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Effect of Hyperprolactinaemia on concentration of immunoglobulins and complement components in a series of infertile Iraqi women

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Abstract

The present study was designed to detection the imunoglobulins concentration and complement compenent effects by hyperprolactinaemia The sample included ٦٠ patients, who were attending the Institute for Embryo Research and Infertility Treatment for diagnosis and treatment In Bagdad city during the period 200٧-April -to -200٧-Septemper .

primary infertile females patients with hyperprolactinaemia (age range: 20 – 40 years) were investigated.The collected serum was tested for the determination of prolactin levels using a miniVidas system. (serum level of prolactin ≥٢٠ ng/ml), the total patients were divided into three groups; I (٢١patients), II (٢٤patients) and III (١٥patients), Their serum prolacin levels were

22-29, 30-39 and ≥ 40 ng/ml, respectively.

(single radial immunodiffusion test) , used to Quantitative Measurement of Immunoglobulins (IgA, IgG and IgM) and Complement components C3 and C4 which was developed by(Mancini *et al*. 1965**).**

The immunoglobulins (IgA, IgG and IgM) showed a significant increased total level in the sera of total patients when compared with controls, (265.5 *vs*. 225.3, 1423 *vs*. 1076 and 266 *vs*. 188 mg/dl, respectively..

With respect to complement components, the serum levels of C3 and C4 were slightly decreased in the patients, but the difference did not reach a statistical level .

Introduction:

Hyperprolactinaemia is the most common endocrine disorder of the hypothalamic-pituitary axis. Although the clinical syndrome resulting from hyperprolactinaemia has been recognized in women since ancient times, the biochemical condition is a relatively new disorder as human prolactin was only purified and verified to be distinct from human growth hormone in 1971 (Delitala, 1998). Pathological hyperprolactinaemia is defined as a consistently elevated serum prolactin level when physiological causes of prolactin hypersecretion have been excluded (Molitch, 2001). Prolactin secretion is controlled by the predominantly inhibitory effect of the hypothalamus through one or more prolactin inhibitory factors (PIF) that reach the pituitary via the hypothalamic-pituitary portal vessels (Kathryn *et al*., 2007). Dopamine is the main physiological PIF, which acts on surface membrane dopamine D2 receptors on lactotroph cells. Disruption of the pituitary stalk and therefore the transport of dopamine to the lactotrophs, or blockade of endogenous dopamine receptors by a variety of drugs, leads to a moderate increase in prolactin secretion. Prolactin secretion is also influenced by prolactin-releasing factors such as vasoactive intestinal peptide and thyrotropin-releasing hormone, but their precise physiological roles are not clear (Brue and Delemer, 2007).

Theaetiology of hyperprolactinaemia may be physiological, pharmacological, pathological. ysiological hyperprolactinaemia, which occurs during pregnancy, lactation, hypoglycemia, myocardial infarction, and surgery, is usually only mild or moderate (Biller *et al*., 1999). Pharmacologically, any drug that affects the hypothalamic dopamine system and/or pituitary dopamine receptors can result in an elevated prolactin level (Luciano, 1999). Pathological hyperprolactinaemia can be caused by nonhypothalamic-pituitary disease. Forty percent of patients with primary hypothyroidism have mild elevation of prolactin levels, and about 30% of patients with chronic renal failure and up to 80% of patients on hemodialysis have elevated prolactin levels (Sievetsen *et al*., 1980). However, a fourth aetiology may also be of importance, it is the immunogenetic predisposition to develop hyperprolactinaemia. Such predisposition is presented by alleles of the major histocompatiblity complex (MHC), which is known in human beings as human leucocyte antigen (HLA) system (Klein and Sato, 2000). The HLA alleles are controlled by genes located on a region of the short arm of chromosome 6, and the prolactin gene is also located in the proximity of such region, an observation that may suggest a genetic or/and functional relationship between the products of these genes (Johnston and Schroeder, 2007). Furthermore, the role of HLA system in the immune response is well documented, and the antigens of such system are involved in the immunological recognition of non-self antigens (Shankarkumar, 2004). Equally important, prolactin is known to regulate cellular functions including proliferation, differentiation, angiogenesis, and protection against apoptosis and inflammation. The initial step of prolactin action is the binding to specific membrane receptors. Prolactin receptors are distributed throughout the immune system and are included as members of the cytokine receptor superfamily such

