Comparative Biochemical Study of Interleukin -35 and Some Sex Hormones in MS Female Patients with Duration of the Disease*

Ali K. Baden
Bushra H. Ali
drbushra750@yahoo.com
Dept. of Chemistry/College of Education for Pure Science(Ibn Al-Haitham) / University of Baghdad

Received in : 9 September 2013 , Accepted in :24 September 2013

Abstract

Multiple sclerosis (MS) is a chronic disorder of the central nervous system (CNS) characterized by autoimmune inflammation, demyelination, and axonal damage. The present study aimed to shed a light on the contribution of interlukin-35 and its relation to some sex hormones in the pathogenesis of multiple sclerosis. Sixty six female patients with age range (20-40) years were taken from Baghdad Teaching Hospital through the period from Nov. 2012 to –April 2013 and 20 apparently healthy subject as control group matched age as group G1. The patients were divided into three groups depending on duration of MS diseases G2 from 3 months to 2 years, G3 from 2 years to 4 years, G4 from 4 years to 10 years and more. Investigations included estimation of serum levels of Interlukin-35 (IL-35), Testosterone (TEST), Progesterone (PROG), follicle stimulating hormone (FSH), luteinizing hormone (LH) and Prolactin (PRL). Serum IL-35 levels were significantly higher in MS patients as compared with control subject, also significant increase appeared in TEST levels in G3 compared to control in for MS female patients. No significant differences were found between PROG and FSH with duration also non-significant difference levels G2 compared to G1 in LH, on the other hand a significant increase levels for LH in G3 and G4 compared to control subject , a significant difference in prolactin levels for G2 and G4, but non-significant difference for G3. From this study a conclusion was drawn, that evaluation of concentration of a new super family cytokines IL-35 can be considered as a clinical biomarker in multiple sclerosis female patients. This finding may indicate that MS might influence cytokine e.g. interleukin-35 production in these patients.

Keywords : Interleukin-35- Multiple Sclerosis- Steroid hormones

*This paper is part of MSc thesis of the first author

Introduction

Multiple sclerosis (MS) is a chronic disorder of the central nervous system (CNS) characterized by autoimmune inflammation, demyelination, and axonal damage [1,2]. MS, also known as " disseminated sclerosis " or "encephalomyelitis disseminata", is an inflammatory disease in which the fatty myelin sheaths around the axons of the brain and spinal cord are damaged, leading to demyelination and scarring as well as broad of signs and symptoms [3] . The etiology of MS remains elusive; however, it is assumed that both a complex genetic background and environmental factors contribute to disease manifestation [4]. The clinical course of MS classified into Relapsing-remitting MS (RR-MS), Secondary Progressive MS (SP-MS), Primary Progressive MS (PP-MS) and Progressive-relapsing MS (PR-MS) [5]. Regulatory treg cells use a broad range of mechanisms to suppress immunity, including suppressive cytokine such as IL-10, IL-35, and Transforming growth factor beta (TGF-β) [6]. IL-35 belongs to IL-12 family of cytokines. IL-35 (composed of p35 and EBI3) is distinct from other family members in that it is produced by Treg cell populations and is suppressive. *In vitro* and *in vivo*, IL-35 has two known biological effects: suppression of the proliferation of conventional T cells, and the conversion of naïve T cells into a strongly suppressive induced Treg cell population, called 'iTr35' cells; which function in via IL-35 [7]. Steroid hormones are crucial substances that mediate a wide range of vital physiological functions of the body these protective effects are thought to be mediated by testosterone's immunomodulatory properties such as decreasing the production of pro-inflammatory cytokines TNFα and IL-1β by macrophages [8] and monocytes [9] as well as increasing production of the anti-inflammatory cytokine IL-10 by T cells [10]. Several *in vitro* studies have shown that testosterone can also be more directly neuroprotective [11]. It is likely that the immunomodulatory, anti-inflammatory and neuroprotective actions of testosterone contribute to this effect [12]. Prolactin (PRL) has an important role in the innate and adaptive immune-response, by regulating the maturation of CD4- CD8- thymocytes toCD4+ CD8+ T cells via IL-2 receptor expression [13]. There is a correlation between PRL level and the number of B and CD4+ T lymphocytes [14]. PRL changes Th1 and Th2 type cytokine production. PRL up-regulates IL-6 and INFγ production and has an immune regulatory role on IL-2level [15–17]. In addition, Hyperprolactinemia (HPRL) affects dendritic cell (DC) maturation, skewing DC function from antigen presentation to pro-inflammatory phenotype with increased interferon α production [18].

(HPRL) have been described in many autoimmune diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis(RA), the organ specific autoimmune-diseases associated with HPRL including multiple sclerosis (MS) [19].

Aim of the study

The present study aimed to shed a light on the contribution of interleukin-35 and its relation to some sex hormones in the pathogenesis of multiple sclerosis.

