They are used to treat diseases that feature high bone resorption - multiple myeloma, osteolytic bone metastases, osteoporosis, Paget's disease , fibrous dysplasia , McCune-Albright syndrome, hypocalcaemia of malignancy and are most commonly administered by intravenous infusion(IV).

Bisphosphonates are classified in two groups based on their chemical structure (24):

- Aminobisphosphonates contain nitrogen in the side atomic chain of their molecule, with a much stronger effect
- Non-aminobisphosphonates - metabolized by osteoclasts to inactivate non-hydroxylysine adenosine triphosphate-analogues that have a direct cytotoxic effect which lead to apoptosis (40).

Although they have a good safety profile, these drugs can also cause some serious complications after oral and especially long-term intravenous use. Treatment with bisphosphonates may result in short or long-term side effects such as renal failure, upper intestinal tract disorders, esophageal cancer, acute early phase reaction, uveitis and other ocular inflammatiory diseases , arthralgia, fever, muscle pain , hypocalcaemia, bone turnover suppression, risk of atypical femoral fractures. In recent years, reports of the occurrence of cases of bisphosphonate-related osteonecrosis of the jaw (BRONJ) have increased significantly (31).

HISTORY

Pure phosphorus was first obtained from animal bone tissue in the early 19th century. The compound immediately found use in the matchmaking industry in the form of white phosphorus (yellow phosphorus).

The infamous epidemic of a disease called "phossy jaw" began around 1858 and continued until 1906, after which only occurred sporadic cases. The epidemic was linked to the "yellow phosphorus", key ingredient in the matches. Depending on the duration of exposure, many workers

exposed to heated fumes, containing phosphorus, developed a condition characterized by a painful bone exposure in the oral cavity, whereas the people, working in the office environment didn't have that condition. After finding effective and relatively safer substitutes, the European countries signed an agreement (the Berne Convention, 1906), obliging the participants not to produce or import matches containing white (yellow) phosphorus (23).

Yellow phosphorus has a simple chemical structure (P4O10) and in a medium rich in oxygen and carbon dioxide environment, such as the oral cavity, and in the presence of amino acids (lysine), the compound can form bisphosphonates almost identical to alendronate (Fosamax, Novartis Pharmaceuticals , East Hanover, NJ) and pamidronate (Aredia, Novartis Pharmaceuticals). A century later, the cause of the "phossy jaw" disease was established, and it was verified that the 19th-century epidemic actually represented cases of bisphosphonate-related osteonecrosis of the jaws, long before the pharmaceutical industry began to market and sell these drug. The contemporary cases of BRONJ represent a second "phossys jaw" epidemic (23).

More recent data on BRONJ dates back to 2002, when 9 months after the intravenous bisphosphonate zoledronic acid was approved by marketing regulators, the FDA received reports of nine cancer patients who were treated with zoledronic acid and unexpectedly developed a condition characterized by exposed jawbone (15). The first official report was published in 2003 by Marx, who reported 36 cases of "painful exposure of the lower and upper jaw bone in patients undergoing bisphosphonate treatment with pamidronate and zoledronate (25). That same year Migliorati reported five cases (27), Carter and Gross - four (12), Wang - three (39). In 2004, Ruggierro and co. published 63 cases of osteonecrosis of the jawbone in patients treated with bisphosphonates (35). In 2004, Novartis, the manufacturer of Aredia and Zometa, reported to healthcare professionals about additional warnings to these products, regarding the risk of development of osteonecrosis of the jaws (19). In 2005, the manufacturer officially declared 475 cases of BRONJ and the precautions were changed for all members of the bisphosphonate group, including oral bisphosphonates.

Information about BRONJ continues to be published in the scientific literature, but unfortunately, the number of cases both in Bulgaria and globally remains unknown.

The first practical guidelines on how to ensure safe dental treatment for patients on bisphosphonate therapy are published in the Journal of Clinical Oncology Practice in January 2006 (34).

