MODERN MEDICINE AND PRACTICE
https://inovatus.es/index.php/ejmmp

EUROPEAN JOURNAL OF MODERN MEDICINE AND PRACTICE
Vol. 4 No. 8 (Aug - 2024) EJMMP ISSN: 2795-921X

EFFECT OF PROLACTIN ON WOMEN INFERTILITY

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Abstract: Background: Acne in adult female may start during adolescence and persist or have an onset in adulthood. Acne has various psychosocial effects that impact patients' quality of life. Treatment of acne in adult women. This study aimed to evaluate the effect the of some trace element levels such as Zinc, Copper, Manganese, and iron (Zn, Cu, Mn, and Fe) and the correlated antioxidant enzymes, SOD1 and GRx in Iraqi women with acne vulgaris.

Subjects and Methods: This study enrolled of 50 woman with acne vulgaris completed diagnosis and 40 woman without any apparent skin disorders as control group. The two groups were compared in light of the measured variables. Determination of Zn, Cu, Mn, and Fe were done by spectrophotometric kits while GPx and SOD were estimated by ELISA.

Results: The results of present study suggesting significant differences in levels of Zn, Cu, and Mn (p-vale<0.05) between both groups but also the results not showing any statistically differences in levels of iron (p-value>0.05). from the current study we suggesting statistical significant differences in antioxidant enzymatic activity of SOD1 and GRx between AV and control group (p-value<0.05).

Conclusion: The levels of trace element Zn, Cu, and Mn decrease in AV group but Fe not change, the SOD1 and GRx activity decrease in AV group compare to control.

Keyword: Acne, Iraqi women, trace elements, antioxidant.

1-Introduction:

Prolactin (PRL) is one of several hormones that are produced by the pituitary gland. PRL has many different roles throughout the body, and most of those are clearly shown as clinical symptom. Perhaps the most important classical role of prolactin is to stimulate milk production in women after the delivery of a baby. Prolactin levels increase during pregnancy causing the mammary glands to enlarge in preparation for breastfeeding and ready to secrete colostrums closely after delivery. Later on the elevated prolactin levels help with the sustained production of milk during nursing. The somatomammotrop cells of the anterior pituitary gland synthesize and secrete prolactin, which is under the control of hypothalamic factors, mainly the tonic inhibition of Dopamine (DA). There are several other sources of PRL-like substances in the periphery such as placental lacto gens, (similar to pituitary PRL), mammary gland (produced within the

mammary epithelial cells), or PRL variants of immune cell origin (that modulates the immune system). (Gellersen,1989;Andersen 1990; Lkhider, 1996; Kurtz,1993; Gala, 1994, Montgomery,1990; Ben-Jonathan1996; Yu-Lee LY 1997).

It is important to underline that serum PRL in normal individuals is considered as almost entirely pituitary PRL sources, the above mentioned extra pituitary-PRL may contribute significant amounts but either carries as specific function and target mainly to the local environment acting via paracrine/autocrine manner (Yu-lee 1997; Bachelot 2007).

During the first several months of breastfeeding, the higher basal prolactin levels also serve to suppress ovarian cyclicity, through the inhibition of pituitary hormones, mainly via LH suppression (Taya 1982) This is the reason why women who are breastfeeding do not get their periods and therefore do not often become pregnant. In actively breastfeeding mothers the related hyperprolactinaemia persisting even over a year. It was observed that extended lactation amenorrhea is associated with low LH levels, and interestingly suckling inducedPRL elevation as a response has a positive effect on prolongation (Diaz 1991; Diaz 1995). Menstruation and ovulation may only occasionally occur before the drop of elevated basal PRL levels. As time goes on with less frequent breastfeeding, e.g. during weaning however, the PRL levels do not stay as high and the woman may start to ovulate. In cases of no lactating/ no breastfeeding mothers, that may happen between 2-3 month after delivery (Baird 1979).

Similarly, elevated PRL levels are shown during gestation, but mechanisms to inhibit ovulation is related to elevated estardiol and progesterone levels and a consequent depression of pituitary FSH secretion (Marrs 1981).

