Vitamin D Deficiency: Causes & Treatment

Chapter 2

Enviromental Factors and Multiple Sclerosis

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1. Introduction

Multiple sclerosis (MS) is a socially significant immune-mediated disease with unknown etiology. The first symptoms are usually registered in the reproductive age-between the 20th and 40th year, with first peak between the 21st and 25th year, and the second peak between the 41st and 45th year. In less than 5 to 10% of the cases the onset of the disease is before the 10th and the after the 50th year. Results from different studies of the disease evolution show that women suffer from it more frequently than men-ration women/ men 2:1, 3:1 [1,2,3,4,5]. The three main forms of progress of the disease: relapsing-remitting (RRMS), secondary-progressive (SPMS) andprimary-progressive (PPMS), reflect the chronic development of the process, disabling the patients for a different period of time. The relapsing-remitting MS is observed in 85-90% of all patients; typical are oligoclonal bands in the cerebrospinal fluid, the clinical development in on average of 90% of the cases correlates with the MRI findings. The fast development of new symptoms or deterioration of old ones, with duration of over 24 hours, is followed by remission and recovery to a different degree. The relapsing-remitting phase progresses into the therapeutically unfavorable secondary-progressive phase in 50-70% of the patient after 10 years after the onset of the disease, on the average [6,7,8,9]. A joint study of the WHO and the World Bank, *Global Burden of Disease Study* (1998), shows that "MS is one of the hundred most severe diseases in the world, and thus it is listed among the ovarian and prostate cancer, trachoma and leprosy" [10]. A comprehensive study of the aspects of the immune-mediated process of CNS, the proving of scientific facts on the participation of specific factors in the pathogenesis of the disease are a premise for the optimization of the therapeutic methods, through inclusion of new immune-effective drugs.

2. Immunology

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Multiple sclerosis (MS) is an immune-mediated disease, which is characterized by demyelination, axonal transection and oligodendropathy in the central nervous system (CNS). The modern vision on the immune-mediated genesis is based on the analyses of the changes in the immunological indices in the blood and the CSF of the patients, in the focal lesions and on the results obtained during experimental allergic encephalomyelitis (EAE). The interaction between peripheral autoreactive antigenic cells (ag) of unknown origin causes clonal expansion of proinflammatory populations and mediators of the inflammation, which pass the blood-brain barrier (BBB) and cause perivascular demyelination, neurodegeneratuion and axonal disorders in the CNS [11,12].

Today MS is considered as organ-specific disease with the following characteristics:

- Specificities of the etiology, including assumed viral agent, genetic factors and environmental factors;

- Specificity of the autoantigens from the target organ: myelin basic protein (MBP), myelin-oligodendrocyte glycoprotein (MOG), proteolipid protein (PLP), myelin-associated glycoprotein (MAG);

- Aberrant immune response from Th1- and Th17-mediated, disseminated inflammation of the central myelin structures with cytokine production imbalance from Th1,Th17,Th2 и CD4+CD25+FoxP3 subpopulations in the CNS and the periphery [11,13,14,15,16].

2.1. Activation of peripheral antigen-specific immunocompetent cells

In healthy individuals there are low concentrations of autoreactive T and B lymphocytes, which due to suppression remain tolerant to the individual's own antigens. In MS, it is assumed that the impaired immune tolerance and molecular mimicry is the reason for the immune-mediated reactivity in the peripheral circulation and the CNS [17]. Another hypothesis suggests transportation of antigens from the CNS to the peripheral flow after injury and/or infection [18,19,20]. This belief is supported by experimental results-inoculation of MBP in the brain and the CSF of animals causes cellular and hormonal responses in the peripheral flow. It is believed, that the cerebrospinal and the interstitial fluid carry the antigens from the CNS, expressed on the membranes of the antigen-presenting cells (APC) [11]. APC monocytes, macrophages, B-lymphocytes and dendritic cells and parenchymal microglia cells express on their surface highly immunogenic part of the antigen, costimulatory molecules and class I/ II molecule of the major histocompatibility complex (MHC). The T-lymphocytes recognize the antigens through two types of T-cell receptors (TCR)–$\alpha\beta/\gamma\delta$ [21]. The trimolecular complex (MHC class I/II molecule, antigen and TCR) is the first signal that induces immune

response, however, it is not sufficient for complete T-cell activation. Complete sensitization occurs after interaction between costimulatory molecules, expressed on the membranes of T-lymphocytes(CD28, CTLA-4, CD40 ligand) and APC (B7.1(CD80), B7.2(CD86), CD40 (22). After complete T-cell sensitization, the aberrant immune response is maintained by the APC cytokine secretion-IL12, IL23, which stimulates the T-cells to secrete inflammation inductors IL2, IL17, IFNγ and proteins with key role for their transfer through the BBB-sphingosine-1-phosphate receptor [22]. In MS it is assumed that the Th1-dependent synthesis of IFNγ, TNFα, IL2, IL12 andthe Th17-mediated production of IL17, IL22, are inflammation inductors, while the Th2-mediated secretion of IL4, IL5, IL6, IL13 has anti-inflammatory effect [18,23,24,25,26,27].