Methods

The present study was performed on a group of 66 female patients attending from Baghdad Teaching Hospital in MS unit during Nov.2012 to April 2013 .They were diagnosed by physician at the hospital using Magnetic Resonance Imaging (MRI) examination .The patients were divided into three groups depending on duration of MS diseases (G2) from 3monthes to 2years ,(G3)from 2yers two four years, (G4) from 4years to 10 years and more . In addition, to group (G1) of 20 healthy were enrolled in the study as a control group.

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 which separated the serum and stored at -20 $^{\circ}$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, TEST determination by kits supplied from Accu Bind, USA, PROG, FSH, LH and PRL supplied from Human, Germany determinations.

Statistical analysis

Results were expressed as Mean± SEM. Student-test was used to show the difference between groups variation was considered significant when P-values are ≤ 0.05. The correlation coefficient (r) test is used to describe the association between the different studied parameters.

Results

Table (1) shows the levels of IL-35,TEST, PROG,FSH,LH and PRL concentration in sera of G1,G2,G3 and G4 for control and patients respectively. The interleukin-35 (IL-35) levels showed a high significant increase ($p<0.001$) in sera of group 2 (G2) (34.7± 1) pg/ml, group 3 (G3) (35±0.66) pg/ml compared to group 1 (G1) (23.6±0.82) pg/ml, while there was a highly significant decrease ($p<0.001$) for group 4 (G4) (35.5±0.83) pg/ml compared to group 1 (G1) (23.6±0.82) pg/ml, while a non significant difference ($p>0.05$) was noticed among the patient groups. Testosterone showed significant increase ($p<0.05$) in (G3) (2.9±0.38) ng/ml compared to (G1) (0.56±0.04) ng/ml, while it showed no significant difference ($p>0.05$) in (G4) (1.2±0.34) ng/ml and (G3) (2.9±0.38) ng/ml compared to (G2) (1.6±0.59) ng/ml, also there was a non significant difference ($p>0.05$) in (G4) (1.2±0.34) ng/ml and (G2) (1.6±0.59) compared to (G1) (0.56±0.04) ng/ml. Progesterone level showed highly significant decrease ($p<0.001$) for (G3) **(**5.3±1.2) ng/ml compared to (G1) (0.6±0.08) ng/ml, also there was a significant increase ($p<0.05$) in (G2) (9.5±2.47) ng/ml compared to (G1) (0.6±0.08) ng/ml, while there was a non significant difference ($p>0.05$) in (G4) (4.1±1.68) ng/ml compared to (G1) (0.6±0.08) ng/ml, also there was a non significant difference ($p>0.05$) in (G4) (4.1±1.68) ng/ml and for (G3) **(**5.3±1.2) ng/ml compared to in (G2) (9.5±2.47) ng/ml. Results of FSH showed a no significant difference in (G4) (6.2±0.76) IU/l, (G3) (4.7±0.7) IU/l, and (G2) (12.08±4) IU/l compared to group 1 (G1) (5±0.35) IU/l, also in (G4) (6.2±0.76) IU/l, (G3) (4.7±0.7) IU/l compared to (G2) (12.08±4) IU/l. While Luteinizing hormone levels showed highly significant increase ($p<0.001$) in group 3 (G3) (2.6±0.4) IU/l compared to (G1) (11.7±1.31) IU/l, on other hand there was highly significant decrease (G4) (2.9±0.42) IU/l compared to (G1) (11.7±1.31) IU/l, while there was a significant decrease ($p<0.05$) in (G3) (2.6±0.4) IU/l, and (G4) (2.9±0.42) IU/l compared to (G2) (7±1.87) IU/l, but there was a non significant difference ($p>0.05$) in (G2) (7±1.87) IU/l compared to (G1) (11.7±1.31) IU/l. Prolactin levels showed significant increase ($p<0.05$) in (G4) (20.5±4.41) ng/ml compared to (G1) (8.2±0.67) ng/ml, while there was a non significant difference ($p>0.05$) for (G3) (9.1±1.44)ng/ml and (G2) (13.3±2.62) ng/ml compared to (G1) (8.2±0.67) ng/ml, also there was a non significant difference ($p>0.05$) in (G4) (20.5±4.41) ng/ml and (G3) (9.1±1.44)ng/ml compared to (G2) (13.3±2.62) ng/ml. Table (2) shows positive correlation for IL-35,PROG,PRL and duration of diseases (r=0.139,0.162 ,0.327 respectively) as well as the positive correlation in G3 for LH (r= 0.021) ,G4 for TEST,FSH,LH and PRL(r=0.046,0.265,0.0841 and 0.026) as shown in figures (1,2,3,4,5) respectively, while there was negative (-ve) correlation in G3,G4 for IL-35 (r=-0.142, -0.178) ,G2,G3 for TEST(r=-0.17,r=-0.134),G3,G4,for PROG(r=-0.112,-0.22),G2,G3 for FSHR(r=-0.404,-0.118), G2 for LH(r=-0.283) , G3for PRL(r=-0.092) & duration.