as receptors for interleukin (IL)-2 beta chain, IL-3, IL-4, IL-6, IL-7, growth hormone (GH), and erythropoietin (Bole-Feysot *et al.*, 1998; Yu-Lee, 2001). Thus, the profiles of immunity, humoral and cellular, are probably consequently affected by hyperprolactinaemia, and some autoimmune conditions may be pictured (Orbach and Shoenfeld, 2007)

Material and method :

Primary infertile female patients with hyperprolactinaemia (age range: 20 – 40 years) were investigated. Based on serum level of prolactin, the total patients (٦٠ hyper-prolactinaemic females) were divided into three main groups, I (٢١patients), II (٢٤patients) and III (١٥patients). Their serum prolacin levels were 22-29, 30-39 and ≥ 40 ng/ml, respectively. with ٤5 fertile females (their husbands were infertile and had normal serum prolactin level)

The collected serum was tested for the determination of prolactin levels using a miniVidas system. The technique of enzyme immunoassay (Enzyme Linked Fluorescent Assay; ELFA) was employed for such determinations (Kunio *et al.*, 1993). The assay was performed on cycle days 2, 10, 21. The laboratory staff of the Institute for Embryo Research and Infertility Treatment carried out the laboratory determination of this hormon.

It is a single radial immunodiffusion test , used to Quantitative Measurement of Immunoglobulins (IgA, IgG and IgM) and Complement components C3 and C4 which was developed by (Mancini *et al*. 1965**)** for quantitive determination of proteins in the serum. Test sample is added to a well in an agarose gel containing a monospecific antiserum. The sample diffuses radially through the gel and the substance being assayed forms a precipitation ring with the monospecific antiserum. Ring diameter is measured and the concentration is determined from the reference standard curve.٥ml of blood were obtained by venepuncture, using ٥ml disposable syringe. The blood sample, dispensed in a plain tube, and left for 15 minutes at 4ºC to clot. Then, it was centrifuged at 3000 rpm for 10 minutes to collect serum.

Before starting procedure, the plates were opened and left for 5 minutes at room temperature, and then 5 µl of serum was dispensed into a well in the plate. The plate was incubated in flat position at room temperature (20-25°C) for 48 hours (IgA, IgG, C3 and C4) or 72 hour (IgM). The ring diameter was measured by an immune viewer and the concentration was obtained from the reference curve.

RESULT :

The total level of three immunoglobulins (IgA, IgG and IgM) and two complement components (C3 and C4) were assessed in the sera of hyperprolactinaemic patients and controls.

Immunoglobulin A (IgA)

Total patients, as well as, groups II and III showed a significant ($P \leq 0.001$) increased mean serum level of IgA (265.5, ٢٣٢.7, 274.4 and 297.1 mg/dl, respectively) as compared to control subjects (225.3 mg/dl), while group I of patients showed a non-significant increase (Table -1)

Table - ١ - : Total serum level (mean ± S.E.) of IgA in hyperprolactinemic patients (total and groups) and controls.

Groups		Number	IgA Serum Level (mg/dl)			Probability ≤
			Mean ± S.E.	Minimum	Maximum	
Controls		45	225.3 ± 9.9	200.0	240.0	
Patients	Total	60	265.5 ± 3.5	212.0	265.6	0.001
	Group I	21	232.7 ± 12.1	219.0	242.0	N.S.
	Group II	24	274.4 ± 3.6	230.0	295.0	0.001
	Group III	15	297.1 ± 8.3	212.0	350.0	0.001

N.S.: Not significant

Immunoglobulin G (IgG)

The total patients, as well as, their three groups showed a significant ($P \leq 0.01$ and 0.001) increased serum level of IgG (1423, 1330, 1353 and 1665 mg/dl, respectively) as compared to control subjects (1076 mg/dl). The highest increase was observed in group III of patients (Table-٢)

Table - ٢- : Total serum level (mean ± S.E.) of IgG in hyperprolactinemic patients (total and groups) and controls.