2.2. Blood-brain barrier-transfer of immunocompetent cells and inflammatory antigens from the periphery to the CNS

The blood-brain barrier, composed of endothelial cells and extracellular matrix, is impermeable for large molecules and for the majority of cells. The transfer of immunocompetent cells and other inflammatory antigens through the BBB determines immune inflammation in the CNS with subsequent destruction of the myelin [28].

The influx of inflammatory cells through the BBB includes several stages:

• Attachment of the leukocytes to the vascular endothelium through adhesion molecules-E-selectin and P-selectin, integrin, expressed on their surface. The process is realized through the connection-chemokine receptor on the leukocyte surface / ligand on the endothelial membrane;

• Activation of integrins, mediated by G-protein production;

• Adhesion of the leukocytes to the endothelium after bonding of the leukocyte receptors with own ligands on the membranes of endothelial adhesion molecules-ICAM1, ICAM2; increased leukocyte adhesion following repeated connection of late leukocyte activating factor-VLA-4 with the receptor of the endothelial molecule VCAM1;

• Influx of leukocytes in the CNS [29].

2.3. Immune-inflammatory process and neurodegeneration in CNS

The influx of CD4+ T-cells into the CNS is followed by intensive immune inflammation. The process is triggered after presentation of autoantigens in complex with MHC molecule from local cells-microglial cells and dendritic, as well as from macrophages and monocites in the CNS [17,22,30]. The last stage finishes with tissue damage in the CNS. The tissue disorders have different mechanism: immune-inflammatory, degenerative (Wallerian degeneration),

ischemic, oxidative. Recent studies show results for axonal disorders in the early stages of the disease. Axonal transection is found in patients with disease progression from 2 weeks to 27 years [31]. The number of axons with transection in the lesion areas exceeds 11000 mm³ [32]. The registered correlation between the immune-inflammatory activity in the focal lesions and the axonal damage is a reason to assume immune mechanism of the axonal transection, however, currently there is no proof for direct disorders caused by specific immune mediators [22]. Immunopathomorphological analyses show heterogeneity of the focal lesions with respect to the inflammatory infiltrate, the degreeof remyelination and the oligodendrocyte damages. Today, there are 4 models of myelin destruction. Samples 1 and 2 are typical for the experimental disease with classical autoimmune inflammation-T-cell and antibody-mediated destruction of myelin. Sample 3 is observed in the virus-induced experimental disease and suggests viral etiology, cytotoxic T-cell and proinflammatory cytokines, which mediate the cell death. Sample 4 is found during autopsy of patients with primary-progressive MS. It is assumed that this is caused by genetic disorders or metabolic defect. Sample 1 and 2 are present in all types of MS, 3 in aggressive progression, sample 4-in primary-progressive progression [33,34,35]. The notion of dependence between the sample of the myelin destruction, the clinical manifestations and the therapeutic response potential, is a ground for studying the participation of different factors in the immune-mediated process-genetic, hormonal and environmental, in order to improve the control of the disturbed immune regulation.

3. Results from Epidemiological and Population-Genetic Studies, Which Favor the Hypothesis on the Participation of Environmental Factors in the Etiopathogenesis of the Disease

3.1. Epidemiological studies results

3.1.1. Geographical areasof distribution of the disease:

In 1982, J. Kurtzke et al define geographical areas with high, average and low frequency of the MS disease. The areas of high frequency, disease rate over 30/ 100 000 people, are regions in Northern Europe (north latitude from 44º to 64º), Northern USA, South Canada, Southeast Australia, New Zeeland, etc. The areas with average frequency, disease rate between 5 to 20/ 100 000 (north latitude 32º-47º), include territories around the Mediterranean basin, the south regions of the USA and Africa and vast areas of Australia. Low disease rate, under 5/ 100 000 people, is observed in the regions with tropical and subtropical climate –Latin America, Japan, China, etc [22,36].

Bulgaria is located between 41,5º and 44º north latitude and the index ''geographical area'' suggests average disease rate. Epidemiological study conducted in the country by O. Kalafatova in 1983, shows disease rate of 21.3/ 100 000 people. The most recent study, N. Topalov 1999, registers double increase of this index for the last 17 years – 44.5/ 100 000 people

[37,38,39]. The correlation coefficient between the disease rate and the geographical area is high (r = 0.79), however, the disease rate in some countries like Korea and China, is significantly lower compared to the same geographical latitudes in Europe and North America [37]. The MS disease rate in France is lower than some European territories at the same latitude, such as Spain and Italy. In the northern regions of Europe, the rate is higher compared to the southern areas [36,39].

The MS distribution in the world is characterized with north-south gradient. Moving away from the Equator the disease rate increases, affecting larger groups of the population and reaching 100/ 100 000 in some northern regions. The epidemiological studies show decrease of the gradient in the northern hemisphere, in north-south direction and increase of the index in the same direction in Australia [22]. A study conducted in 1979 in the USA among 5305 war veterans, shows south-north gradient of the disease rate on the territory of the country. The results indicate variation of the index in the area of the 37th parallel – increase and decrease of the disease rate above and below the 37th parallel. In the eastern regions of the country, the 39th parallel divides the areas with high disease rate from those with average rate [40,41].

