

The Role of Cytomegalovirus (CMV) and Vitamin D in Multiple Sclerosis (MS)

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Abstract

Multiple sclerosis (MS) is one of the autoimmune demyelinating diseases involved in the central nervous system (CNS) and its etiological factors believed to be both environmental and genetic factors . The disease affected brain and spinal cord with various characteristic involved defect of myelination , chronic inflammation , pathological effect on axon and oligodendrocyte . The herpesvirus family is play a vital role in pathogenicity of disease . Cytomegalovirus (CMV) from these family members which detected to have a role in MS and our results investigated a significant relation of CMV with MS between patients and controls . Besides a previous data which approved the effect of the level of vitamin D on MS development and modifications in patients . In this study the results achieve significant differences in the levels of vitamin D between the two groups under study .

Key words: *multiple sclerosis (MS), Cytomegalovirus (CMV), (25- hydroxyvitamin D), Vitamin D.*

Introduction

One of the diseases involved in the central nervous system (CNS) is multiple sclerosis (MS) ¹ . The disease affected brain and spinal cord with different clinical features such as blemish of myelination , chronic inflammation , pathological effect on axon and oligodendrocyte ² . Generally , the disease pathogenesis may related to genetic factors of an individual or due to environmental conditions surrounding the patients ³ . The environmental factors can be suggested to related to distribution of the disease , in some population like Jordanians the disease incidence , is ranged from 20-60 cases per 100,000 persons ⁴ . In spite of World Health Organization , showed the incidence of MS reported to be 4 in 100,000 ; in South Asia reported to be region with a medium to high risk for MS ⁵ . While the prevalence in northern Europe or Canada is about 0.1 - 0.2% in population ³ . Multiple sclerosis is considered as idiopathic disease, and many factors can be associated with its progression ; from these are the genetic factors such as the role of the genes responsible of DNA repair in activation of MS and lesions formation ⁶ . As well as the role of polymorphisms in human leukocyte antigen (HLA) genes class II ⁷ . Numerous of genes associated with the disease are the regulatory genes affected the immune cells and their function which lead to

dysregulation of the immune system ⁸ . Auto-reactivation of T cells against myelin may contribute to pathogenesis, and there is increasing attention of the role of human viruses in autoimmune diseases and its progression ⁹ . There is a significant increasing damage in nuclear and mitochondrial DNA in lesions of MS comparing with normal brain white matter and this highlights on the role of repair genes in DNA ⁶ .

Studies shown that multiple genes such as base excision repair genes involved in DNA repair may expressed in lymphocytes and lesions of MS when compared with tissues of control ¹⁰ . The environmental factors are strongly approved to be associated with the triggering of MS , like viral infection , high radiation of UV , climate and temperature , fat intake from animal sources , consumption of dairy products and levels of vitamin D ¹¹ . genotoxic agents ,like cigarettes and nitric oxide may support and involve in MS induction ¹² .

The Role of Vitamin D :

Vitamin D concedes as essential nutrient for the bone homeostasis and plays a role in many disorders like cardiovascular and autoimmune diseases ¹³ . Although the deficiency of vitamin D is common in all ages but it hard to occur in the young and elderly ; and not only bone disorders , but many disorders can be related to

it , such as cardiovascular , autoimmune and neuro-related diseases such as schizophrenia ¹⁴ . In the immune system it is proposed to play a vital and supportive role in controlling of several diseases, as a cancer and neurodegenerative disease and possible relation with MS ¹⁵ .

Enhancement the function of vitamin D is by binding to the vitamin D receptor (VDR) on the cells ; by the ability to converting 25(OH)D to the active form 1,25(OH)2D. The VDR concedes as a mediator for the signaling, and its regulation is unclear until now ¹⁶ More than 2,000 genes can be regulated directly or indirectly by 1,25(OH)2D ; as well as there are at least eighty various metabolic processes regulated by these genes such as repair of DNA , apoptosis , alteration of function of the immune system , oxidative stress ¹⁷ .