Discussion

The results in the present study showed that the serum level of IL – 35 was significantly higher in patients with MS than in healthy control. But other study suggested that MS is a debilitating neuro inflammatory disease that occurs when auto reactive T cells gain entry into the central nervous system (CNS) and destroy myelin-producing oligodendrocytes. T cell-derived cytokines, including Th1, Th17, IL-17 and IFN-γ, are primarily responsible for the disease symptoms that occur when the myelin sheath that insulates the neurons is damaged during MS [20]. Inflammatory dendritic cells (DCs) and macrophages also contribute to disease induction and progression by activating these auto reactive T cells and secreting inflammatory cytokines in the CNS. Recent studies have identified that chemical mediators, interleukin-23 (IL-23) and granulocyte macrophage colony-stimulating factor (GM-CSF), contribute to the autoimmune characteristics of these T cells. Data suggests that absence of these pro-inflammatory signals was sufficient to prevent inflammation in the brain. This suggests that therapeutic strategies directed at blocking the production of inflammatory mediators could be effective for treating MS [21]. Other study provides that IL-35 can be used to induce the conversion of conventional CD4+ T-cells into induced regulatory T-cells that have potent suppressive capacity *in vitro* and *in vivo* [22]. These cells have been designated iTr35 cells by the inventors. This approach can be used to generate regulatory T-cells to treat autoimmune and inflammatory diseases, such as (but not limited to) type 1 diabetes, MS, arthritis, asthma and allergic diseases, IBD, etc.

The autoimmune diseases involve both genetic and environmental factors and the autoimmune process is characterized by the breakdown of tolerance in subjects with genetic susceptibility, with subsequent target (organ-specific and not) injury, that, in turn, elicits repairing mechanisms. Increasing evidence in the literature suggests an important role of the microenvironment on the immune system activity and of sex steroids in this context. Sex steroids may act at the multiple steps of the autoimmune process with different (and contrasting) effects, depending on the type of the steroid (natural or synthetic), the concentration and co-presence of the ligands and the binding to their specific receptors. Regarding the effectors of the immune system, different actions of sex steroids depend on the type of immunocytes involved (with final stimulatory or inhibitory actions). To complete the scenario, sex steroids may influence the whole process through multiple, contrasting and time-dependent pathways, for instance, modulating the autoimmune destroying process and/or stimulating the reparation. Increasing evidence in the literature indicates an immunomodulatory role of sex steroids in the pathogenesis of autoimmune diseases. The results of the clinical trials will give the basis in order to better define the use of sex steroids in combination with current therapeutic drugs in autoimmunity [23]. Sex steroid receptor modulating drugs are a promising class of therapeutic agents that will provide new approaches in these pathologies. As far as to our knowledge, this is the first in the field at IL-35 and its relation to steroid hormones in MS patients.

References

1. Rossalind , C. and Kalb,R.L. (2012) Multiple sclerosis: The questions you have the Answers you need, 5th Ed. Bradford& Bigelow.
2. Manual, C.; and Samia, J.K. (2012) Immunopathgenesis of multiple sclerosis. J. Clinically immunology. 142(1):8-12.
3. Compston, A. and Coles, A. (2008) Multiple sclerosis, J, Lancet. 372 (9648): 1502–17.
4. Benjamen, W.; Richard, N. and Omer, M. (2012) Multiple Sclerosis. J. Med. 40(10):523-528.
5. Confavreux, C. and Vukusic, S. (2006) Natural history of multiple sclerosis: a unifying concept. J. Brain, 129 (pt 3): 606-16.