NOMENCLATURE AND DEFINITION

In Bulgaria, the most common acronym, used for describing the complication is BAONJ (BP associated osteonecrosis of the jaws). Various abbreviations are available in the English-language literature - BRONJ, BRON, BON, BAONJ, ONJ. The recognition of jaw necrosis as a complication associated with the use of other drugs, excluding BP, provoked the American Association of Oral and Maxillofacial Surgery (AAOMS) to recommend the term "medication-associated osteonecrosis of the jaw" (MRONJ) (6).

Two working definitions for BRONJ are proposed. The first one is presented by a special task force convened by the American Society for Bone and Mineral Research defines BRONJ as the presence of exposed bone in the maxillofacial region that does not heal within eight weeks after identification by a health care professional. (21) In the worldwide and in the Bulgarian literature , the second defitition , provided my AAMOS, and upgraded in their last position paper from 2014 (6), is adopted: „necrosis of the jaw bone, related or unrelated to dental procedures, persisting for more than 6 to 8

weeks, refractory to conservative treatment, in patients having no history of prior radiotherapy in the affected area, treated intravenously with amino-containing bisphosphonates for at least one year, or orally for a much longer period, for a general disease causing bone resorption." (25). This definition is also adopted by an international task force on osteonecrosis of the jaw (20). Some authors consider this definition to be inaccurate. Fedele et al. believe that using the traditional definition could result in 25% of patients remaining undiagnosed (16).

EPIDEMIOLOGY AND RISK FACTORS

The epidemiology of BRONJ still remains unclear due to the inconsistency and limitations of available studies, including the lack of specific ICD (International Classification of Diseases) code, unrealistic and reduced reporting in drug surveillance systems, the recent introduction of preventive measures, short-term monitoring and lack of cumulative rates of morbidity in the long term. It is surprising that BRONJ was not found in the initial testing of BF and the first cases were officially reported and published in 2003 (11).

Most authors present statistical studies on patients with multiple myeloma, breast cancer, prostate cancer and lung carcinoma, as these are the largest groups of patients treated with bisphosphonates. Sook-Bin Woo et al. quote a study of 1203 cases, in which 7 % of the 904 patients with multiple myeloma and 4 % of the 299 patients with lung carcinoma developed osteonecrosis of the jaws, related to the usage of BF (40). Kohl et al. report a wide incidence of BRONJ from 0 to 27.5% , with/at an average incidence of 7% (22). In one of the latest reviews on the topic, Rugani et al. conducted a search in PubMed and found a total incidence of 2.09% in breast cancer patients, 3.8% in prostate cancer patients and 5.16% in multiple myeloma patients (33).

Very little information can be found about the incidence of BRONJ in patients with osteoporosis. Solomon et al. found a morbidity rate of 0.028% to 4.3% in the literature, and in their own cohort studies, the incidence was 0.02% (95% CI 0.004% - 0.11%) (37).

Risk factors related to bisphosphonates

Type of BF: There is no absolute unanimity in the literature on which BF causes more frequent development of BRONJ, but scientific evidence supports the prevailing view that Zoledronic acid has highest risk-potential (6, 9, 11, 26).

Route of administration: The risk is higher with intravenous BP (6, 9, 11, 26) than with oral BP, but this factor may be closely related to their widespread use in patients with malignant neoplasms that receive significantly higher overall doses and their treatment usually has longer duration (11). According to the literature, the lower risk for oral BP intake is due to poor intestinal absorption (0.64%) (11).

Administration schedule: The frequency of administration is also recognized as a risk factor by many oncologists who now prescribe intravenous zoledronate every 3 or 6 months, which is different from the manufacturer's recommendation for 3 weeks to one month. This reduction in the frequency of administration has led to a decrease in the number and severity of the cases (26).

Dose: It is well known that for almost any pharmaceutical product, increased dosing results in a greater effect, and respectively, to a greater and more significant severity of the complications. Available data shows a higher risk of developing osteonecrosis of the jaw by increasing the total dose of BP administered intravenously monthly to cancer and hematology patients (11).