Many factors and there accurate roles must be examined because they preventing and treating many disorders related to with VDR and its function by facilitating and improving strategies ¹⁸ . Many studies indicate the protective role of vitamin D on MS patients, and the risk of disease is reduced by Vitamin D supplements and exposure to sunlight ¹⁹ . Moreover, expression of VDR induced by 1,25(OH)2D in both resting and activated cells ; and for homeostasis of T cell during MS , vitamin D plays crucial role , and supplementation of it should be appropriate to treat the disease severity ²⁰ .

Multiple Sclerosis and Viral Association :

Studies connect between viral etiology and MS; that due to the association of viral infection and encephalomyelitis , demyelinating processes in the nerves and axonal damage ; all that may precede the lesions of MS; as well as plaques of active demyelinating caused by T CD8 lymphocytes which involved in viral immunity ²¹ . Processes of cortical and axonal damage with lesions absence are detected in viral demyelinating , are seen generally in MS , and there is a resemblance of relapsing remitting MS form and periods of reactivation and latency of many viruses, such as the herpes virus ²² .

Cytomegalovirus (CMV) , Epstein- Barr Virus (EBV) , Human Herpes Virus 6 and 7 (HHV-6 and HHV-7) are members of the family *Herpesviridae*, and are from plausible viral agents associated with triggering of MS, as well as endogenous retroviruses also considered to be significant agents in MS ²¹ . The infection by CMV may activate the reaction of the immune system

against the constituents of myelin of peripheral nerve ²³ . A wide of inflammatory diseases and brain cancer , CMV was concerned to be as a co-etiological agent ²⁴ . Furthermore a wide range strategies can be caused by CMV can be targeting the host defenses, from disturbing the pathways processing of antigen to modulating of cytokines ; and this establishing success coexistence of CMV during active infection , and in many tissues and organs this virus can be found , as well as in fluids of body ²⁵ .

Types of MS :

There are 4 clinical types according to the course of disease; relapsing-remitting MS (RR-MS), secondary-progressive MS (SP-MS), primary progressive MS (PP-MS), and progressive-relapsing MS (PRMS) ; the first type is the major one and approximately 85% of patients are identified with it , in which exacerbations symptoms is noticeable and remission period is followed by , and the symptoms either progress or withdraw ; in a number of patients RR-MS types of disease may developed into the second type (SP-MS) in which the disease continues to be deteriorate with remission period or without ⁴ . The third types of disease (PP-MS) about 10 percent of total patients be affected ; and the symptoms gradually worsening without remissions or relapses, the rare types of MS is the last one (PR-MS) which affecting less than 5 percent of total patients and from the onset of disease it is progressive intermittent flareups with no remission periods and the symptoms are worsening the way ²⁶ . In the adolescence the disease onset initiates and the peak reaches among the persons in late 20s and the beginning of early 30s , and the females developed the disease in doubling than males ²⁷ . In Arabic populations , all ratio of gender designate that the females affected more over than males ²⁸ .

Materials and Method

In this case control study sixty patients participating . They had MS from all types ,who attendance the MS center in medical city, their ages range between (19-53) years . And the confident diagnosis was confirmed according to examination clinically and neurologically by neurologists . Sixty persons ; females and males included in this study as a control with normal clinical and neurological status and with no history of MS , and their ages approximately matched to that of patients .venous blood samples were collected and the serum separated from patients and control . Vitamin D immediately

examined while the rest of serum stored in -4°C until Anti-CMV antibodies examined . CMV IgM ELISA kit provided by (CALBIOTECH / USA company) .

used to compare means of (Vitamin D and Anti-CMV antibodies) serum level between two groups (patients and controls) . A p-value ≤ 0.05 was considered as significant ²⁹ .

Statistical Analysis

The statistical analysis of this study included the calculation of mean values and percentage. The association between Vitamin D and Anti-CMV antibodies was done by calculated of Chi square and P value with 95% confidence interval (95%CI). T-test was

Results and Discussion

In present study 60 for each of patients and control group taken . All the types of MS included in this study and 38 (63%) were detected with the first type (RR-MS) . The results obtained is illustrated in the Table (1) .