Generally, the lactogenic hormones play role also in regulation of reproductive function. On one hand, PRL is essential to maintain regular oestrus cycles. PRL knockout mice are completely infertile (Horsemann 1997). One of the other actions of PRL is to stimulate ovarian production of progesterone. That is required in the process of preparation for embryo implantation and it is dependent on a continued estrogen and progesterone secretion by the corpus luteum, which is supported by a functional pituitary during the first half of pregnancy in rodents. (Binart 2000)On the other hand, high prolactin levels are associated with an ovulation or may cause directly or indirectly infertility. In young women, hyperprolactinemia is probably one of the most common endocrine disorders related to pituitary function. Women who are not pregnant and are not breastfeeding should have lower levels of basal PRL (typically 10–28μg/L in women and 5–10 μg/L in men are defined as “normal levels”) If a non-pregnant woman has abnormally high levels of PRL, it may cause her difficulty in becoming pregnant. It is considered as the most frequent cause of anovulatory sterility, although spontaneous pregnancy may occur occasionally. The prevalence of hyperprolactinemia varies in different patient populations, stays below 1% (0.4% in an unselected normal population) but can be as high as 17% of women with reproductive disorders shown at the clinics (Crosignani 1999).

The suppression of pituitary hormones by PRL, similar that described during lactation has an indirect anovulatory effect. PRL however, acts also directly on the ovary to inhibit the hCG-induced follicle rupture, resulting in the inhibition of ovulation. (Yoshimura 1989).

Clinically significant elevation of PRL levels may cause infertility in several different ways.

First, prolactin may stop a woman from ovulating. If this occurs, a woman’s menstrual cycles will stop. In less severe cases, high prolactin levels may only disrupt ovulation once in a while. This would result in intermittent ovulation or ovulation that takes a long time to occur. Women in this category may experience infrequent or irregular periods. Women with the mildest cases involving high prolactin levels may ovulate regularly but not produce enough of the hormone progesterone after ovulation. This is known as a luteal phase defect.

Deficiency in the amount of progesterone produced after ovulation may result in a uterine lining that is less able to have an embryo implant. Some women with this problem may see their period come a short time after ovulation (Shibli-Rahhal, 2011)Hyperprolactinemia is commonly found in both female and male patients with abnormal sexual and/or reproductive function or with galactorrhea. If serum prolactin levels are above 200 μg/L, a prolactin-secreting pituitary adenoma (prolactinoma) is the underlying cause, but if levels are lower, differential diagnoses include the intake of various drugs, compression of the pituitary stalk by other pathology, hypothyroidism, renal failure, cirrhosis, chest wall lesions, or idiopathic hyperprolactinemia. When a pituitary tumour is present, patients often have pressure symptoms in addition to endocrine dysfunction, such as headaches, visual field defects, or cranial nerve deficits (Wang, 2012). The objectives of therapy are to improve the symptoms associated with high PRL levels and to reduce the size of a pituitary tumour.

Pharmacotherapy is available to reduce the tumour size and consequently decrease PRL levels. The large majority of patients with prolactinomas, both micro-and macroprolactinomas, can be successfully treated with dopamine D2 receptor agonists as first-line treatment, with normalization of prolactin secretion and gonadal function, and with significant tumour shrinkage in a high percentage of cases, to prevent the need for surgery. In cases when the only cause of infertility is chronic anovulation due to hyperprolactinemia, a 60-80% pregnancy rate can be achieved. Surgical resection of the prolactinoma is the option for patients who may refuse or do not respond to long-term pharmacological therapy. Radiotherapy and/or estrogens are also reasonable choices if surgery fails. In patients with asymptomatic microprolactinoma no treatment needs to be given and a regular follow-up with serial prolactin measurements and pituitary imaging should be organized (Asa 2002; Crosignani 1999, Molitch 2003).

The most commonly used dopamine agonists are bromocriptine, pergolide, quinagolide and cabergoline. When comparing the plasma half-life, efficacy and tolerability of these drugs are different, there is also important to evaluate the risk/ benefits profile of each product. As the current clinical practice, pharmacological treatment with dopamine agonist plays an important role. The recommendations on the most effective dosages and the advantages of a long term efficacy of products have been evaluated summarizing the results of case histories of the last decades.

1-2. Clinical diagnosis of hyperprolactinemia

A variety of etiological factors including disorders of the hypothalamo-pituitary axis, interruption of dopamine synthesis, stress, pituitary tumours, polycystic ovary syndrome, primary hypothyroidism, and various medications may lead to hyperprolactinemia.