Duration of treatment: The incidence of development of BRONJ is higher with longer treatment duration, especially when the duration of therapy exceeds four years. This period may be reduced by the presence of certain concomitant diseases, chronic glucocorticosteroids, or co-administration of BF with angiogenesis inhibitors (9).

Risk potential: Alendronate and zoledronate have the highest potency and cause the majority of the cases of BRONJ (26).

Risk factors associated with the dento-alveolar system

Anatomical comorbidity

The alveolar process, both in the lower and upper jaw, is the most vulnerable part. Typically, cases of spontaneously occurring necrosis are within alveolar bone (26).

Mandible / Maxilla: Most of the studies based on a large number of cases show a tendency for BACCP to appear more frequently in the lower jaw than in the upper jaw (2: 1) (26). Rarely, both jaws are affected. (4.5%). (6).

Torus: The mandibular torus, palatine torus and bone exostosis are places, that are more easily locally traumatization in the course of day-to-day activities,and are often affected. (11, 26).

Dental diseases and procedures

Dental procedures: According to the literature, the prevalence of bisphosphonate-associated osteonecrosis of the jaws associated with previous dental procedures is predominant compared to the so-called spontaneous BRONJ. Woo Sook-Bin and call. found that between 33% and 86% of the cases were manifested after various dental procedures (21). Dental extraction (6, 9, 11, 26) has the leading role - initiator in up to 62% of the cases (26).

Hyper oclusion / Prosthetics: Excessive chewing forces initiate cases of BRONJ, due to the higher velocity and remodeling rates in the alveolar bones (26).It is assumed that the mechanism of development of necrosis after putting dentures is analogous (6, 11).

Periodontal diseases: Active periodontal inflammation is associated with the development of osteonecrosis of the jaw in patients receiving BP (6, 9, 11, 26). This is due to further increased bone remodeling stimulated by the inflammatory process.

Surgical interventions in the alveolar bone: Surgical manipulations, other than tooth extraction - dental implants, endodontic, periodontal and peri-implant surgery, also increase the rate of bone remodeling and can therefore lead to the appearance of BRONJ. (6, 9, 11,26).

Other risk factors

Many authors discuss the role of additional to the BP intake therapy, such as chemotherapy (9, 26), corticosteroids (6, 9, 11, 26), antiangiotensive agents (6, 9, 11), as well as the influence of age and gender (9, 11), geographic location (9), smoking, alcohol, obesity (6, 9, 11, 26), genetic factors (6, 9, 11), type of tumor (6, 9, 11, 26) and accompanying diseases - hypocalcemia, hyperparathyroidism and disturbances in bone mineralization (11), diabetes, anemia (6, 26).

However, about 25% of the BAONC cases are spontaneous (26).

Abu-Id et al have proposed a predictive scale for risk of development of BRONJ:

1. At high risk: patients with malignancy receiving intravenous BF (Zoledronate or Pamidronate) and / or with a history of chemotherapy, radiotherapy or ongoing exogenous steroid use.

2. At low risk: patients receiving oral BF without a history of chemotherapy, radiotherapy or ongoing exogenous use of steroids (mostly patients with non-steroid-induced osteoporosis) (1).

PATHOPHYSIOLOGY

Although the first cases of BRONJ were reported more than a decade ago, the pathophysiology of this disease has not been fully elucidated. A source of debate among clinicians and researchers is the potential mechanisms underlying BRONJ (36). The leading hypotheses for explaining the unique location in the jaws include the role of remodeling or excessive suppression of bone resorption, inhibition of blood flow, permanent microtrauma, suppression of innate or acquired immunity, deficiency of vitamin D, BP toxicity on soft tissues and infection / inflammation. None of these hypotheses can explain all the cases.

