



Study effect of IL-35 and some sex hormones in male patients who have multiple sclerosis (MS)

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Abstract

Multiple sclerosis (MS) is a disease characterized by inflammation and demyelination. Currently, the cause of MS is unknown. An early and accurate diagnosis of multiple sclerosis (MS) is very important, since it allows early treatment initiation, which reduces the activity of the disease. The present study aimed to focus a light on the contribution of interleukin-35 (IL-35) and its relation to some hormones such as testosterone, progesterone, and prolactin (PRL) in the pathogenesis of multiple sclerosis. Twenty two enrollers (samples) attended from Teaching hospital of Baghdad were divided into two groups. Ten enrollers were described as healthy subject as control group as group 1 (G1) in range ages (20-30) years. Twelve male patients as group 2 (2) have been relapsing remitting multiple sclerosis (RRMS) matched in age with group 1 (G1).

Keywords: Multiple Sclerosis; Interleukin-35; Testosterone; Progesterone; Prolactin.

1. Introduction

Multiple sclerosis (MS) is the most common demyelinating disease of the central nervous system (CNS) occurring at an incidence of approximately 2.5 million in worldwide. MS is characterized by acute, focal demyelination and dysfunction at central nervous system (CNS). Pathologically, the hallmark of MS is the plaque, occurring in the white matter (myelin) of the CNS (Francisco et al, 2013). The presence of CD4+ and CD8+ T lymphocytes, macrophages and activated microglia have been involved in MS brains (Codarri, et al, 2010), and it is considered that this inflammatory infiltrate contributes to the demyelination and axonal loss present in this disease (María et al, 2012). The set of causes of MS remains elusive; however, it is assumed that both a complex genetic background and environmental factors contribute to disease manifestations (Benjamin et al, 2012). While most of these factors are associated with the susceptibility to developing MS, more recent studies explained that some of these factors also impact on the MS disease course (Marcus et al, 2013). Regulatory T cells (Treg) use a wide range of mechanisms to inhibit immunity, including suppressive cytokine such as IL-10, IL-35, and Transforming growth factor beta (TGF- β) as shown in fig1 (Mattson et al, 1995). IL-35 belongs to IL-12 family of cytokines. IL-35 (composed of p35 and EB13) is featured from other family members in that it is produced by regulatory T cells and is suppressive. In vitro and in vivo, IL-35 has two known biological effects: suppression of the propagation of conventional T cells, and the conversion of naive T cells into a highly suppressive induced Treg cell population, called 'iTreg35' cells (Goodman et al, 2009). The immune, endocrine and nervous Systems communicate with each other through a myriad

of molecules, including cytokines, hormones and neurotransmitters. The steroid hormones' testosterone that is the victorious circulating androgen in the bloodstream and is produced by leydig cells in testicles, and plays an important role in normal development of male characteristics (Thualfeqar, 2010). These preventative effects are thought to be mediated by testosterone's immunomodulatory properties such as cutting off the production of pro-inflammatory cytokines, tumor necrosis factor- α (TNF α), and interleukin-1 β (IL-1 β) by macrophages (Agostino et al, 1999) and monocytes (Danis et al, 1993), as well as increasing production of the anti-inflammatory cytokine interleukin-10 (IL-10) by T cells (Liva et al, 2001). While in spite of progesterone and prolactin are smaller secretions in male than female, but they having light roles in multiple sclerosis respectively. Progesterone appears to cause a switch from a Th1 to Th2 immune responses (Whitacre et al, 1999). Progesterone has an effect that improves outcomes and decrease mortality following brain damages and may help those suffering from central nervous system dysfunctions and multiple sclerosis (Judy G, (2010). Prolactin (PRL) is polypeptide hormone with 190 amino acids with three intra-chain disulphide bridges that is synthesized and excrete from significant cells of the anterior pituitary gland (Thualfeqar, (2010). The cytokines IL-1, IL-2 and IL-6 stimulate prolactin secretion, while interferon- γ (INF γ) and endothelin-3 are inhibitory cytokines (Chikanza, 1999). Prolactin has a critical role in the innate and adaptive immune-response, by order the ripening of CD4- CD8- thymocytes to CD4+ CD8+ T cells via IL-2 receptor expression (Grattan et al, 2008).