Groups		Number	IgG Serum Level (mg/dl)			Probability ≤
			Mean ± S.E.	Minimum	Maximum	
Controls		45	1076 ± 39	1015	1113	
Patients	Total	60	1423 ± 163	1200	1799	0.01
	Group I	21	1330 ± 112	1200	1398	0.01
	Group II	24	1353 ± 66	1295	1405	0.01
	Group III	15	1665 ± 27	1360	1799	0.001

Immunoglobulin M (IgM)

The total patients showed a significant ($P \leq 0.05$) increased serum level of IgM as compared to controls (266 *vs*. 188 mg/dl). A similar picture was shared by groups II and III of

Table - ٣ - : Total serum level (mean ± S.E.) of IgM in hyperprolactinemic patients (total and groups) and controls.

Groups		Number	IgM Serum Level (mg/dl)			Probability ≤
			Mean ± S.E.	Minimum	Maximum	
Controls		45	188 ± 9	175	205	
Patients	Total	60	266 ± 55	180	369	0.05
	Group I	21	201 ± 16	180	220	N.S.
	Group II	24	283 ± 38	250	319	0.001
	Group III	15	330 ± 58	265	369	0.001

N.S.: Not significant

The Third Component of Complement (C3)

A non-significant decreased serum level of C3 was observed in total patients, as well as, groups I and II (106, 115 and 104 mg/dl, respectively) as compared to control subjects (124 mg/dl).

Such decrease was more pronounced in group III of patients (98 *vs*. 124 mg/dl), in which the difference reached a significant level ($P \leq 0.01$) (Table-٤)

Table -٤ - : Total serum level (mean ± S.E.) of C3 in hyperprolactinemic patients (total and groups) and controls.

Groups		Number	C3 Serum Level (mg/dl)			Probability ≤
			Mean ± S.E.	Minimum	Maximum	
Controls		45	124 ±12	103	149	
Patients	Total	60	106 ±11	85	125	N.S
	Group I	21	115 ± 8	104	125	N.S.
	Group II	24	104 ±16	90	123	N.S
	Group III	15	98 ± 4	85	122	0.01

The Fourth Component of Complement (C4

A non-significant decreased serum level of C4 was observed in total patients, as well as, groups I and II (27.9, 28.4 and 30.2 mg/dl, respectively) as compared to control subjects (31. 9 mg/dl). Such decrease was more pronounced in group III of patients (23.2 vs. 31.9 mg/dl), in which the difference reached a significant level ($P \leq 0.05$) (Table-٥)

Table -٥ - : Total serum level (mean ± S.E.) of C4 in yperprolactinemic patients (total and groups) and controls.

Groups		Number	C4 Serum Level (mg/dl)			Probability ≤
			Mean ± S.E.	Minimum	Maximum	
Controls		45	31.9 ± 4.7	25.0	39.0	
Patients	Total	60	27.9 ± 5.3	16.0	38.0	N.S
	Group I	21	28.4 ± 5.7	22.0	34.0	N.S.
	Group II	24	30.2 ± 7.8	20.0	38.0	N.S
	Group III	15	23.2 ±1.1	16.0	31.0	0.05

Disscution :

The hormone prolactin has its own particular allocation within this bidirectional pathway in that it is produced by both pituitary and immune cells (Ben-Jonathan *et al*., 1996) and exerts its influence on the immune system by endocrine and paracrine/autocrine mechanisms (Matera *et al*., 2000). Therefore, differentiation of the individual immunoregulatory roles of pituitary and non-pituitary prolactin is important for understanding the significance of central nervous system stimulation in host defence.

prolactin receptor is a member of the cytokine-hemopoietin receptor superfamily, which includes receptors for IL-2 (β and γ chains), IL-3, IL-4, IL-6, IL-7, IL-9, IL-12, IL-15, GM-CSF, G-CSF and IFN-γ. Description of a cross-talk between prolactin and cytokine/hemopoietin receptors reinforces its many roles in the immune network.