6. Mattson,D.; Petrie, M .and Srivastava, D.K. (1995) Multiple Sclerosis: Sexual dysfunction and its responses to medications. J. Arch Neurol. 52(9):862-868.
7. Goodman, A.D.; Brown, T.R.; Krupp, L.B. (2009) Sustained-release oral fampridine in multiple sclerosis: a randomized, double-blind, controlled trial. J. Lancet. 373: 732-738.
8. Neema, M.; Stankiewicz, J. and Arora, A. (2007). MRI In multiple sclerosis: what inside the toolbox?, J. Neurotherapeutics. 4:602-617.
9. Bruck, W.; Bitsch, A, and Kolenda, H. (1997) Inflammatory central nervous system demyelination: correlation of MRI findings with lesion pathology. J. Ann. Neurol. 42:783-793.
10. Fisher, E.; Chang, A. and Fox, R.J. (2007). Imaging correlates of axonal swelling in chronic multiple sclerosis brains. J. Ann Neurol. 62:219-228.
11. Van, W.; Lycklama, A .and Jongen P.J. (2001) Hypointense lesions on T1-weighted spin-echo magnetic resonance imaging: relation to clinical characteristics in subgroups with multiple sclerosis. J. Arch. Neurol. 58:76-81.
12. Ulf, Z.; Mathias ,W. and Elke, H. (2012) Development of biomarkers for multiple sclerosis as a neurodegenerative disorder. J. Progress in Neurology Sci. 95:670-685.
13. Bettelli, E.; Carrier, Y.; Gao,W.; Korn, T.and Strom, TB. (2006) Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. J. Nature. 441(7090):235-8.
14. Siffrin, V.; Radbruch, H.; Glumm, R.; Niesner, R.; Paterka, M. and Herz, J. (2010) In vivo imagingof partially reversible th17 cell-induced neuronal dysfunction in the course of encephalomyelitis. J. Immunity. 33(3):424-3.
15. Mosmann, T.R.; Cherwinski, H. and Bond, M.W. (1986) Two types of murine helper T cell clone: I. Definition according to profiles of lymphokine activities and secreted proteins. J. Immunol.136(1):2348–2357.
16. Johnson, A.J.; Suidan, G.L. and McDole, J. (2007) The CD8 T cell inmultiple sclerosis: suppressor cell or mediator of neuropathology, J. Int Rev Neurobiol. 79 :73–97.
17. Skulina, C.; Schmidt, S.; Dornmair, K. and Babbe, H, (2004) Multiple sclerosis: brain-infiltrating CD8+ T cells persist as clonalexpansions in the cerebrospinal fluid and blood. J. Proc Natl Acad Sci. 101:2428–2433.
18. Stromnes, I.M.; Cerretti, L.M.and Liggit D.(2008) Differential Regulation of central nervous system autoimmunity by Th1 and T(H17) cells. J. Nat Med. 14(3): 337-42.
19. Brennan, F. and Feldmann, M. (2000) Cytokines networks. In: Balkwill F (ed.), the cytokine network, Oxford University Press, New York.pp 49-70.
20. Howard, L.W. and James, M.S. (2012) Multiple Sclerosis Diagnosis and Therapy A John Wiley & Sons, Ltd.pp 18-21.
21. Jock, M.; Carlos S.S. and Nancy .H.(2013) A Guide for the newly diagnosed of Multiple Sclerosis, 4th Ed. Domes Medical Publishing, LLC. 15-6.
22. Zhao, N.W. (2010) Interleukin (IL)-35 is raising our expectations. J. Rev Med Chile. 138: 758-766.
23. Martocchia, A.; Stefanelli, M.; Cola S. and Falaschi, P. (2011) Sex Steroids in Autoimmune Diseases. J. Curr Top Med Chem. 11: 1668-83.

Table (1): Levels of IL-35, Testosterone, Progesterone, Prolactin, FSH, and LH in sera of the four studied groups.

	Studied Groups								
Parameter	**Control (G1) mean±SEM**	**MS (G2) mean±SEM**	**MS (G3) mean±SEM**	**MS (G4) mean±SEM**	**G2 vs G1**	**G3 vs G1**	**G4 vs G1**	**G3 vs G2**	**G4 vs G2**
IL-35Pg/ml	23.6±0.82	34.7±1	35±0.66	35.5±0.83	HS	HS	HS	NS	NS
TEST ng/ml	0.56±0.04	1.6±0.59	2.9±0.38	1.2±0.34	NS	S	NS	NS	NS
PROGng/ml	0.6±0.08	9.5±2.47	5.3±1.2	4.1±1.68	S	HS	NS	NS	NS
FSH IU/l	5±0.35	12.08±4	4.7±0.7	6.2±0.76	NS	NS	NS	NS	NS
LH IU/l	11.7±1.31	7±1.87	2.6±0.4	2.9±0.42	NS	HS	HS	S	S
PRL ng/ml	8.2±0.67	13.3±2.62	9.1±1.44	20.5±4.41	NS	NS	S	NS	NS

P values< 0.05 considered significant (S)
P values< 0.001 considered highly significant (HS)
P values>0.05 considered non- significant (NS)

Table (2): Correlation analysis between biochemical parameters among three studied groups.

		Group2 (G2) MS	**Group 3 (G3) MS**	**Group 4 (G4) MS**
IL-35&YEARS	**r**	0.139	-0.142	-0.178
	p	P<0.05	P<0.05	P<0.05
TEST& YEARS	**r**	-0.170	-0.134	0.046
	p	P<0.05	P<0.05	P<0.05
PROG& YEARS	**r**	0.162	-0.112	-0.220
	p	P>0.05	P>0.05	P>0.05
FSH& YEARS	**r**	-0.404	-0.118	0.265
	p	p>0.05	p>0.05	p>0.05
LH& YEARS	**r**	-0.283	0.021	0.0841
	p	p>0.05	P<0.05	P<0.05
PRL& YEARS	**r**	0.327	-0.092	0.0260
	p	P<0.05	p>0.0	P<0.05

