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Diagnostic biomarkers of multiple sclerosis

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ABSTRACT

Multiple sclerosis (MS) is a chronic and inflammatory disease of the central nervous system (CNS) that is common in people between the ages of 20 and 40. Although the exact cause of MS is still unknown, evidences shows that genetic and environmental factors have a role in activating immune cells to attack the myelin sheath of the neurons, and causing them to be damaged. In order to control and reduce the disease prevalence, early identification of at risk people and patients in the early stages of the disease is important. Measurement of interferon gamma (IFN-γ), vitamin D, melatonin, neurofilaments, micro RNA-132 (miR-132), miR-155, determination of antibody titer against Epstein-Barr virus (EBV) and human leukocyte antigen (HLA) typing are appropriate methods to diagnose the disease, and ultimately prevent its recurrence.

Keywords: multiple sclerosis, vitamin D, interferon gamma, microRNA, neurofilament, melatonin

INTRODUCTION

MS is a chronic and inflammatory CNS disease that causes myelin destruction, and worsens the patient's disability. MS affects about 2.5 million individuals worldwide [1]. The disease is the most common neurological disorder between the ages of 20 and 40 [2]. In more patients, the disease presents as a periodic disorder and over time becomes a progressive disease [3]. Although the exact cause of MS is not known, it is believed that the disease may be due to genetic and environmental factors that activate the body's immune cells against the CNS myelin antigens [4], resulting in inflammatory lesions in the system [5]. Activated autoimmune cells such as T helper1 (Th1), Th-17(Th17) and B cells, which are attached to the endothelium of the CNS by crossing the blood-brain barrier (BBB), enter the brain and target the myelin sheath directly and indirectly [6]. One of the most important autoimmune cells that play a critical role in the pathology of MS is T cells, so MS is known to be a disease caused by T lymphocytes attacking the CNS [7]. In this disease, the balance between the regulatory T cells (Tregs) and the Th cells is disturbed [8]. MS is classified into four subgroups: relapsing / remitting MS (RRMS), secondary progressive MS (SPMS), primary progressive MS (PPMS), and progressive-relapsing MS (PRMS) [9]. The most common type of the disease is RRMS [10] and is on the rise in Iran, so diagnosis of at risk people is important. Identifying reliable biomarkers is essential for the diagnosis and follow-up of the MS patients. These biomarkers can be examined in blood, urine, saliva, and CSF [11]. The aim of this review is to introduce appropriate biomarkers to diagnose MS and prevent disease progression and recurrence.

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VITAMIN D MEASUREMENT

Vitamin D is a cholesterol derivative that plays a hormonal role. This vitamin is obtained mainly from food as well as skin 7-dehydrocholesterol exposure to solar ultraviolet B (UVB) radiation [12]. Upon entering the target cell, this vitamin alters the transcription rate of several genes associated with the immune system, so plays the critical role of modulating the immune system [13]. Vitamin D deficiency can lead to inappropriate immune responses, followed by a range of inflammatory and autoimmune diseases, one of which is probably MS. Epidemiological evidences also suggest a link between vitamin D levels and the disease progression [14]. Vitamin D serum levels are affected by nutrition, environmental and genetic factors [12]. About 85% of the world's population lives in countries that are more exposed to sunlight [15], while the remaining 15% are less exposed and are more likely to develop MS [16]. Therefore, people who spend more time in direct sunlight during young ages are less likely to develop MS [17]. Pregnant mothers who are less exposed to sunlight during the first three months of pregnancy have an increased risk of developing MS in their newborns [18]. The cytochrome P450 family 2 subfamily R member 1 (CYP2R1) gene codes for one of the most essential enzymes in the liver that transforms vitamin D to 25(OH)D [19]. Based on the results of an experiment examining the relationship between serum levels of 25(OH)D and MS recurrence in patients before and after receiving 3,000 international units (IU) of vitamin D daily, there is a strong association between high levels of the vitamin and reduced MS recurrence [20]. In general, it can be concluded that the serum level of vitamin D will be effective in the development of MS and its progression [21]. Therefore, since there may be a link between vitamin D levels in serum and the risk of developing MS, it is recommended that people with a family history of MS be tested for vitamin D deficiency.