The immunoglobulins (IgA, IgG and IgM) showed a significant increased total level in the sera of total patients when compared with controls, (265.5 *vs*. 225.3, 1423 *vs*. 1076 and 266 *vs*. 188 mg/dl, respectively.. Prolactin enhances immunoglobulin production, which may contribute to increased autoreactivity. A variety of autoantibodies was observed in patients with hyperprolactinaemia including antibodies to prolactin, endothelial cells, cardiolipin, Krause *et al*., 1998) and in systemic lupus erythematosus (SLE) prolactin may have effect on autoantibody production through the up-regulation of T-helper cytokines, and in this regard prolactin triggers IL-1, IL-6, IL-12, and INF-γ production and increase the effect of IL-2 on lymphocytes. Some of the cytokines affect B cell function and may contribute to the development of autoimmunity (Vera-Lastra *et al*., 2002).

The present study showed prolactin is effective in increasing the levels of immunoglobulins. The effect is certainly on the cells that produce cytokines, and the latter due to deviations from normality in hyperprolactinaemic patients. Therefore, a correlation between prolactin and immunological functions is expected. A higher level of prolactin has been demonstrated to have a possible pro-inflammatory role in chronic inflammatory diseases, and consequently, it stimulates T and B-lymphocytes which lead to a more pro-inflammatory situation with elevated serum level

some imunoglobulins, which showed a significant increased in hyperprolacinaemic patients (Cauci *et al*., 2003).

complement components, the serum levels of C3 and C4 were slightly decreased in the patients, but the difference did not reach a statistical level. The complement system plays a major role in host defense and the inflammatory process

Therefore , some of the complement mediated defence mechanisms due to intraction

of the all components , which have short active life and found serum inhibitors e.g C1 inhibitor , C3b inactivater , activation of these inhibitors can result in certain diseases .

further complement deficiencies especially C2 , C4 are associated with a syndrome resembling SLE .(Roitt ., 1998) .

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دراسة تاثير زيادة هرمون الحليب عند النساء العقيمات على تراكيز الامينوكلوبيولينات المناعيه ومكونات المتمم

ماجدة غازي مكطوف الجوراني

الخلاصة

صممت الدراسه لكشف تراكيز الامينوكلوبيولينات ومكونات المتمم عند ارتفاع هرمون الحليب لعينة من النساء العراقيات العقيمات شملت الدراسه ٦٠ مريضة واللاتي كن يراجعن معهد بحوث الاجنه وعلاج العقم في مدينة بغداد راجعن للتشخيص والعلاج للفتره من نيسان ٢٠٠٧ الى ايلول ٢٠٠٧ و بعد اجراء الفحوصات من قبل كادر المختبر في المعهد المذكور وحسب طريقة miniVidas system قسم العدد الكلي للمريضات وبالاعتماد على ارتفاع المستوى المصلي لهرمون الحليب (≤٢٠نانوغرام/مليليتر) الى ثلاث مجاميع : مجموعة I(٢١مريضة)، مجموعة II(٢٤مريضة)، مجموعة III(١٥مريضة)، 22-29, 30-39 and ≥ 40 ng/ml, على التوالي.

اعتمدت طريقة[Mancini *et al*. 1965–] لتقدير تراكيز الامينوكلوبيولينات ومكونات المتمم في مصل الدم. ارتفع معنويا المستوى المصلي للكلوبيولينات المناعية IgA و IgG و IgM في العدد الكلي للمريضات مقارنة مع السيطرة. و في هذا الصدد تناسب ذلك طرديا مع زيادة المستوى المصلي لهرمون الحليب .

المستويات المصلية لمكونات المتمم انخفضت قليلا عن سيطرة المستويات المصلية لبروتيني المتمم الثالث و الرابع في المريضات الا ان الفرقين لم يكتسبا الدلالة الاحصائية.