Table (1) : The ages , mean of ages and gender of patients and control groups .

Groups	Ages	Mean of ages	P value	Females	Males
Patients (n=60)	(19-53) years	34.73±8.9	0.9	43(72%)	39(65%)
Control (n=60)	(17-56) years	34.93±10.4		17(28%)	21(35%)

P-value ≤ 0.05 is significant

In this study we found that there is no significant differences of ages between two groups and , the prevalence of females is more than males , our results matched with previous studies who told that , generally MS presents in women higher than in men ; and this may be due to variations in the nervous or the immune system , differences in hormones , variations in the genetic , differences in the environment and modern lifestyle between men and women ³⁰ . And there are markedly increasing of this disease in women than men in last years ³¹ .

This increasing possibly pointed to unclear changes in nutrition or environmental conditions which lead to predominance of this disease in women ³² . Also there is a different response of females and males against factors in the environment ; such as the sun light and supply of Vitamin D ³³ . Moreover , increasing intake of dietary salt may be suggested to develop autoimmune diseases by activation of T-helper 17 cells ³⁴ .

The role of CMV in MS patients :

The level of anti-CMV antibodies of patients and control groups were detected by ELISA kit and our results represented that , there is a significant differences of anti-CMV antibodies between two groups . And CMV showed to have a positive role in MS disease ; table (2) .

Table (2) : Anti-CMV antibodies of patients and control group .

Groups	Anti-CMV antibodies positive (number and %)	Anti-CMV antibodies negative (number and %)	Mean of Anti-CMV antibodies positive (IU/mL)	Border line (number and %)	P value
Patients (n=60)	40(66.66%)	16(26.66%)	2.21±1.5	4(6.66%)	0.005
Control (n=60)	26(43.33%)	29(48.33%)	1.46±1.2	5(8.33%)	

P-value ≤ 0.05 is significant

Many studies about *Herpesviridae* family, talk about the role of these family members in triggering and pathogenesis of MS; and most of these studies recorded the significant role in induction of MS; these viruses have periods of latency and exacerbations within CNS; Epstein-Barr, cytomegalovirus, human herpesvirus 6 and human herpesvirus 7 viruses from these viruses which are possibly triggering MS²¹. Several studies proved a high rates of infection with CMV in MS patients; but with more benign characteristic of this disease²³. In comparison, the high titers of antibodies against CMV, the patients have disease reactivation in lower rates and cerebral atrophy in fewer signs with those patients who had lower antibodies titers³⁵.

Sanadgol *et al.*, support the hypothesis of contribution of CMV with MS and establishment of systemic infection by CMV in induction of immune response²³. As well as induction of demyelinating lesions may be caused by myelin-specific lymphocytes which triggering by CMV³⁶. In contrast of that Najafi *et al.*, revealed, there is no significant differences between the anti-CMV antibodies and MS based on statistical analysis³⁷.

Many surveys correlate between the role of infection by CMV and some types of autoimmune disease; and these studies demonstrated the role of CMV infection in attacking of the immune system to own cells of patient's by antigenic mimicry, and a high titers of IgG antibodies against CMV found in certain autoimmune disease³⁸. In other hand probably CMV is capable to evade the immune system and induce the mechanisms of immune regulation, which may decrease the reactivity of MS disease in patients with MS³⁹.

In general There is controversy about the role of pathogenesis of CMV in MS patients; some studies suggested a positive relationship between MS activity disease and infection with CMV; while others investigated negative role between MS and CMV infections⁴⁰. Many studies needed to improve the role of CMV in MS induction by taking larger samples of patients of MS and healthy control.

The role of vitamin D in MS :

In this study the level of Vit. D is measured and the results of two groups shown in the following table.

Table (3) : the level of vitamin D of patients and control group .