3.1.2. Migration and MS:

A study of the migration factor shows dependence between the area of residence during the years of adolescence (10-15 years of age) and the risk of developing the disease. If individuals from one geographical area move, before they reach 10 or 15 years of age, to area with different disease rate, the migrants would assume the risk rate of the new area. This index does not change in case of migration after that age. This finding is supported also by a study among population of European immigrants in South Africa. In case of permanent resettlement before the age of 15 years, the immigrants adopt the risk of the new area-13/ 100 000 people, if the immigration occurs after that age, the individuals keep the risk of the previous area-30-80/ 100 000 people [42]. These results suggest etiological significance of exogenic factors during the childhood years, with subsequent long latent period until the first onset of the disease in later years.

3.2. Data from genetic studies

The genetic etiology of the disease has been a subject to numerous studies. The first studies with families with MS date back from the end of the 19th century. Charcot presents evidence of genetically determined risk of the disease, confirmed by modern studies. In the first generation of the family with MS proband, the risk is 20 to 40% higher compared to the general population. The risk evaluation in twins from families with MS, there is concordance of 20% to 30% in the monozygotic twins, and 2-5% in the dizygotic twins. In genetically identical individuals, the discordance in the onset of the disease reaches 70% in both individuals. These results suggest that the effect of certain environmental factors plays a key role for the onset of

MS. [8,43,44,45,46]. The arguments in favor of the genetic predisposition are based on epidemiological observations of racial clustering of MS cases, and prevalence of the disease among ethnic groups from high-risk areas. There is low disease rate among black Africans, American Indians, Asian, etc [22,41]. In North Europe, the representatives of European ethnicities are at higher risk of developing the disease, than the individuals with different descent [36,43]. In Bulgaria, I. Milanov et al, 1999, establish low disease rate (18.4/100 000) among the Roma population, compared with the average for the country (44.5 / 100 000) [47]. Today, we believe that the genes of the major complex of the tissue compatibility (Major Histocompatibility Complex, MHC) in chromosome 6p21.3 control the response to the different antigens, as well as the hereditary risk in 10 to 50% of the MS cases in North Europe [48,19]. Three large-scale genetic studies among families with MS in USA, Canada and Australia identify 13 regions in chromosomes 5,6,17 and 19, associated with susceptibility to the disease; however, there is no definition for the term "risk population" [43]. The genetic tests confirm the polygenic nature of MS, and show intra-individual heterogeneity, which determines the variation in the severity of the immune respons, the type of demyelination and the response to the immune-stimulation therapy [29]. Today, the general believe is that MS is a disease with moderate hereditary risk, modifies to a different extend by environmental factors. The majority of the results are indicative of the participation of exogenic factors in the etiopathogenesis of the disease. In the last years, subject of scientific interest is the role of Vitamin D, as environmental factor, in the distribution of MS.

4. Synthesis and Metabolic Activation of Vitamin D

Vitamin D is secosteroid hormone. In the human body, there are two forms: endogenous, Cholecalciferol (Vit D_3), and exogenous Ergocalciferol (Vit D_2). The serum concentration is a result of: synthesis of cholecalciferol (D_3) from 7-dehydrocholesterol in the skin after UV irradiation, 70-80% of the total amount; synthesis ofergocalciferol (D_2) from plant plant sterol-ergosterol, following UV irradiation, 10-20% of the total amount [50,51,52,53,54]. In the circulation, there are metabolites: 25-hydroxyvitamin D (25(OH)D, calcidiol),1,25dihydroxyvitamin D ($1,25(OH)_2D$, calcitriol) and 24,25-dihydroxyvitamin D ($24,25(OH)_2D$). The serum levels of 25(OH)D are result of the Vitamin D_2 and D_3 hydroxylation in the liver, through cytochrome P450 (CyP) 27A1. $24,25(OH)_2D$ has high concentration in the circulation, insufficiently known biological properties and results from the metabolizing of 25(OH)D in the kidneys. $1,25(OH)_2D$ is biological active form with properties of hormone, obtained after hydroxylation (1α-hydroxylase, CyP 27B1) of 25(OH)D in the kidneys. Vitamin D and the metabolites are inactivated in the liver, through conjugation or oxidation, to glucuronides and sulfates [50]. They are transported to the target cells by serum glycoprotein. The biological effects are mediated by nuclear receptor VDR-a member of the nuclear hormone receptor superfamily [26]. More than 30 tissues in the human body express VDR: parathyroid, bone, renal, the B-cells of the

pancreas, keratinocytes, oligodendrocytes, astrocytes, macrophages, neurons, lymphocytes, etc [50,54,55,56,57]. The scientific evidence shows that 3% of the human genome depends on the Vitamin D Receptor, which explains the pleiotropic effects and the possible participation in the pathogenesis of different diseases (demyelinating, cardiovascular, colorectal carcinoma, etc). The pleiotropic effects, autocrine/ paracrine, occur at 10 nmol/l fold higher concentrations of $1,25(OH)_2D$ than the physiological levels, maintaining the Ca balance. Recent studies find intracellular synthesis of $1,25(OH)_2D$ through local hydroxylation of the 25(OH)D entering the target cells. The local synthesis provides high concentrations of the active metabolite, necessary to achieve the pleiotropic effects. The active 1α-hydroxylase is contained in the monocytes and macrophages, the renal and parathyroid cells and with intracellular synthesis $1,25(OH)_2D$ achieves immune-modulating effects [58].