HUMAN LEUKOCYTE ANTIGEN TYPING

The encoding sequences of HLA are located in the short arm of chromosome 6 at p21.3, which contains about 4,000 Kb of DNA [22]. HLA molecules are expressed at the surface of various cells in the body, which is important in differentiating self-cells and non-self-cells. HLA genes are classified into three classes including class I (with loci A, B, C), class II (DR, DQ, DP), and class III. In some studies, the association of HLA with MS has been observed. Some class I alleles mainly play a protective role against MS, while the risk of MS is higher in some class II alleles [23]. The HLA class II molecules are expressed by antigen presenting cells such as dendritic cells, macrophages, and B cells, and play a role in antigen presentation to T cells and antigen recognition by them [24]. HLA-DRB1 gene expression is increased in MS patients [25]. In positive patients for HLA-DRB1*15 allele, T cells produce large volumes of TNFα, which plays an important role in initiating inflammation and demyelination [26]. Among the HLA-DRB1 alleles, ca is the allele responsible for genetic risk in the MS population, that triples the risk of MS [27]. The presence of this allele reduces the age of the onset of the disease [28]. In patients with HLA-DRB1*1501alleles, N-acetyl-aspartate (NAA) levels decrease in the white matter of the brain, leading to brain atrophic outcomes and cognitive impairment [29]. Vitamin D is also said to be effective in regulating HLA-DRB1 allele expression [30].

MELATONIN MEASUREMENT

Melatonin is a hormone related to sunlight that is secreted by the pineal gland in response to darkness. This hormone, as the most important substance secreted by the pineal gland, has antiseptic and antioxidant properties and acts as a neuroprotective against cerebral ischemia [31]. It regulates immune cell formation and function and also has anti-inflammatory actions on nerve cells [32]. Depending on the stage of inflammation, this hormone can have both pre-inflammatory and anti-inflammatory properties, so low melatonin secretion is associated with neurological disorders such as MS [33]. Melatonin secretion is impaired in MS patients as its serum level is lower than healthy individuals [34]. Elevated levels of this hormone causes the production of anti-inflammatory cytokines such as interleukin 4 (IL-4) and IL-10, in contrast, reduces inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and IL-1β [35]. Because active microglia and lymphocytes are involved in the destruction of myelin, melatonin secretion has significantly reduced the number of active microglia and lymphocytes in the spinal cord of experimental autoimmune encephalomyelitis (EAE) models [36]. Melatonin reduces the ratio of Th1 to Th17 and modulates immune responses by increasing IL-10 secretion and regulatory T cell development [37]. The hormone also affects oligodendrocyte metabolism as well as the production and regeneration of myelin proteins including myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG) and myelin-associated oligodendrocytic basic protein (MOBP) [35]. Night work, especially in young people, reduces serum level of the hormone, which is associated with a higher risk of MS [38]. Due to the role of melatonin in the pathogenesis of MS, measurement of it can be helpful in early diagnosis of MS.

NEUROFILAMENTS

Neurofilaments are the primary components of neuronal skeletal protein, which consist of three main chains of different sizes including; neurofilament light chain (NfL), medium (NfM) and heavy (NfH) [39]. The amount of neurofilaments in blood and CSF rises after autoimmune attacks on the CNS or peripheral nervous system (PNS) [13]. The levels of both NfH and NfL chains increase at all stages of MS in the blood and CSF [40,41], but analyzing the NfL is much more effective than NfH in patients with various forms of MS [42]. The NfL might be a reflection of axonal damage caused by inflammatory processes, or it could be a prognostic factor for MS before its progression to clinically isolated syndrome (CIS) [43]. So it is likely to indicate neurodegeneration and predict future brain atrophy [44]. Measurement of NfL levels in serum can be a good marker for axonal damage [45,46], also its concentration in CSF increases 3 to 10 times after clinical recurrence, so that it reaches to peak in the first two weeks after the onset of clinical relapse symptoms [44]. This condition persists for 15 weeks after the onset of symptoms and returns to baseline after this period [47]. Serum concentration of NfL is 50 to 100 times lower than in CSF [48] however, due to the high similarity of CSF content with serum, serum NfL can be measured [49]. Another factor that increases in this disease is NfH, as the level of NfH is significantly related to the progression of the disease. High levels of NfH in more advanced patients indicate persistent NfH release, thus reflecting an irreversible neural process in these patients [50]. However, by measuring the amount of neurofilaments in the serum and CSF before the onset of clinical symptoms, the disease can be predicted to some extent.