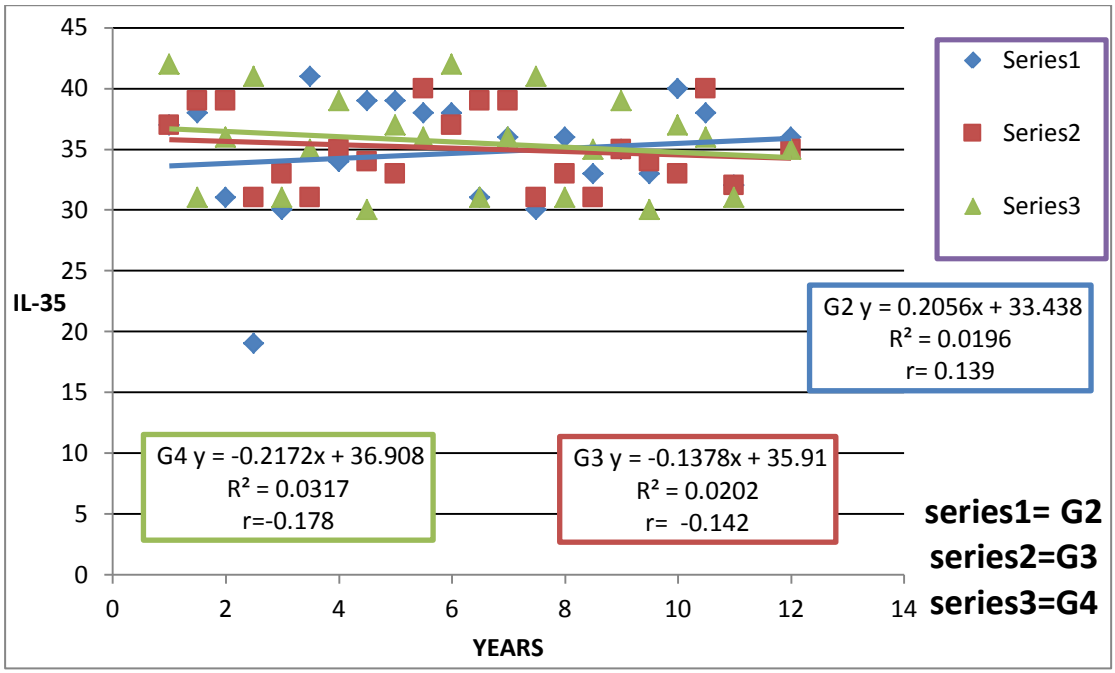

Fig. (1): Correlation between IL-35 and duration of disease in years

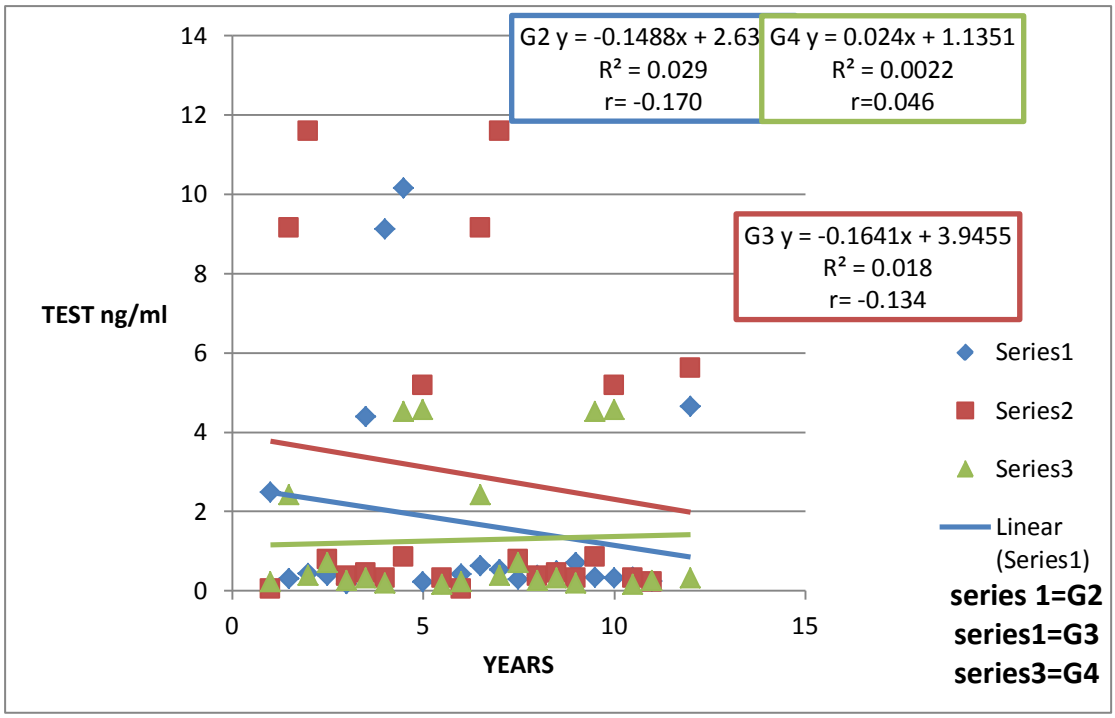

Fig. (2): Correlation between Testosterone and duration in years.