5. Exogenic Factors Effecting the Vitamin D metabolism and their Relation to the Disease Rate of MS

5.1. Solar exposure/UV radiation

The main source for Vitamin D synthesis is the sunlight. After twenty minutes exposure to the sunlight, the synthesized Vitamin D is between 15-20 000 IU (199). In geographical areas with sunlight of less than 2000 hours annually, the disease rate of MS is higher, compared to areas with sunlight of more than 2000 hours [55].

Study results show that after maximum exposure to sunlight, the amount of the synthesized Vitamin D is comparable to oral intake of 250 μg Vitamin D [59,60].

The intensive exposure to sunlight between the age of 6 and 15 years reduces the risk of MS [54]. The intensity of the UV radiation and the Vitamin D synthesis depend on number of factors: geographical latitude, the altitude above sea level, seasonality, ozone saturation of the atmosphere, the Solar Zenith Angle (SZA), cloudiness, etc.

5.2. Latitude

The Vitamin D deficiency correlates to the geographic latitude and the daily hours of sunlight. In Northern European countries – Denmark, Finland, Ireland, there is deficiency in 50% of the children aged 12.5 yearsincluded in a study, with 50-75 nmoll/serum l25(OH)D being considered as sufficient. In South America there is deficiency in 53.4% of the studied individuals, in comparison to the general population [61,62].

5.3. Altitude

Study results show that the disease rate of MS varies depending on the altitude above the sea level of the place of residence-in reduces in areas at more than 1000 m above the sea level,

and increases in areas below 1000 m [59]. This suggests a correlation between the amount of synthesized Vitamin D and the changes in the intensity of the sunlight and UV irradiation. There are indicative results showing increased with 41% risk in white American adolescents with low serum levels of Vitamin D, as well as evidence of risk decrease when the serum concentrations are increased with 50 nmol/l [59,63]. According to D. Pierrot et al, the risk of MS is associated with low serum levels of Vitamin D in the childhood years [64]. In our country, in 1989 B. Yordanov analyzes the MS distribution in flat and mountain regions and finds lower rate in the flat regions. The results of N. Topalov (1999) regarding the geographical features of the patient's place of residence, until the first symptoms of the disease, confirm that the flat terrain is a "protective" factor (L:OR-0,55; 55% CI-0,30 до 0.99), [37,39].

5.4. Seasonality

There are scientific observations showing seasonal fluctuations in the clinical activity of MS-higher during the spring months compared to the winter. It is assumed that the reduced Vitamin D synthesis in the autumn and winter, caused by the less days of sunlight during these seasons, is the reason for the activation of the disease in the spring [55,65]. A study in Canada including 40 000 MS patients shows much higher number of individuals born in May than in November. CJ Willer et al report higher frequency of MS episodes during the warm months compared to the cold [66,67].

5.5. Ozone

The ozone saturation of the atmosphere is a specific factor determining the geographical area. The ozone reduces the UV rays reaching the Earth, and decreases the Vitamin D synthesis [68].

5.6. Solar Zenith Angle (SZA)

The size of the solar zenith angle changes the intensity of the UVradiation. When the zenith angle is big, the amount of the UV rays reaching the Earth surface is less, as they travel longer distance through the atmosphere with significant absorption/ dispersion. SZA is biggest early in the morning and during the winter, and it is smallest in the summer and at the Equator [68].

5.7. Cloudiness

The clouds absorb and reflect the UV rays and in some circumstances they cause high intensity of UV radiation. With the increase of the altitude above sea level, the UV radiation and the Vitamin D synthesis also increase [68].

Epidemiological and ecological studies show reverse causality between the UV rays and

the MS rate-at the Equator the disease has the lowest rate, the SZA there is the smallest and the UV radiation intensity is the highest [56,69].