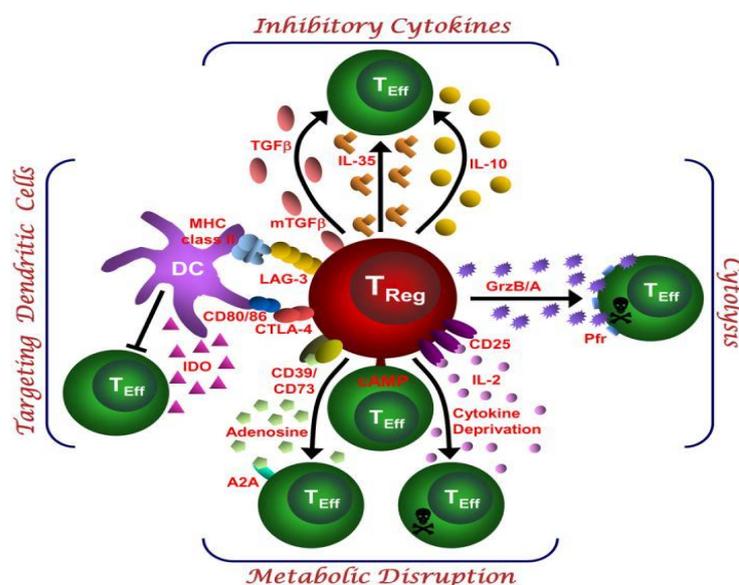


Fig. 1: Regulatory (Treg) T Cells in Immune System Responses.

2. Methods

The present study was performed on male patients suffering from disease symptoms about 2.5 years attending from teaching Baghdad hospital in MS unit during two months. They were diagnosed by physician at the hospital using Magnetic Resonance Imaging (MRI) examination. The samples were divided into two groups. Group 1 (G1) consist of ten enrollers considerate as a health group with range ages about (20-30) years. Group 2 (G2) that have multiple sclerosis numbered twelve enrollers matched in age with control group, which they have relapsing-remitting MS (RRMS) according to physicians diagnosis. Five ml blood were drawn from all subjects enrolled in this study, and kept in plain tubes left to clot at room temperature for 15 min separate the serum and stored at -20°C until used to 3500 rpm for 10 min to estimate IL-35 and sex hormones. Interleukin 35(IL-35) has been estimated by using enzyme-Linked Immuno Sorbent Assay (ELISA) technique using the manufacturer instruction as supplied with kit from Cusabio, China, testosterone determination by kits supplied from Accu binds, USA, progesterone, and prolactin supplied from Human, Germany's determinations.

3. Statistical analysis

Student's t-test was applied to compare the significance of the difference in the mean values of any two groups, and ($p < 0.05$) was considered statistically significant, while ($p < 0.001$) was con-

sidered highly significant. The correlation coefficient (r) test was used to describe the association between the different studied parameters. Standard Error of Mean (SEM) is the standard deviation of the sample mean estimate of a population mean. It is usually calculated by the sample estimate of the population standard deviation.

4. Results

Table (1) shown the level of interleukin (IL-35), progesterone, testosterone, and prolactin in sera of group 2 (G2) compared to group 1 (G1). The interleukin-35 (IL-35), and progesterone levels were showed a high significant increase ($p < 0.001$) in sera of group 2 (G2) (35.5 ± 3.22) (0.76 ± 0.28) respectively compared to group 1 (G1) (23.8 ± 3.99) (0.40 ± 0.21) respectively. Testosterone level was shown significant increase ($p < 0.05$) in sera of group 2 (G2) (6.03 ± 2.97). Prolactin level was shown non-significant in sera of group 2 (G2) (7.5 ± 2.99) ($P > 0.05$) compared to group 1 (G1) (8 ± 2.83). Table (2) shown negative (-ve) correlation in IL-35, testosterone, and progesterone and duration of disease ($r = -0.172$, -0.186 , -0.160 respectively). While table shown positive (+ve) correlation in prolactin ($r = 0.116$) with duration of disease.