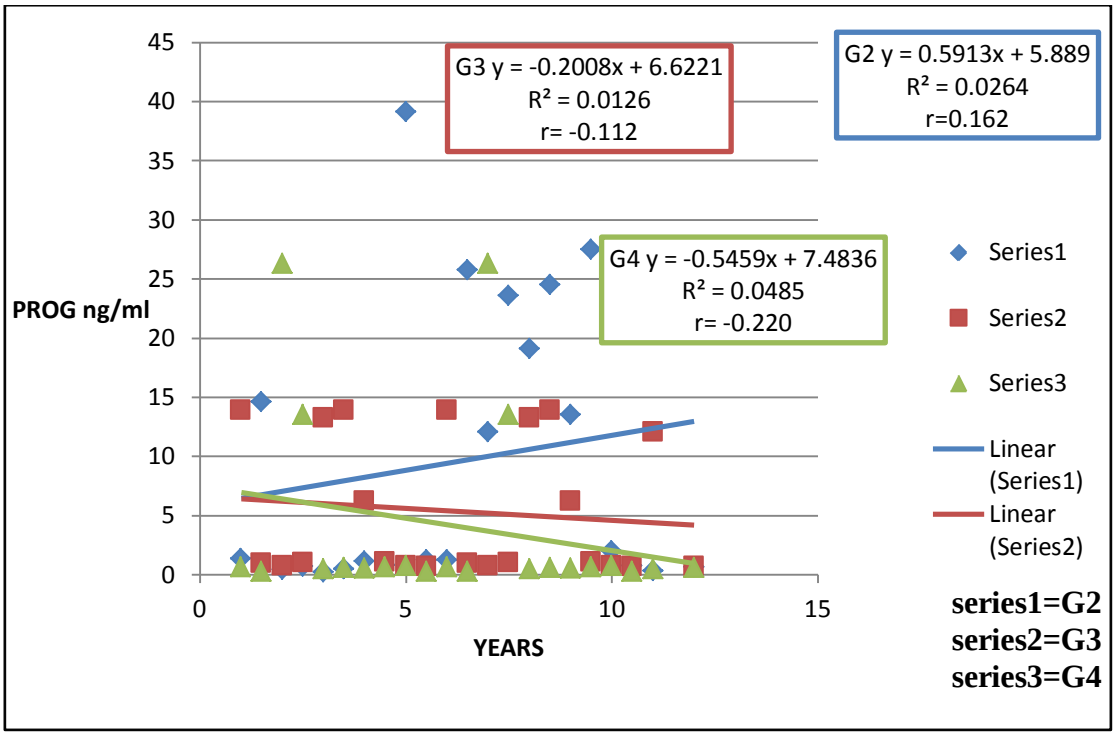

Fig.(3):Correlation berween Progesterone and duration of diseases in years

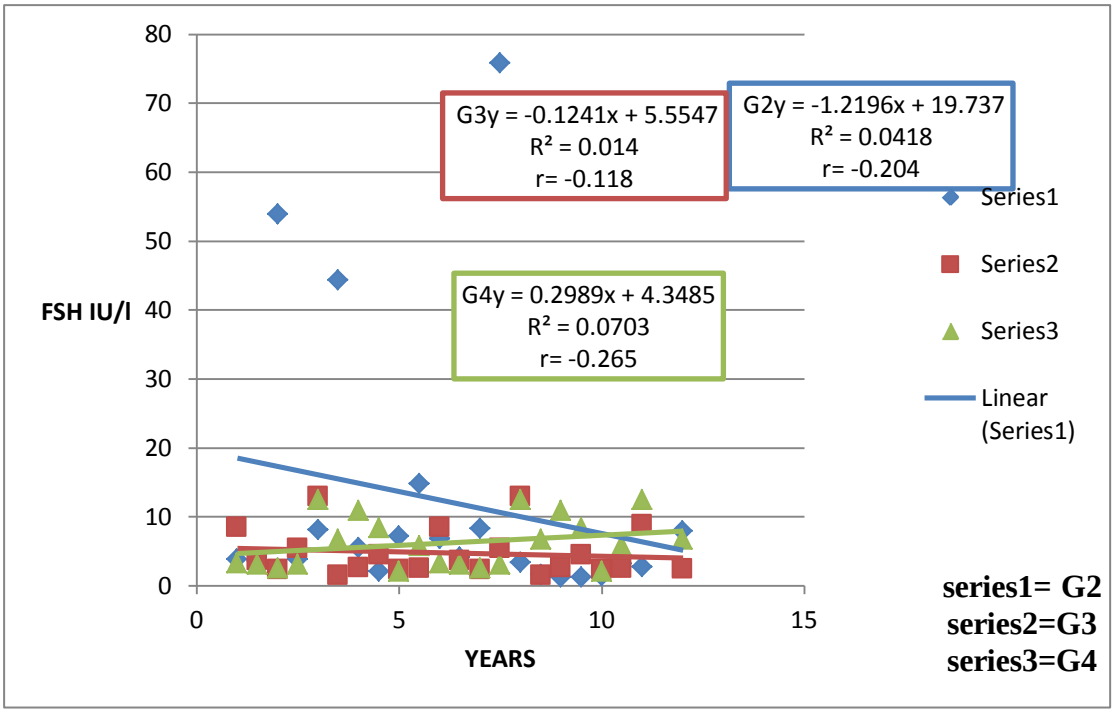