CLASIFFICATION

In 2006, Ruggiero et al. proposed an ONJ classification comprising three stages : stage 1 = bone exposure but without signs or symptoms of infection; stage 2 = bone exposure/necrosis with clinical evidence of infection; stage 3 = the above manifestations and also alterations such as pathological fractures, extraoral fistulas or osteolysis extending to the inferior mandibular margin (34).

In 2007 the AAOMS adopted this classification, though in addition to the group of patients with BRONJ (with its three stages), it included another group of patients comprising individuals at risk. These patients were defined as subjects without evident exposed or necrotic bone or symptoms, but who have been treated with oral or intravenous BPs (4).

In the year 2009, the AAOMS added a stage 0 to its classification, involving alterations (pain, tooth mobility, fistulas, radiographic changes, etc.) that may have been due to treatment with BPs, but without exposed bone. The risk of progression towards more advanced stages of the disease was not known at that time (5).

In 2014, AAOMS updated its classification (6):

At risk category - No apparent necrotic bone in patients who have been treated with either oral or IV bisphosphonates

Stage 0 - No clinical evidence of necrotic bone, but non-specific clinical findings, radiographic changes and symptoms

Stage 1 - Exposed and necrotic bone, or fistulae that probes to bone, in patients who are asymptomatic and have no evidence of infection

Stage 2 - Exposed and necrotic bone, or fistulae that probes to bone, associated with infection as evidenced by pain and erythema in the region of the exposed bone with or without purulent drainage

Stage 3 Exposed and necrotic bone or a fistula that probes to bone in patients with pain, infection, and one or more of the following: exposed and necrotic bone extending beyond the region of alveolar bone, (i.e., inferior border and ramus in the mandible, maxillary sinus and zygoma in the maxilla) resulting in pathologic fracture, extra-oral fistula, oral antral/oral nasal communication, or osteolysis extending to the inferior border of the mandible of sinus floor

n 2016, Bakardjiev, as a result of his clinical observations, proposed the formation of sequesters as an addition to the AAOMS Stage III criteria (8).

CLINICAL MANIFESTATION

Clinically, intraoral lesions at BRONJ look like spaces of yellow-white hard bones, with a soft or thickened board. Their dimensions may vary from a few millimeters to a few centimeters. These areas of exposed and necrotic bone may remain asymptomatic for weeks, months, or even years, and show no tendency for spontaneous recovery and diminishing, which is a hallmark of BRONJ

(3) Clinical manifestation occurs in the development of inflammation in surrounding soft tissues. The main symptom is a protracted, dull, irritating pain in the jaw, which is suppressed by analgesics, but does not disappear completely. Usually, mobility of teeth is observed, which can lead to their loss, periosteum, gingivitis. The surrounding mucous membrane is hyperemised, painful, in some places distorted and covered by necrotic scarring (3). The presence of extra- and intraoral fistulas is typical. As the process progresses, the lesions grow and engage vast spaces in the oral cavity, leading to eating, speech and oral hygiene difficulties. Cases of pathological fractures of the jaw bone have been observed.

In the literature are described some cases of a subclinical form of BRONJ, which occurs without bone exposure. The clinical manifestations of this form of BRONJ includes unexplained jaw pain, fistula formation, edema, mobility of the teeth, and pathological fractures (30).

PARACLINICAL TESTS

Radiological /X-ray findings in patients with BRONJ include osteosclerosis (most commonly) , osteolysis, dense woven bone, thickened lamina, subperiosteal bone deposition, and inability to post-operative remodeling.

In the early stages of disease development, X-ray changes may be absent (40). Some authors recommend computed tomography (10). Chiandussi et al. believe that late changes in the jaws in bisphosphonate-associated osteonecrosis are visualized by X-ray, and CT scanning or nuclear magnetic resonance is better for earlier detection of lesions (13). Other authors recommend scintigraphy of the jaw with Tc-99m methylene diphosphonate (28).

Microbiological testing most commonly finds Actinomyces druses. In a 10-year review of the literature on microbiological data, Hinson et al. report presence of actinomyces in 68.8% of the cases, followed by Streptococci, isolated in 54.7% of the oral lesions reported in the review (17).