Hyperprolactinemiain girls causes delayed puberty, hypogonadotropichypo gonadism, primary or secondary amenorrhea, and galactorrhea (Fideleff, 2000). Hyperprolactinemia in men may result in as a first signs of decreased libido or impotence, however also cause inefficient sperm production and infertility (Colao, 2004).

As one of the fist signs in women with high prolactin levels may have irregular periods or no periods at all. Another common symptom is “galactorrhea”, which is the occurrence of a milky discharge from the breast in a woman who has not recently been pregnant. The 150 Prolactin discharge is the result of persistant high PRL levels stimulating the mammary gland for milk production. Some women may see galactorrhea occur spontaneously. Others may see it only if they squeeze their nipples.

As diagnostic practice, after signs and lab tests have been evaluated the magnetic resonance imaging (MRI) of the pituitary gland should be performed in all patients. A pituitary adenoma with a diameter of less than 1 cm is defined as “microadenoma” and one above 1cm in diameter as “macroadenoma”.

1-3. Measurement of prolactin

Prolactin can be measured with a simple blood test drawn at the fertility doctor's office. In order to get accurate results, prolactin should be drawn first thing in the morning. Since PRL may serve as a hormone to affect reproductive functions, sexual contact, stimulation of nipples in human may cause a not just immediate but also next-day-long alterations of the PRL secretory pattern. (Kruger 2012) These fluctuations are measured on the next day to produce a PRL elevation around noon, additional of the regular circadian rhythm of PRL levels, as the peak on the morning. Accordingly it is important to note that the woman should have the instructions to eat nothing from the night before and to avoid any stimulation of the breast and nipples, included sexual intercourse as well, from the day before also.

Since stimulation of the breast /nipples (stress such as physical exam) may cause immediate release of PRL one common mistake that doctors make is to draw a prolactin blood test immediately after a patient has had a breast exam in the office. These women will have high prolactin levels because of the exam and therefore they may show false (i.e. transient)increase of PRL levels. Prolactin should also be drawn early in the menstrual cycle – before ovulation. This is because prolactin levels are naturally higher after ovulation.

A prolactin level of 5-20 ng/mL is considered normal in both sexes, according to some laboratories and test references the male and female (a bit higher) normal range may differ.

A level above 20 ng/mL in two successive measurements is defined as hyperprolactinemia (7). According to WHO standards: 1 μg/L = 21.2 mIU/L. PRL levels > 250 ng/mL usually indication for prolactinoma, when PRL > 500 ng/mL it is considered as diagnosis for macroprolactinoma. (Melmed, 2011)

There are cases when false positive and elevated PRL levels are measured: two high molecular mass forms of prolactin (PRL) in serum have been identified: macroprolactin (bigbig PRL, > 100 kDa) and big PRL (40-60 kDa). Big PRL is a consistent “normal” component of total serum PRL but rarely cause of hyperprolactinemia. Macroprolactin is usually acomplex of PRL and IgG in composition, it is formed in the circulation from monomeric PRL with a molecular mass of 150-170 kDa, but may have some additional variability in composition. In labor tests the PRL in the complex remains reactive to a variable extent in immunoassays. Individuals may show a different pattern of % of these variants, or even can be a predominant immune reactive component of circulating PRL and the cause of apparent hyperprolactinemia, but it has minimal bioactivity in vivo and is not of pathological significance. As necessary the reference technique of gel filtration chromatography at the laboratory should be available for confirmation and request on investigation of samples to avoid confusion of diagnostics. (Fahie-Wilson 2005).

1-4. Causes of high prolactin levels

1-4.1. Pituitary tumours

Pituitary adenomas are the most common tumour type in the pituitary gland. There is approximately 10% incidence was shown obtained by post-mortem autopsy, with similar ratio of male and female patients. The most frequently detected tumours (over 39%) are sparsely granulated PRL cell adenomas. The others types are GH cell or mixed PRL/GH adenoma, ACTH cell adenoma/Crooke’s cell adenoma (~14%) ; Gonadotroph cell adenoma (6.6%); Null cell adenoma/oncocytoma (~32%) and other or unclassified types (Buurman,2006).