5.8. Diet

The European Action on Nutrition and Health Survey, 1996, a study of adults from Southern and Northern Europe, shows lower serum level of 25(OH)D (20-30 nmol/l) in the residents of the South-European territories, than in those from the northern latitudes (40-50 nmol/l). The unexpected difference is associated with the diet habits-consumption of fish oil and cod liver (400 IU or 10μg daily) in Northern Europe, as well as the light skin pigmentation, increase the Vitamin D synthesis [50]. An interesting finding is the difference in the MS rate in Norway – lower compared to the other Scandinavian countries. It is assumed that this is a result of the specific diet habits in the country-high consumption of foods containing Vitamin D [56]. There are various factors effecting the synthesis and the metabolic activation of Vitamin D: limited exposure to sunlight, due to the geographical features of the region, or traditional clothing; the skin pigmentation; diet; slow metabolism with the advancement of age; gene polymorphism of 7-dehydrocholesterol reductase in the skin; cytochrome P450 25-hydroxylase in the liver; the Vitamin D-binding protein in the circulation; renal and liver diseases, etc [70]. Today it is believed, that the individual characteristics are a key factor in the correct evaluation of the multiple effects of the endo/exogenic factor in the synthesis and metabolism of Vitamin D. In this respect, there is an interesting study of Hawaiian surfers, following 15 hours/ week exposure to sunlight, for a period of 3 months. The studied population showed great variation of the serum concentrations-from 15 to 75 ng/ml. It is assumed that this difference depends on the individual characteristics [70].

6. Vitamin D Status Evaluation

An indicator of the Vitamin D status in the body, is the serum concentration of 25(OH)D, which correlates to the biologically active metabolite with the properties of hormone $1,25(OH)_2D$ [71]. The following criteria are used:

- The level necessary for maximum intestinal Ca absorption;

- The level necessary for highest bone mineral density;

- The level of minimum bone loss and reduced fracture risk [50,60].

In 2011 the US Institute of Medicine (IOM) accepts as optimal 25(OH)D concentration levels of ≥ 50 nmol/l, as insufficient 30-50 nmol/l, and as deficit 30 nmol/l [72]. Based on epidemiological observations, the American Endocrine Society recommends serum 25(OH)D concentrations from 75 to 110 nmol/l, as beneficial for the public health-reducing the risk of autoimmune, cardiovascular and infectious diseases, type 2 diabetes, etc [72]. A multicenter

research in Bulgaria, conducted in 2012 by A.M. Borisova et al, defines for the Bulgarian population the following concentrations: ≥ 50 nmol/l-sufficient; 25-49,99 nmol/l-insufficient, and < 25 nmol/l-deficit. The accepted levels are identical with those specified in the Consensus Conference in Germany in 2012, which allows comparative analysis of the study results of Bulgarian and international teams and cohorts [61]. The study includes 2032 individuals aged 20 to 80 years. Of them 47% men and 53% women, all residing in regions located from 41° to 44° northern latitude. In the studied population, the serum 25(OH)D concentrations ≥ 50 nmol/l reach optimum-PTH suppression and are defined as sufficient level, necessary for maintaining the optimal health status of the skeletal system. In 54.5% of the studied individuals, the results show Vitamin D deficiency at 24.2% insufficiency. Severe deficiency is observed two times more often in the women, compared to the men, within the age range 20 to 44 years (p<0.007) [37]. These facts suggest gender differences, modulated by the estrogens [73,74]. The results are of great scientific interest, following comparison with the last epidemiological survey in the country: twofold increase of the MS disease rate for the period 1983-1999; ratio women/men-1.9/1; average age of first symptoms 30.1 years [39].

The analysis of the results from the multicenter study brings the following conclusions: the gender is leading factor for the Vitamin D synthesis; second is the lifestyle and third is the place of residence, related to the environmental conditions (smog, number of sunny days in the year, dust, etc). The evaluation of the seasonal dynamics of Vitamin D in the studied group, shows significant differences in one season compared to the other three-winter (24.14 nmol/l, 95% CI : 25.70-28.58), spring (43.56 nmol/l, 95 CI: 41.96-45.17), summer (61.74 nmol/l, 95% CI: 58.95-64.55), autumn (52.75 nmol/l, 95% CI: 50.63-54.88),(p<0,001) [50]. These results suggest the necessity of year-round prophylactics of the individuals with deficiency or insufficiency, so the negative consequences for the health of the population could be prevented. The literature review shows that one the discussed topics is the Vitamin D replacement dose [75,76,77,78,79,80,81]. For adults, the American Endocrine Society recommends daily intake of 1500-2000 IU Vitamin D, for serum 25(OH)D levels >75 nmol/l, necessary for the mineralization of the skeletal system. Many researchers believe, that the definition of sufficiency, insufficiency and deficiency of Vitamin D, must be determined according to the health consequences [50].

7. Experimental Evidence Substantiating the Hypothesis of Vitamin D Participation in the Pathogenesis of MS

There are experimental data proving the immune-modulating activity of Vitamin D in the CNS and the peripheral organs of the immune system. There is established expression of Vitamin D receptor (VDR) on APCs, dendritic cells, T and B-lymphocytes, macrophages, $1,25(OH)_2D$ synthesis from astrocytes, involvement of the vitamin in the myelin production in the CNS [25,82]. The treatment of suitable test animal with Vitamin D metabolites prevents

the development of experimental allergic encephalitis (EAE), if applied before the induction of myelin protein, and delays the progressive course when applied after the first clinical symptoms. In respect to EAE, M. Cantonara et al (1996) find the following: the application of $1,25(OH)_2D$ 24 hours before the induction with myelin protein, terminates the development of the pattern; the treatment of EAE with $1,25(OH)_2D$ in dose of 300 ng after the first symptoms, holds the progressive development [83]. An experiment of M. Cantorna et al (2000) with IL4-/IL4+ mice, finds: the treatment of IL4-mice with $1,25(OH)_2D$ has lower efficiency in holding the progressing clinical symptoms, compared with IL4+ test animals. The interruption of the pattern in the IL4- mice by high doses of $1,25(OH)_2D$ does not change the IL4 levels, but causes intensive production of TGFβ1. These results are grounds to assume that the high $1,25(OH)_2D$ doses are key factor for the differentiation of the regulatory subpopulation, associated with the production of the anti-inflammatory cytokine TGFβ1 [84].