Histopathological analysis shows the absence of a finding comparable to the bisphosphonate-treated disease, but the presence of a necrotic bone surrounded by bacteria that do not enter it (32).

TREATMENT

Despite the significant number of publications on the subject, a particularly vague aspect, and certainly one of the most controversial, is the treatment of BRONJ. There is still no prospective evidence-based study with long-term follow-up to guide treatment.

In particular, it is not known whether the affected persons should receive symptomatic therapy with pain and infection control and possibly minimally invasive debris of the surface of the necrotic bone, or resective surgical methods should be used. The subject is complicated because some patients have minimal symptoms and can therefore benefit from minimally invasive therapy. In contrast, there are others, with a painful, advanced disease that does not respond to symptomatic therapy and require more aggressive intervention.

With respect to surgical treatment, there is still controversy among researchers on the type of surgery and the time of performing it. One group of authors (25) recommend superficial curettage and prolonged conservative treatment. Another group of authors (2) recommend hyperbaric oxygenation, superficial debridement, laser treatment, and in advanced cases – aggressive resection of the diseased bone and soft tissue. Hoff, et al (18), reported healing process in 23% of the patients treated with conservative therapy only. Some authors completely remove the diseased bone, covering the site with mucosa, and report good results in 85% to 100% of the patients (38). Similar results were reported by anot-

her author (29), who identified the boundaries of the diseased bone using fluorescent light. This technology has been used in Bulgaria from Bakardjiev for two years. The analysis of a number of publications shows that in the early stages of the disease (AAOMS 1 and 2) most authors administer medication therapy and conservative treatment, and in stage 3 - resection and/or sequestrectomy are performed. The method proposed by Otto S et al. (29), reported by Assaf AT et al. (7), uses fluorescent light for visualization of vital and non-vital bone, is a new and promising method for demarcation when performing resection of the jaw

Eventually, regardless of the stage of the disease, areas of necrotic bone that are the source of chronic soft tissue irritation and sequestration should be removed or reconstituted so that the soft tissue healing is optimized.

In Bulgaria, Pechalova et al. (31), by summarizing the information found in the literature, provided a algorithm for treatment of patients, suspected to have BRONJ:

1. A careful clinical examination to find the location and volume of exposed necrotic bone
2. Imaging diagnostics of the affected jaw - X-rays, CT scans, MRI, bone scintigraphy
3. Providing material for histopathological analysis to exclude the presence of a systematic

process in the jaw (in case of multiple myeloma, a metastasis in case of oncological diseases or a primary neoplasm)

4. Providing material for microbiological examination with emphasis on fungal or other

pathological bacterial infections

290 A Textbook of Advanced Oral and Maxillofacial Surgery

5. Treatment- conservative and surgical

There is no consensus on the need for discontinuation of bisphosphonate treatment after diagnosing osteonecrosis of the jaw. Marx suggests that behavior in terms of bisphosphonate therapy in cancer patients shall be discussed with the oncologist on the purpose of determining the benefit to risk ratio, in view of the long half-life (10 years) of bisphosphonates(24). Dunstan et al. suggest that bisphosphonate therapy be discontinued (14). Expert group guidelines are also divergent (9). The decision should be taken individually on a case-by-case basis, taking into account the expected benefits and harm to the individual patient.

PREVENTION

To date, there is no evidence-based therapeutic strategy with regard to BACCP and the condition is considered irreversible, so the focus of the medical community's focus is on its prevention capabilities (9, 11 ,40).

Prior to initiation of bisphosphonate therapy

The American Association of Clinical Oncology (ASCO), AAOMS, the International Task Force on Osteonecrosis of the Jaw and the European Medicines Agency (EMA) recommend, that all patients planning antiresorptive or angiogenesis inhibitor therapy should undergo a full dental examination prior to beginning the therapy. Preventive dental activities include extraction of the non-prospective teeth, endodontic treatment and retreatment, periapical surgery, the main purpose of which is that the patient does not require dental manipulation as long as possible (20).