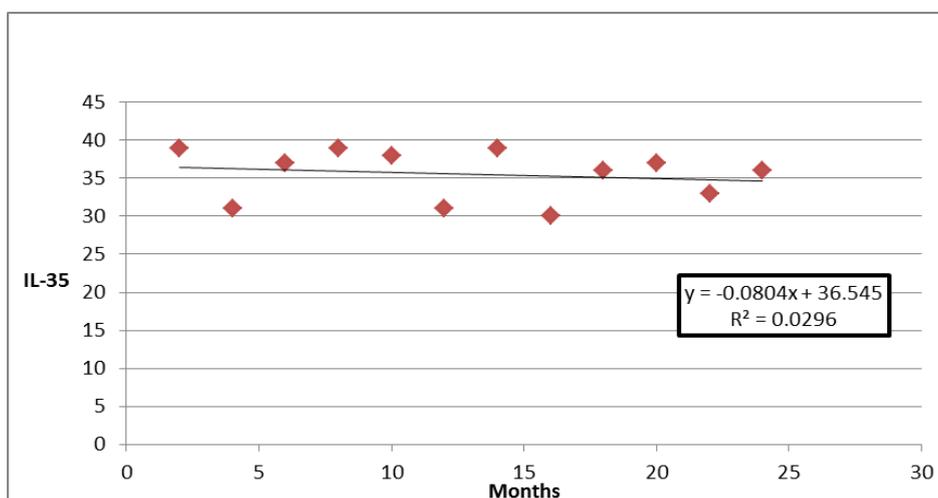


Fig. 2: Correlation between IL-35 with Duration of Disease

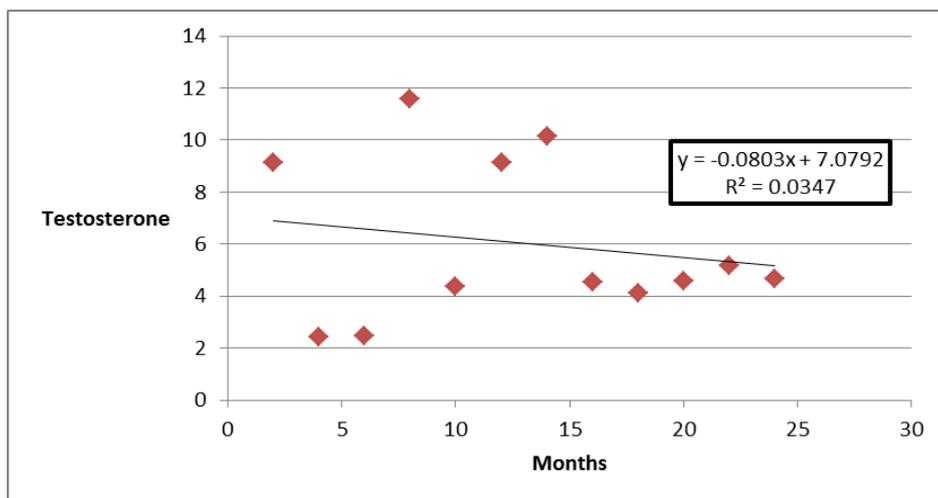


Fig. 3: Correlation between Testosterone with Duration of Disease

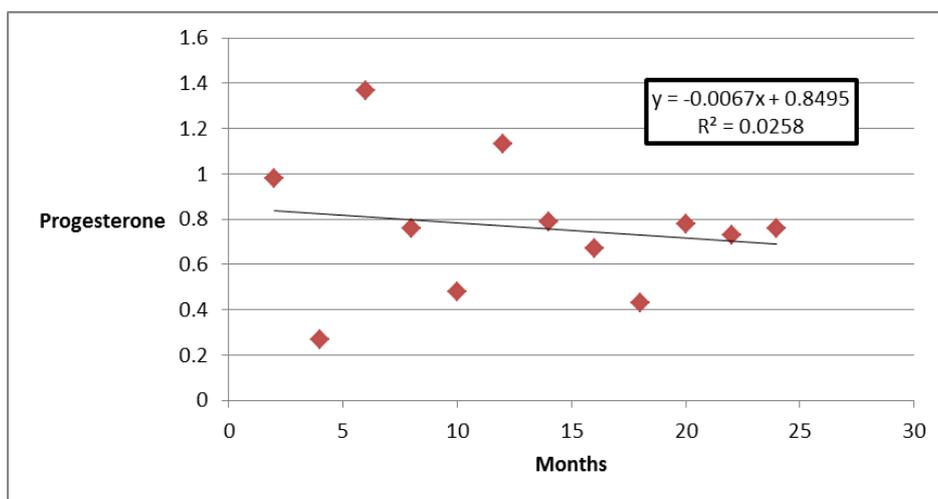


Fig. 4: Correlation between Progesterone with Duration of Disease

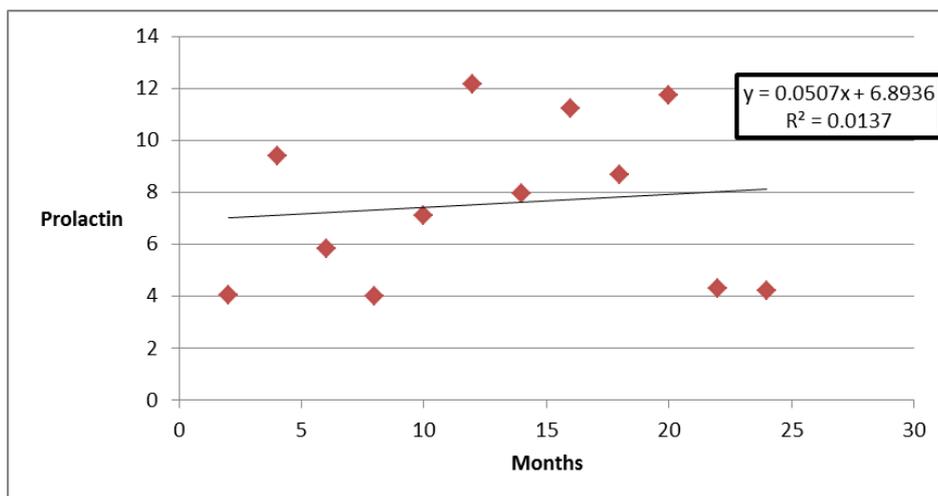


Fig. 5: Correlation between Prolactin with Duration of Disease

5. Discussion

Interleukin-35 (IL-35) is not substantially expressed in tissues (Li X et al, 2012), and is made primarily by Treg cells (Chaturvedi et al, 2011). The gene encoding IL-35 is also transcribed by vascular endothelial cells, smooth muscle cells and monocytes after activation with pro-inflammatory cytokines and lipopolysaccharide (Li X et al, 2012). IL-35 induces the transformation of CD4⁺ effector T cells into Treg cells that in turn express IL-35 but lack expression of Foxp3, TGF- β and IL-10 (iTreg35 cells) (Lauren et al, 2010). Studies have also shown that testosterone can reduce the in

vitro production of inflammatory cytokines such as TNF α and IL-1 β by human macrophages. Mechanisms of action of testosterone involve both immunomodulatory and neuroprotective pathways thus suggesting that sex hormones represent novel treatment options that could help affect the inflammatory as well as the dysfunction of CNS component of the MS disease (Stefan et al, 2009). Progesterone has been observed to have both neuroprotective and pro-myelinating effects on CNS. It preserves neurons from following brain blighter or vascular harm (Stein, 2008). In the spinal cord, progesterone raises motoneuron survival after injury, protects cultured neurons against glutamate toxicity and nor-

malizes functional weakness of harmed neurons. Progesterone also influences myelin synthesis both in the Peripheral and Central Nervous System. It increases the propagation and differentiation of oligodendrocyte precursor cells that play a major role in remyelination after toxin-secreted lesions and aging. In addition to neuronal and myelinating effects, progesterone may modify the immune system, shifting a Th1 pro-inflammatory response to a Th2 anti-inflammatory response (Tomassini et al, 2009). Finally, prolactin is a neuroendocrine peptide with strongly immunomodulatory characteristics. It can raise the expression of costimulatory molecules or cytokines secretion from T, B, NK, and dendritic cells. Hyperprolactinemia was found in 30% of MS patients and was consider to be related to hypothalamic lesions (Shahar et al, 2011).

6. Conclusion

Interleukin-35, testosterone, and progesterone which considerate immune-modulator by suppression pro-inflammatory cytokines. Stimulus anti-inflammatory cytokines, therefore, possible moderate of severity of MS, hence diminish in concentrations in conjunction with doses effect of β - interferon drug of MS, which has also immune-modulatory properties. Prolactin was observed rise in RRMS patients for specific period before in return fall level because abundant use of drug in passes of days

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