Fig .(4): Correlation between FSH and duration of diseases in years.

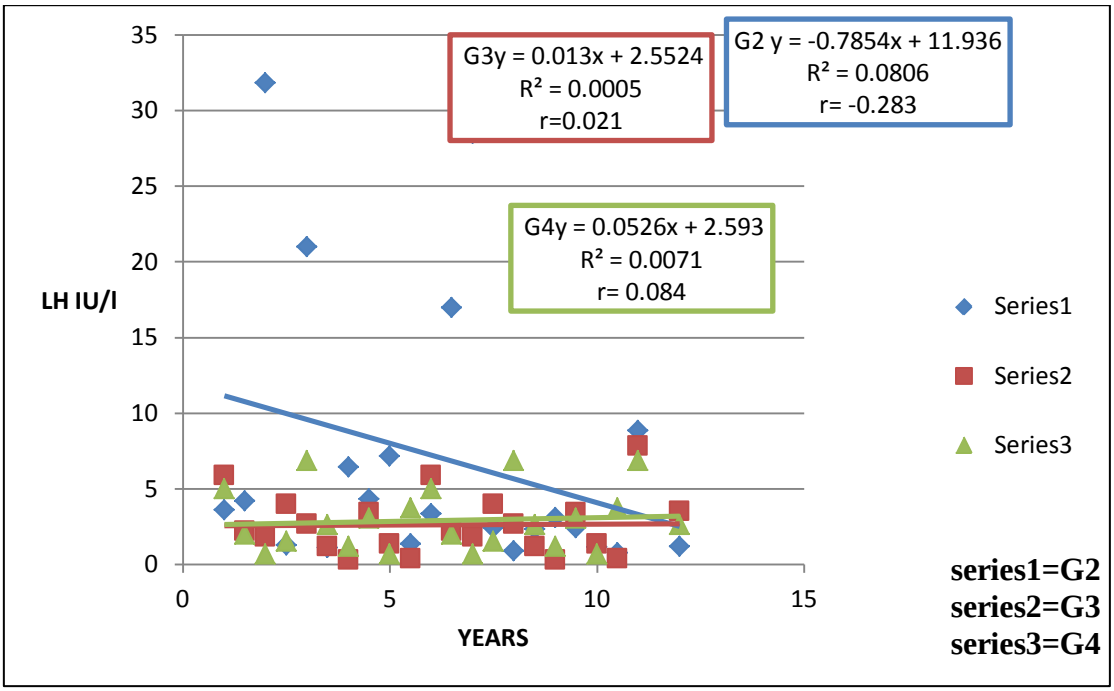

Fig.(5): Correlation between LH and duration of diseases in years.