Pechalova et al. recommend the following plan for behavior (31):

1. A thorough clinical examination of the dentition and oral cavity, panoramic radiograph and, by the discretion of the doctor, targeted periapical radiographs as a mandatory required minimum.
2. Conducting dental treatment aimed at elimination of infection and the need for invasive procedures in the short- and mid-term future

All invasive dental procedures should be performed at least one month before initia-

ting bisphosphonate therapy in order to allow sufficient time for recovery of the jawbone (31).

After the start of bisphosphonate therapy

After the start of bisphosphonate therapy patients are subject to preventive check-ups at every four months, with radiographic examination, which should seek vigilantly for the presence of osteolysis, osteosclerosis, and expansion of periodontium and involvement of furcations. If additional treatment is required, non-invasive dental procedures are preferred (31).

Oral hygiene should be carefully monitored. Patients should be encouraged to quit smoking (9).

Patients should be asked about any planned dental procedures prior to administration of each dose of an anti-resorptive agent and should be reminded to avoid invasive dental procedures (tooth extraction, implants, and any other procedures involving manipulation of the jawbone or perineum) while being treated with these types of drugs (24)

CONCLUSION

BRONJ is a complication, associated with BP intake, that seriously degrades the quality of life of many patients. As the condition is considered to be irreversible, and treatment options are still a complex and controversial subject among the clinicians, our efforts should be directed primarily to its prevention, both before and after the onset of BP therapy. It is crucial to increase the awareness of dental practitioners, oncologists and patients and their willingness to work together as a team to minimize the risk of developing this complication.

REFERENCES

1. Abu-Id MH, Warnke PH, Gottschalk J et al "Bis-phossy jaws" - high and low risk factors for bisphosphonate-induced osteonecrosis of the jaw. J Craniomaxillofac Surg. 2008 Mar;36(2):95-103.
2. Agrillo A, Petrucci MT, Tedaldi M, Mustazza MC, Marino SM, Gallucci C, et al. New Therapeutic Protocol in the Treatment of Avascular Necrosis of the Jaws. J Craniofac Surg. 2006;17(6):1080-3.
3. Allen MR, Ruggiero SL. A review of pharmaceutical agents and oral bone health: how osteonecrosis of the jaw has affected the field. Int J Oral Maxillofac Implants 2014; 29:e45.
4. American Association of Oral and Maxillofacial Surgeons Position Paper on Bisphosphonate-Related Osteonecrosis of the Jaws. Advisory Task Force on Bisphosphonate-Related Osteonecrosis of the Jaws. J Oral Maxillofac Surg 65:369, 2007. =
5. American Association of Oral and Maxillofacial Surgeons position paper on bisphosphonate-related osteonecrosis of the jaws--2009 update. J Oral Maxillofac Surg 67:2, 2009.
6. American Association of Oral and Maxillofacial Surgeons. Medication-Related Osteonecrosis of the Jaw – 2014 Updatee http://www.aaoms.org/docs/position_papers/mronj_position_paper.pdf?pdf=MRONJ-Position-Paper (Accessed on September 04, 2014).
7. Assaf AT, Zrnc TA, Riecke B, Wikner J, Zustin J, Friedrich RE, et al. Intraoperative efficiency of fluorescence imaging by Visually Enhanced Lesion Scope (VELscope®) in patients with bisphosphonate related osteonecrosis of the jaw (BRONJ). J Craniomaxillofac Surg. 2014;42(5):e157-64. doi: 10.1016/j.jcms.2013.07.014.
8. Bakardjiev A (2016) The Challenge for the Dental Community-Bisphosphonate-Related Osteonecrosis of the Jaw. J Dent Oro Surg 1(4): 121. Doi: + http://dx.doi.org/10.19104/jdos.2016.121
9. Berenson J.R. Stopeck A.T. Medication-related osteonecrosis of the jaw in patients with cancer. ©2015 UpToDate.
10. Bilezikian JP. Osteonecrosis of the jaw – Do Bisphosphonates Pose a Risk? N Engl J Med 2006; 2278 – 81.
11. Campisi G, Fedele S, Fusco V, et al. Epidemiology, clinical manifestations, risk reduction and treatment strategies of jaw osteonecrosis in cancer patients exposed to antiresorptive agents. Future Oncol. 2014