Groups	Deficiency (number and %) of Vit.D level	Sufficiency (number and %) of Vit.D level	Mean of Vit.D deficiency (ng/ml)	P value
Patients (n=60)	47(78.33)	13(21.66)	21.78±13.1	0.000
Control (n=60)	19(26.66)	41(68.33)	36.43±14.1	

P-value ≤ 0.05 is significant

The result revealed that, there is a significant differences between two groups in levels of vitamin D. The patients with MS suffering from deficiency in vitamin D level and Sintzel *et al.* pointed to relationship between vitamin D levels in serum and development and activity modifications of MS in patients; besides the lower level of vitamin D, the higher risk of MS development⁴¹.

Besides the levels of (25- hydroxyvitamin D) in many cases of MS are much lower when compared with healthy peoples, as well as the patients with active form

of disease have lower levels of vitamin D than those with the disease with less active state, and this indicated a significant inverse relationship between serum 25(OH) D and the activity of clinical state of MS⁴².

Evaluation the relationship between the intake of vitamin D from diet or supplements and the risk of development of MS showed that, the women with highly intake of vitamin D supplement had a lower incidence with 33% less of MS development in comparison with women who taking low or no supplements of vitamin D; moreover, the women that used supplements of vitamin D had a 41% reduction in development of MS disease in comparison with non-user women; and that predict to

reducing the onset of MS risk ⁴³ .

Furthermore , an individual etiological factor for MS , vitamin D reported to interact with other causes of MS like , genetic factor as HLA-DR1 to environmental factor as a smoking ⁴² . Decrease in serum level of vitamin D due to genetic causes supposed to increase the susceptibility to MS by fifty percent ; while increasing the level of 25(OH)D by 50% may decrease getting MS at about 50% ⁴⁴ . On the other hand a vitamin D daily dose or a gestational dose per day ; may “*keeps the MS doctor away*” is not proven until now ; in addition in a large majority , the level of serum 25(OH)D may not prevent MS of many peoples ⁴⁵ .

Runia *et. al.* told that risk of relapse may reduce significantly in individuals with medium (20-40 ng/mL) or high as (> 40 ng/mL) levels of vitamin D in comparison to those individuals with levels lower than ⁴⁶ . The association between MS lesions development and the levels of vitamin D showed that individuals with high levels of vitamin D are developing much lower newly lesions and of enhancing lesions gadolinium (Gd+) ⁴⁷ . Moreover increased level of 25(OH)D related to less active rate of new lesions and relapse rate at 57% ⁴⁸ .

The receptors of vitamin D especially for 25(OH)D found in many cells of immune system like macrophages and ; and stimulation of these cells through the receptors can inhibit the immune response and cytokine production ; and in patients with MS , vitamin D supplements can the level of IL-2 mRNA in mononuclear cells ⁴⁹ . In patients with MS, the immunomodulation by 25(OH) D ,can increase the anti-inflammatory cells and inhibit the pro-inflammatory cells , which reduce the severity disease ; after the intake of vitamin D dietary(1000 IU/day) also the cytokine profile modification can reduce inflammation and this can be demonstrated in patients with MS ⁵⁰ .

As well as in the genomic level vitamin D receptor (VDR) gene polymorphisms have been demonstrated to be link to numbers of diseases such as MS ⁵¹ . Furthermore a similar result showed the significant differences between the patients and controls of genotype distribution of (VDR) genes ⁵² . On the other hand , Cakina *et al.*, showed that , there is no significant differences between MS patients and control groups ⁵³ .

Conclusion

In spite of the controversy role of CMV in triggering

of MS ; this virus play a major role in this disease either by induction of disease by activation of immune system against the myelin of CNV by molecular and antigenic mimicry or by reduce the reactivity of disease and lesion formation due to evasion of immune system by periods of latency of these viruses. Vitamin D is not only act a vitamin it is a gene and cell regulator . It is play a crucial role in induction of many diseases like cardiovascular disease , diseases related with the nervous system and immune system . And its role in MS induction and activation is clear , and its role of it and its receptors need for more investigations.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Kerbala – Veterinary college / Microbiology Dept. /Iraq and all experiments were carried out in accordance with approved guidelines.

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