The treatment of EAE with $1,25(OH)_2D$ registers reduction of the clinical symptoms, decrease of the infiltrates in some areas of the focal lesions, suppressed expression of MHC class II molecules, reduced number of APCs (monocytes, macrophages, microglial cells), reduction of the CD4+ lymphocytes and NOSII-Nytric oxide synthase [85]. After treating EAE mice with $1,25(OH)_2D$, JH. Chang et al discover: reduced number of lymphocytes in the CNS, suppressed expression of CCR6-CC120, facilitating the transfer of Th17-lymphocytes from the periphery to the CNS; increased production of IL10, mediated by TGFβ1; suppressed differentiation of Th17 phenotype by VDR. In vitro studies show: $1,25(OH)_2D$ inhibits the Th1 proliferation and the production of the anti-inflammatory cytokines IL2, IL6, IL12, TNFα, IFNγ; suppresses the expression of MHC class II molecules, suppresses the functional activity and holds the maturation of the dendritic cells; increases the expression of IL4. It is believed that $1,25(OH)_2D$ suppresses the differentiation to Th1/Th2 phenotype and induces the synthesis of regulatory T cells population. Some authors maintain that the immune-regulatory potential of $1,25(OH)_2D$ dominates the suppressive effects [27]. In case of combined application of Dexamethazone with $1,25(OH)_2D$, the CD4+ T-cells differentiate to Th2 phenotype with intensive production of IL5, IL10, and inhibited synthesis of IFNγ. The treatment with Dexamethazone alone suppresses the IFNγ secretion, without changing the levels of IL10 [86]. A research from 2007 studies combined treatment with IFNβ, cyclosporine and TX 527-hypocalcemic analogue of $1,25(OH)_2D$. The combination TX 527/ IFNβ has synergic and immune-modulating activity-inhibition of the antigen presentation, induction of the Th2-mediated cytokine secretion. The clinical effects-delay of the clinical symptoms, extending the paralysis-free period is significantly improved, compared to the application of TX 527 and IFNβ separately [87]. Based the results from experimental model with mice, A. Boonstra et al (2001) assume that $1.25(OH)_2D$ directly transforms the ratio Th1/Th2 subpopulations to domination of the Th2-mediated secretion of IL4. After stimulation with T-cell receptor, $1,25(OH)_2D$ affects the CD4+ T-cell activity, and causes suppression of the polarization to Th1 phenotype and in-

duction of the differentiation to Th2 phenotype. This and other results suggest that CD4+ T-cells are targets for $1,25(OH)_2D$ in the process of Th2 differentiation. The mechanism of the immune-modulating action of the Vitamin D metabolites is yet to be explained. Today, we assume that the biologically active metabolite with characteristics of hormone $1,25(OH)_2D$ achieves its effects through VDR, however, some authors find non-genomic mechanisms of its action. In this respect, there is an interesting study of two groups of test animals-VDR+/VDR- mice. The model includes 54% VDR+ mice, and 27% VDR- mice. Following treatment with $1,25(OH)_2D$, the VDR+ animals do not develop experimental disease, while EAE is registered in 35% of the VDR- animals [88].

The experimental evidence substantiate the hypothesis for CD4+ T-cell mediated myelin destruction with imbalance of the cytokine secretion of TH1, TH17, TH2 subpopulations. During the exacerbation, the imbalance in the periphery is characterized by decrease of the regulatory CD4+CD25+FoxP3 subpopulation, associated with the production of the anti-inflammatory cytokines TGFβ1, IL10, inhibited IL4 synthesis and increased secretion of the proinflammatory cytokinesIFNγ, IL17, TNFα.

8. Evidence from Clinical Observations of the Participation of the Vitamin D Metabolites the MS Pathogenesis

In MS, large portion of the evidence of the immune potential of the Vit-D metabolites is obtained through experimental observations, and smaller portion from studies of MS patients. M Soilu-Hanninen et al (2005) compare the serum 25(OH)D concentrations during the summer and winter season, in the relapse-remission phases, in 40 RRMS patients and 40 clinical healthy individuals. During the winter there are no significant differences between the levels of patients and the controls. In the summer, the patients show significantly lower serum levels of 25(OH)D (58nmol/L), compared to the healthy controls (85 nmol/L). During MS relapse, the serum concentrations of 25(OH)D tend to decrease, compared with the remission phase, but remain within referent limits (196). In 15 RRMS patients, treated with 25(OH)D in increasing doses from от 0,5 μg/day to 2.5 μg/day and oral intake of Ca up to 800 mg/day for 48 weeks, there is significant decrease of the relapse rate, compared to previous period, and there is no hypercalcemia in dose of 2.5 μg/ day [69].