Invasive tumours with multiple recurrences are only classified as aggressive tumours or "atypical adenomas". Tumours with systemic metastasis must be considered as carcinomas,and “only” make up 0.1% to 0.2% of all pituitary tumours, but with very poor prognostics of 66% mortality (Oh, 2012). However it was suggested that a full picture inlcuded clinical signs (gender, DA-resistant hyperprolactinemia, etc) , radiological status (invasive macro or giant tumour) and histological signs of angiogenesis, mitoses level, vascular invasion and molcular biology parameters (Ki-67 > 3 %, p53 positive, up-regulation of genes

related to invasion and proliferation, and allelic loss of chromosome 11) should be taken into account considering the potential malignancy, prognosis of prolactin secreting tumours and identify the optimal therapy as early as possible. The key question is to identify factors associated with tumour aggressiveness.

The approach combined genomic and transcriptomic analysis focus to the subtype of pituitary tumour able to identify molecular events associated with the aggressive and malignant phenotypes. Allelic loss in certain loci of chromosome 11 has been detected in tumours with signs of malignancy, potentially responsible for triggering the aggressive and malignant phenotypes. Within the recent years there are an increasing number of genes or molecular signs that has been associated with pituitary tumor genesis to develop predictive and potential prognostic markers (Zemmoura, 2012; Dworakowska,2012; Wierinckx, 2011).

About one-third of all pituitary tumours are not associated with hypersecretory syndromes but, rather, present with symptoms of an intracranial mass, such as headaches, nausea, vomiting or visual field disturbances. Only rare cases of pituitary tumours are considered a malignant prolactinoma. Tumours that produce growth hormone (GH) may also secrete prolactin in nearly 25% of cases. This is a common source of misdiagnosis, as the features of prolactin excess may capture attention while the more subtle features of GH excess go unnoticed.

1-4.1. Characteristics of pituitary adenomas

In some people, a small group of cells may form a cyst in the pituitary gland which produces elevated levels of prolactin. These cysts are called prolactinomas or pituitary adenomas. It is unclear exactly how these cysts get started. Recent investigations on pituitary tumours reported that approximately 12% of pituitary glands (obtained by autopsy of 3048 patients) are shown histologically diagnosed but clinically in apparent adenoma.

Among the mean tumour size is approx 1.9mm. According to published data two-thirds of adenomas has a tumour size <3 mm, half of them were smaller than 1 mm in diameter and ~23% was between 3-10mm. In this study only few (3/76) tumours were identified as macroadenomas corresponding to a tumour size >10 mm. (Buurman, *2006)*

The prevalence of clinically apparent prolactinomas ranges from 6–50/ 100,000 in reported populations (Daly, 2006; Fernandez 2010). The prevalence of "ever-treated'' hyperprolactinemia is approximately 20 /100,000 in male patients and approximately 90 /100,000 in female patients. (Kars, 2009)

The adenomas can be seen and measured using MRI and classified based on their size.

Small adenomas are known as microadenomas. They measure less than one centimetre in diameter. This is the most common type of adenoma found. Microadenomas can even be present in healthy people who do not have high prolactin levels. Microadenomas can be treated with medication. They do not grow large and do not need to be treated if hormone levels are normal. Microprolactinomas usually follow a benign course and rarely progress to macroprolactinomas. However, in rare cases microadenoma may transform to other tumours.

- A case history it was reported that a microadenoma transformed to macroprolactinoma within 10 month, probably due to estrogen therapy applied. The case report emphasizes the role of dopaminergic agonist in treatment of hyperprolactinemia (Garcia, 1995).
- A case history of a 22 -year-old woman with the signs of galactorrhea and slight hyperprolactinemia , showed 7-mm intrapituitary lesion which responded to treatment with cabergoline. This PRL-secreting microadenoma has a sudden change within 4 years of diagnose. The case represents a rapid evolution from a microprolactinoma initially responding to dopamine agonists to a fatal pituitary carcinoma (Guastamacchia, 2007).