EPSTEIN-BARR VIRUS

Many infectious agents are involved in MS as one of the most important of which is the EBV. The virus has a double-stranded DNA that encodes the latent membrane protein 1(LMP1) and LMP2, which mimics the T and B signals of cells [51]. Many people in the world are infected with EBV. The primary infection usually occurring in early childhood and is often asymptomatic, but when it occurs in adulthood it causes an acute fever known as infectious mononucleosis [52]. People who are clinically infected with mononucleosis are more than twice as likely to develop MS [53]. Due to the association between EBV and MS, people with MS have high levels of antibodies against EBV nuclear antigen1 (EBNA1) [54]. By binding to CD4+, this antigen stimulates the response of T cells in carriers of the virus [55-58]. Immune response factors against EBV in susceptible MS individuals entered the blood and lymph and react with myelin [59]. The EBV-infected B cells have a potential role in the pathogenesis of MS, passing the infected B cells through the BBB, it creates a pro-inflammatory environment. They activate the antibodies produced against EBV to myelin antigens [60,61]. The virus also causes immortalization of B cells, which leads to the production of autoimmune antibodies and antigen presenting to pathogenic T cells [62]. Infection with EBV will be effective in getting MS when it is accompanied by other factors that cause MS in susceptible individuals, including specific major-histocompatibility-complex (MHC) alleles, vitamin D deficiency, and smoking [63]. Since infection with EBV can increase the risk of MS by impairing immune function, it is better to test for EBV antibodies in susceptible individuals.

IFN-γ MEASUREMENT

Interferons (IFNs) are naturally produced by various cells such as leucocytes, fibroblasts, natural killer cells, and epithelial cells in response to bacteria, viruses, parasites, and tumor cells [64]. IFN-γ is the only member of type II IFN family. The IFN is a pleiotropic cytokine involved in innate and acquired immunity which effects on many biological activities, especially host defense and immune system regulation [65]. In low doses, IFN-γ has a protective effect against microglia and oligodendrocytes, while in high doses it has destructive manner. T cells entering the CNS in MS patients, produce and activate IFN-γ, which plays an important role in the pathogenesis of MS [66,67]. A study showed that IFN-γ exacerbates damage to myelin and oligodendrocytes by inflammation, activation of macrophages or microglia, increasing MHC molecules as well as inducing inflammation mediators [68]. This IFN is actually a key component in MS pathology, so that the amount of this factor in the serum and CSF of MS patients shows an increase compared to those under control. Therefore, measuring the IFN-γ level in serum and CSF can be useful in assessing the condition of the patients.

MIRNA ASSESSMENT

MiRNAs are single-stranded, small and non-coding RNA molecules that play an important role in gene expression and protein synthesis. [69]. In addition to intracellular activity, they are released into the extracellular space [70], so they can be assessed in different body fluids [71]. MiRNAs are important for the body immune system and play an important role in innate and acquired immunity system as well as in the development of lymphocytes [72,73].

Changes in the amount of miRNAs can play a role in causing pathological conditions and clinical disorders such as MS [74]. The levels of miR-155 and miR-132 in MS patients are increased in comparison with the healthy individuals [75]. These microRNAs are rapidly increased in inflammatory infections and produced by B and T lymphocytes [76]. Excessive expression of miR-155 has been linked to a variety of inflammatory diseases, such as MS, and through the activation of pathogenic immune cells has led to neurodegeneration, which contributes to MS pathogenesis [77]. By targeting CD47, miR-155 reduces their expression in brain cells and, by releasing macrophages, causes myelin phagocytosis [78]. MiR-132 value also increases in MS which is involved in the transmission of neurotransmitters in mammals and increases the production of lymphotoxin and TNF, which are proinflammatory factors [79]. High levels of miR-155 and miR-132 may be associated with disease progression. So perhaps by measuring the levels of miR-155 and miR-132, we can comment on the susceptibility to the disease and possibly control it. Therefore, it is better to examine miR-155 and miR-132 in suspected of MS.

CONCLUSION

To control and reduce the MS, it is better to measure the biomarkers such as IFN-γ, vitamin D, neurofilament, miRNA, melatonin, EBV antibody titer and HLA typing of high risk individuals and also prevent the recurrence of the disease.

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