Feb;10(2):257-75.
12. Carter GD, Gross AN. Bisphosphonates and avascular necrosis of the jaw. Aust Dent J. 2003; 48: 268.
13. Chiandussi S, Biasotto M, Dore F, et al. Clinical and diagnostic imaging of bisphosphonate-associated osteonecrosis of the jaws. Dento-maxillofacial Radiol 2006; 35: 236 – 43.
14. Dunstan CR, Felsenberg D, Seibel M. Therapy Insight: the risk and benefits of bisphosphonates for the treatment of tumor-induced bone disease. Nature Clinical Practice Oncology, January 2007, vol. 4, №1, 42 – 55.
15. Edwards BJ, Gounder M, McKoy JM, et al. Pharmacovigilance and reporting oversight in US FDA fast-track process: bisphosphonates and osteonecrosis of the jaw. Lancet Oncol 2008; 9:1166.
16. Fedele S, Bedogni G, Scoletta M, et al. Up to a quarter of patients with osteonecrosis of the jaw associated with antiresorptive agents remain undiagnosed. Br J Oral Maxillofac Surg.
17. Hinson A. M. , Smith C. W., Siegel E. R., and Stack, Jr. B. C. , "Is Bisphosphonate-Related Osteonecrosis of the Jaw an Infection? A Histological and Microbiological Ten-Year Summary," International Journal of Dentistry, vol. 2014, Article ID 452737, 7 pages, 2014. doi:10.1155/2014/452737.
18. Hoff AO, Toth BB, Altundag K, Johnson MM, Warneke CL, Hu M, et al. Frequency and Risk Factor Associated with Osteonecrosis of the Jaw in Cancer Patients Treated with Intravenous Bisphosphonates. J Bone Miner Res. 2008;23(6):826-36. doi: 10.1359/jbmr.080205.
19. Hohnecker JA: Dear Doctor. Precautions added to the label of Aredia and Zometa ed. East Hanover, NJ: Novartis Oncology; September 24, 2004:2.
20. Khan AA, Morrison A, Hanley DA, et al. Diagnosis and management of osteonecrosis of the jaw: a systematic review and international consensus. J Bone Miner Res 2015; 30:3.
21. Khosla S, Burr D, Cauley J, et al. Bisphosphonate-associated osteonecrosis of the jaw: report of a task force of the American Society for Bone and Mineral Research. J Bone Miner Res 2007; 22:1479.
22. Kuhl S, Walter C, Acham S, Pfeffer R, Lambrecht JT. Bisphosphonate-related osteonecrosis of the jaws–a review. Oral Oncol. 48(10), 938–947 (2012).
23. Marx RE , Uncovering the cause of "phossy jaw" Circa 1858 to 1906: oral and maxillofacial surgery closed case files-case closed. , J Oral Maxillofac Surg. 2008 Nov;66(11):2356-63.
24. Marx RE, Sawatari Y, Fortin, et al. Bisphosphonate-Induced Exposed Bone (Osteonecrosis/ Osteopetrosis) of the Jaws: Risk Factors, Recognition, Prevention and Treatment. J Oral Maxillofac Surg. 2005; 63: 1567 – 1575.
25. Marx RE. Pamidronate (Aredia) and Zoledronate (Zometa) induced avascular necrosis of the jaws: A growing epidemic. J Oral Maxillofac Surg. 2003; 61: 1115.
26. Marx RE. Risk factors for antiresorptive drug-related osteonecrosis of the jaw. Antiresorptive Drug-related Osteonecrosis of the Jaw (ARONJ)—a Guide to Research Kenneth E Fleisher, Risto Kontio, Sven Otto.2016 ; 39-44.
27. Migliorati CA. Bisphosphonates and oral cavity avascular bone necrosis: J Clin Oncol. 2003; 21: 4253.
28. O'Ryan, Felice S. et al. Intravenous Bisphosphonate-Related Osteonecrosis of the Jaw: Bone Scintigraphy as an Early Indicato. Journal of Oral and Maxillofacial Surgery , Volume 67 , Issue 7 , 1363 – 1372.
29. Otto S, Schreyer C, Hafner S, Mast G, Ehrenfeld M, Stürzenbaum S, et al. Bisphosphonate-Related Osteonecrosis of the Jaws-Characteristics, Risk Factor, Clinical Features, Localization and Impact on Oncological Treatment. J Craniomaxillofac Surg. 2012;40(4):303-9. doi: 10.1016/j.jcms.2011.05.003.
30. Patel S, Choyee S, Uyanne J et al. Non-exposed bisphosphonate-related osteonecrosis of the jaw: a critical assessment of current definition, staging, and treatment guidelines. Oral Dis. 18(7), 625–632 (2012).
31. Pechalova P, Poriazova E, Pavlov N, Bakardjiev A. Chapter 10 Bisphosphonate – Related Osteonecrosis of the Jaws – Diagnosis and Management. Textbook of Advanced Oral and Maxillofacial Surgery, edited by Mohammad Hosein Kalantar Motamedi, ISBN 978-953-51-1146-7. Published: June 26, 2013.
32. Rossi D, D'orto O, Pagani D, et al. Bisphosphonate-associated osteonecrosis of the jaws: a therapeutic dilemma. OOOOE; 103: 1 – 5.