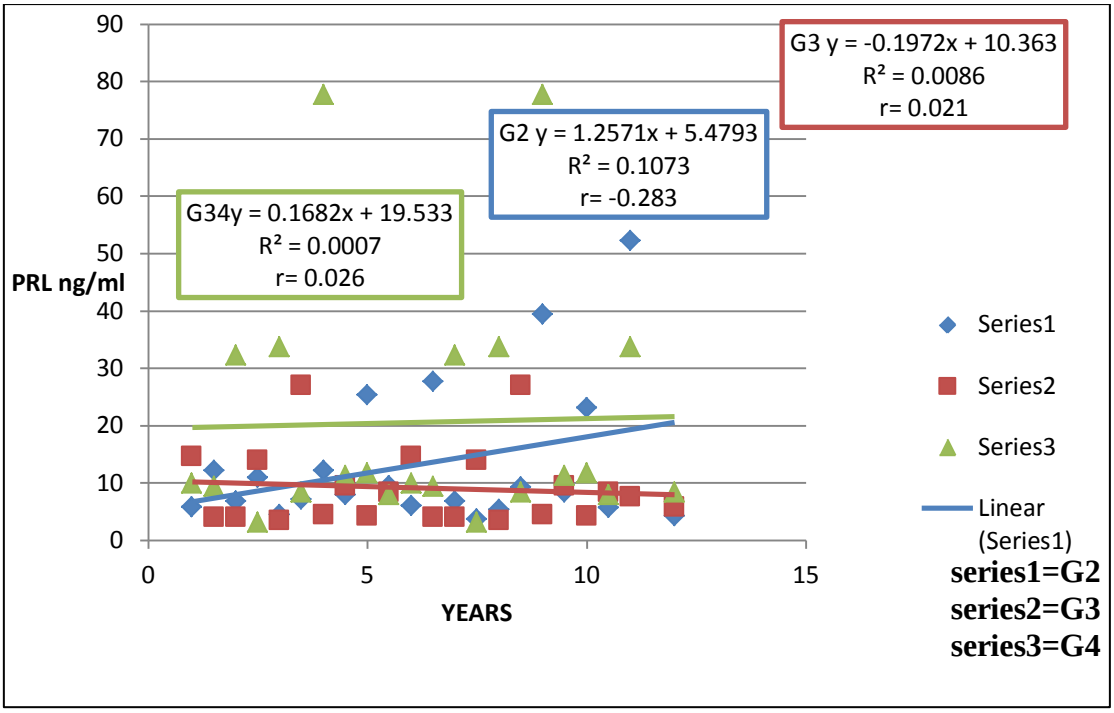

Fig .(6):Correlation berween PRL and duration of diseases in years.

دراسة كيمو حيوية مقارنة للا نترليوكين - 35 وبعض الهرمونات الجنسية عند مريضات التهاب الاعصاب المتعدد مع مدة من المرض

علي كاظم بدن
بشرى حميد علي
قسم الكيمياء/ كلية التربية للعلوم الصرفة (ابن الهيثم) / جامعة بغداد

استلم البحث في : 9 أيلول 2013 ، قبل البحث في : 24 أيلول 2013

الخلاصة

تصلب الاعصاب المتعدد هو اضطراب في الجهاز العصبي المركزي ويوصف بانه احد امراض المناعة الذاتية الالتهابية وفقدان المايلين وتحطم المحاور .هذه الدراسة تهدف الى تسليط الضوء على مساهمة الانترليوكين -35 وعلاقته مع بعض الهرمونات الجنسية الاساسية في نشوء مرض تصلب الاعصاب المتعدد . أجريت التجربة على (66) مريضة بمعدل عمر يتراوح بين (20-40) سنة من مستشفى بغداد التلعيمي للمدة من نوفمبر 2012 ألى أبريل 2013 وكذالك مجموعة السيطرة بالفئة العمرية نفسها وعدت مجموعة أولى. قسمت الحالات المرضية على ثلاث مجاميع بالاعتماد على مدة الاصابة بمرض التصلب العصبي المتعدد ، حيث أعتبرت المدة من 3أشهر إلى سنتين مجموعة ثانية، والمدة من سنتين إلى 4 سنوات مجموعة ثالثة، والمدة من 4 سنوات إلى 10 سنوات أو اكثر مجموعة رابعة. تضمن التشخيص تقدير مستوى ألانترليوكين-35 و التستوستيرون، وهرمون البروجيستيرون ، والهرمون المحفز للجريبات ، و هرمون LH فضلا عن هرمون البرولاكتين. وأتضح وجود أرتفاع جوهري بمستوى الانترليوكين-35 عند مريضات التصلب العصبي التعدد مقارنة بالاصحاء و هذه الحقائق وجدت في مستوى ألانترليوكين-35 و التستوستيرون عند مريضات التصلب العصبي المتعدد. لوحظ عدم وجود اختلاف جوهري بين هرمون البروجيستيرون والهرمون المحفز للجريبات خلال مدة المرض وكذالك لم يلاحظ أختلاف جوهري لهرمون LH خلال المدة المرضية للمجموعة الثانية، فضلا عن وجد فرق جوهري في مستوى هرمون الاتينزنك في المجموعتين الثالثة والرابعة خلال مدة المرض ،أما هرمون البرولاكتين فوجد أختلاف مهم بالنسبة الى المجموعتين الثانية و الرابعة على عكس المجموعة الثالثة التي لم يوجد بها فرق جوهري. من هذه الدراسة التي أجريت نستنتج بأن أرتفاع مستوى تركيز ألانترليوكين-35 يمكن أن يعد دالة كيموحيوية للمصابات بمرض التصلب العصبي المتعدد. وتبين هذه النتيجة أن مرض التصلب العصبي المتعدد يتأثر بالسايتوكين مثل ألانترليوكين-35 الموجود عند تلك المريضات.

الكلمات المفتاحية: أنترليوكين-35، التصلب العصبي المتعدد، الهرمونات الستيرويدية.