In 2006, in the USA is conducted a prospective case-control study among 7 million military personnel. 257 MS patient are registered, selected according to gender, race and age. Two groups are formed based on race including patients and controls. The comparison of the serum 25(OH)D concentrations between the two groups (patients and controls), shows lower serum levels of 25(OH)D in the representatives of the black race. The MS risk evaluation of the two groups with different ethnicity finds significant decrease of the index when the serum levels of 25(OH)D are increased with 50 nmol/L, only in the representative of the white race

[63]. J.Smolders et al (2008) study the connection of the serum concentrations of 25(OH)D, 1.25(OH)$_2$D with the severity of the neurological deficit /EDSS/ and the episode rate in 267 patients with relapsing-remitting, primary and secondary progressive MS. The serum Vitamin D levels are significantly lower in the progressive forms of the disease, compared to the relapsing-remitting form. There is no statistically significant connection between the serum levels of the metabolites and the episode rate. The serum concentrations of 25(OH)Dand 1.25(OH)$_2$D are lower in individuals in relapse, compared to the episode-free patients. No significant connection was found between the 1.25(OH)$_2$D levels and the severity of the neurological deficit. The analysis of the relative risk of relapse in patients with and without exacerbation, shows increase with 51% of the relapse-free patients with increase of the 25(OH)D levels in the serum with 10 nmol/L [60]. A study of J.Kragt et al (2009) evaluates the serum concentrations of 25(OH)D and 1,25(OH)$_2$D during the summer and the winter months, and according to the gender in 110 controls and 103 RRMS patients. Subject of interest are the differences related to the gender, and probably modulated by the estrogens: in the women there is negative correlation between the deficit /EDSS/ and the serum concentrations of 25(OH)D, unlike the men; only in the women the increase of the serum concentrations of 25(OH)D in the serum with 10 nmol/L, reduces the risk of MS with 19% [89]. D. Pierrot et al (2012) study the connection between the relapse rate and the changes in the 25 (OH)D serum after treatment with Cholecalciferol/amp.100 000 IU (average dose 3010 IU/ day in 156 RRMS patients). Until the moment of registration, 76 of the studied patient had been on therapy with drugs modifying the disease course – Copaxone 20 mg. s.c/ day; Avonex 30 μgr. i.m.once per week; βIFN1a 22/44 MIUs.c three times per week. In the other 80 patients, the therapy with Cholecalciferol starts simultaneously with medication modifying the disease course. The serum levels of 25(OH)D in the studied patients increase on average with 49-110 nmol/L. There is negative significant connection between the relapse rate and the serum concentration of 25(OH)D. Each increase of the serum concentrations with 10 nmol/L reduces the risk of relapse with 13.7%. This trends is kept until concentrations of 110 nmol/L are reached, then maintained unchanged above levels of 120 nmol/L. In MS patients with Vitamin D deficit, the authors recommend therapy, which can ensure 25(OH)D serum levels of 100 nmol/L [90]. Studies conducted among nurses (NIHS I, NHS II (2004), evaluate the risk of MS depending on the Vitamin D intake as poly-vitamins with average daily dose of 400 IU. The results show 40% reduction of the risk in the group with intake of poly-vitamins, compared to the other [91]. H.Derakhshand et al (2013) conduct double-blind, randomized study of 30 patients with optic neuritis and 25(OH)D serum levels under 30 nmol/L. The patients were grouped in two: 15 patients take Calcitriol50 000 IU per week for a period of 12 months; the other 15 patients take placebo. Subject to evaluation are: the dynamics of the MRI findings and the risk of another MS relapse. The individuals treated with Vitamin D show significantly lower risk of relapse (RR=0,316, p=0,007) and statistically important smaller number of lesions, both new and Gd enhanced lesions, compared to the placebo patients [92].