33. Rugani, P.; Walter, C.; Kirnbauer,et al.. Prevalence of Medication-Related Osteonecrosis of the Jaw in Patients with Breast Cancer, Prostate Cancer, and Multiple Myeloma. Dent. J. 2016, 4, 32.
34. Ruggiero SL DMD, MD, Gralow J, MD,. Marx RE, DDS. Practical Guidelines for the Prevention, Diagnosis, and Treatment of Osteonecrosis of the Jaw in Patients With Cancer. J OURNAL OF ONCOLOGY PRACTICE •V OL. 2, ISSUE 1. JANUARY 2006.7-14.
35. Ruggiero SL, Mehrotra B, Rossenberg TJ, et al. Osreonecrosis of the jaws associated with the use of Bisphosphonates: a review of 63 cases. J Oral Maxillofac Surg. 2004; 62: 527–534.
36. Simov R., Pechalova, P., Bakardjiev A., Sarachev E., Ivanov G. and Dimitrova S. (2013), "Bisphosphonate-Associated Osteonecrosis of the Maxilla," International Journal of Case Reports in Medicine, Vol. 2013 (2013), Article ID 535319, DOI: 10.5171/2013.535319.
37. Solomon D. H., Mercer E., Woo S. B., Defining the epidemiology of bisphosphonate-associated osteonecrosis of the jaw: prior work and current challenges. Osteoporosis International, 2013, January 2013, Volume 24, Issue 1, 237–244.
38. Stockmann P, Vairaktaris E, Wehrhan F, Seiss M, Schwarz S, Spriewald B, et al. Osteotomy and Primary Wound Closure in Bisphosphonate-Associated Osteonecrosis of the Jaw: A Prospective Clinical Study with 12 Month Follow-Up. Support Care Cancer. 2010;18(4):449-60. doi: 10.1007/s00520-009-0688-1.
39. Wang J, Pogrel MA. Osreonecrosis of the jaws associated with cancer chemotherapy. J Oral Maxillofac Surg. 2003; 61: 1104–1107.
40. Woo Sook-Bin, Hellstein JW, Kalmar JR. Systematic Review: Bisphosphonates and Osteonecrosis of the Jaws. Ann Intern Med. 2006; 144: 753 – 761.

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