D. Golan et al (2013) conduct double-blind, randomized study of 45 MS patients treated with IFNβ in two dose regimens of Vitamin D_3. In 21 patients the daily intake of Vitamin D_3 is 800IU, and in 24 patients 4380 IU. Subject of evaluation are: the serum concentrations of Ca, 25(OH)D, IL17, IL10, IFNγ, the relapse rate, the severity of the neurological deficit (EDSS). In both groups the levels of 25(OH)D significantly increase (48/68 nmol/L: 48/122.6 nmol/L). In the patients with higher dose Vitamin D_3, statistically significant is the increase of IL17, unlike the patients with lower dose [93]. M. Soilu-Hänninen et al (2012) conduct double-blind, randomized study of 66 RRMS patients, treated with IFNβ 1b and additional therapy with Cholecalciferol 20 000 IU once per week. Two groups are formed: 34 patients take Vitamin D_3 in the mentioned dose, 32 patient take placebo. The serum levels of 25(OH)D in the patients treated with Vitamin D_3 reach 110 nmol/L and are significantly higher than the placebo group. There was no hypercalcemia. There were no significant differences between the two groups with respect of: new T2 lesions, number of active lesions, the relapse rate and the severity of the neurological deficit [94]. Study of G. Mossayebi et al (2011) analyzes the effect of Cholecalciferol therapy on the immune reactivity, MRI and clinical indications of the disease activity in 62 RRMS patients treated with IFNβ1b. Two groups are formed: 28 patients receive additional monthly dose of Vitamin D_3-300000 IU i.m.; in 34 patients the therapy is placebo. At the end of the 6th month after the beginning of therapy, there were no significant differences between the groups with respect of: severity of the neurological deficit and Gd enhanced lesions. The patients treated with Vit-D_3 show statistically significant increase of IL10, TGFβ1, compared to the placebo group. In identical comparison, the IFNγ serum concentrations remain unchanged [95]. In open randomized study in vitro, K.Samanta et al (2011) evaluate the potential of $1.25(OH)_2D$ to modulate the immune imbalance in 49 MS patients. The first group includes 25 patients treated with Cholecalciferol 14 000 IU/ day and Ca 120 mg/day, for one year. In the second group of 24 patients, the therapy is placebo. At the end of the first year, in the patients treated with Vit-D_3 the serum levels of 25(OH)D are significantly higher than the placebo group, and there is reduction of the abnormal activity of the antigen-specific peripheral mononuclear cells, without change in the T-cell reactivity, compared to the placebo [96]. Mahon et al (2003) conduct double-blind, placebo-controlled study of 39 RRMS patients and initial serum level of 25(OH)D under 20 ng/ml. A group of 17 patients is treated daily, for a period of six months, with Cholecalciferol and Ca 800 mg. The other 22 patients are treated with Ca 800 mg and placebo for the same period. At the end of the 6th month, the patients treated with Vit-D_3 and Ca show significant increase of the 25(OH) levels and the serum TGFβ1, unlike the patients treated with Ca and placebo. In both groups, the serum concentrations of TNFα, IL13, IFNγ remain unchanged. The authors believe that the Vit-D_3 has the potential to influence the immune-regulatory imbalance in RRMS patients, however, the doses needed for immune tolerance, are subject of further studies [97].

The clinical observations do not present one-directional results on the connection be-

tween the dynamics in the serum concentrations of the Vit-D metabolites and changes of the immune indexes, the severity of neurological deficit, Gd enhanced lesions during MRI test.

Under discussion are questions such as: subgroup of MS patients suitable for treatment with Vitamin D; the doses of application, duration of the treatment; serum levels of 25(OH)D causing suppression of the immune reaction.

Proving scientific facts for the cause-and-effect relationship of the changes in the 25(OH) D levels in the serum, with the dynamics of the immune and clinical indexes of the disease activity, would enrich the available scientific data, and would optimize the therapeutic approach through treatment with Vitamin D.

In Bulgaria, the MS disease rate has increased two fold over a period of 17 years. In this respect, studies of the interaction between environmental factors and their role in the pathogenesis of the disease are substantiated and necessary.

In 2016 was completed a study of 86 individuals from the white race-46 RRMS patients and 40 controls. The variations in the serum concentrations of 25(OH)D, IFNγ, IL17A, TGFβ1, IL4, IL10 were evaluated during the relapse and remission phases. In the patients with relapse and severe neurological deficit, the 25(OH)D levels are reliably lower, compared with the healthy individuals. During the remission, the levels are statistically significantly increased, but they do not reach those of the controls. In the studied population, the 25(OH) D deficit increases the general risk of MS 3.43 times. There is significant negative correlation between the 25(OH)D levels and the severity of the neurological deficit during exacerbation. The comprehensive analysis of the effect of the studies indexes on the degree of EDSS during relapse, shows dependence between the severity of the deficit and the serum levels of 25(OH) D, IL17A, TNFα during that period [98].

10. Conclusion

The multiple sclerosis is one of the scientific problems for the modern neurology. The regulatory immune imbalance has a key role for the chronic-progressive course of the immune-mediated myelin destruction, and for the treatment effect. Many studies are being conducted today, aiming to identify new factors, participating in the aberrant immune response, in order to achieve optimal suppression by combining appropriate immune-efficient agents. The studies of the role of Vitamin D in the pathogenesis of MS are an aspect of the modern conception of the comprehensive control of the pathological process.

The evaluation of the clinically significant changes depending on the degree of impairment of the Vitamin D status-insufficiency/ deficit, is crucial for the prophylactics and treatment of conditions with proven participation of Vit-D in the pathological process.

The persisting immune imbalance is associated with impaired immune tolerance, resulting from insufficient number of known factors, including environmental. The reported evidence on the participation of 25(OH)D, an environmental factor, in the pathogenesis of MS, gives grounds for studying of the therapeutic potential of the metabolite to control the impaired immune regulation and clinically manifested disease activity. Finding scientific proof on the benefits of using new immune-effective drugs in optimal and safe dose, alone or in combination, will increase the potential of the modern methods of therapeutic intervention to modify the outcome of the disease.

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