- Adenomas larger than 1 centimeter are called macroadenomas. If untreated, macroadenomas can grow further and start to compress the nearby tissues and structures causing life-threatening events or even fatal outcome. The closest structures are the optic nerves, internal carotid arteries. If a macroadenoma causes compression of the optic nerves, partial blindness can result. For this reason, it is important to treat macroadenomas whether or not a woman is interested in getting pregnant. Medication can be used to treat them but if that fails, surgery may be necessary.
- According to a recent clinical study in Japan, treatment with Cabergoline achieved a high pregnancy rate with uneventful outcomes in infertile women with prolactinoma, independent of tumour size and bromocriptine resistance or intolerance. Over 90% of patients in the study conceived pregnancies, and one-third of the macroprolactinoma disappeared. Cabergolinemonotherapy could serve as an alternative of the conventional combination bromocriptine therapy with surgery or irradiation in macroprolactinomas (Ono, 2010).

1-4.2. Hypothyroidism

The hyperprolactinemia of hypothyroidism is related to several mechanisms. In response to the hypothyroid state, a compensatory increase in the discharge of central hypothalamicthyrotropin releasing hormone (TRH) results in increased stimulation of prolactin secretion.

Although TRH was originally named for its ability to trigger the release of thyroidstimulating hormone (TSH) in mammals, it became apparent that TRH exerts multiple hypophysiotropic activities also in human. Stimulation with TRH will provide a diagnostic test to demonstrate a TSH release curve typical of the subclinic hypothyroidism. PRL is under tonic inhibition by the hypothalamus by way of the PRL inhibitory factor, DA. PRLreleasing factors include TRH., Increased release of TRH may also cause a sustained stimulation of prolactin release from the pituitary gland. There are several clinical reportspresented the correlation between subclinic hypothyroidism-hyperprolactinemia and sterility. Treatment with thyroid hormone supplements will result in correction of both the thyroid feedback and the high prolactin levels.

1-4.3. Macroprolactinemia

Asymptomatic patients with intact gonadal and reproductive function and moderately elevated prolactin levels may have macroprolactinemia (Vallette-Kasic, 2002).

Hypersecretion of PRL by lactotroph cells of the anterior pituitary cause hyperprolactinemia. Patients with hyperprolactinemia may have radiologically undetected microprolactinomas, but some of them may present other causes of hyperprolactinemiacharacterised as a symptom of macroprolactinemia, with a predominance of higher molecular mass prolactin forms (big-big prolactin, MW > 150 kDa). This term should not be confused with macroprolactinoma, which refers to a large pituitary tumour greater than 10 mm in diameter.

The prevalence of macroprolactinemia varies between 15-46% in hyperprolactinemic populations, and it may because confusing tests results that could not be differentiated from true hyperprolactinemic patients, on the basis of clinical features alone. The pathophysiology of macroprolactinemia is based on a mechanisms of the increased antigenicity of these molecules, leading to the appearance of autoantibodies against PRL,which can consequently reduce the bioactivity of PRL and provide extended half-life.

Therefore macroprolactinemia is manifested with less frequent clinical symptoms in macroprolactinemic patients and the tests results mainly due to the delayed clearance of PRL. According to recent publications of Isik et al, evaluating over 300 hyperprolactinemic patients, over 26% of them resulted in elevated macroprolactin levels, with the less frequent signs of galactorrhea or abnormal MRI results compared those to patients with predominant monomer hyperprolactinemia. The other symptoms and frequency of amenorrhea,infertility, irregular menses, gynecomastia, and erectile dysfunction were similar in both groups. (Isik, 2012)

Macroprolactinemic patients have no clinical symptoms of hyperprolactinemia and may have no pituitary adenomas. It is still controversial whether macroprolactinemia is a benign condition that does not need further investigation and treatment. Patients can be screened form acroprolactinemia by PEG (polyethylene glycol) precipitation as a standard laboratory test with a results of recovery of ≤40% to normal monomeric PRL level is used as an indication of macroprolactinemia (Tamer, 2012). The clinical importance of this test is based on the lower prevalence of pituitary adenomas in this group, compared to "true hyperprolactinemic" patients.

1-4.4. PCOS (polycystic ovary syndrome)

PCOS is a common problem that can cause infertility by inhibiting ovulation, affecting 3.5-10% of the reproductive age of women. For unknown reasons, some women with PCOS may have slightly high PRL levels. PCOS similar to hypoprolactinemic are both common causes of secondary amenorrhea in women. The relationship between PCOS and hyperprolactinemia so far has been reported still with controversial results: it seems that PCOS is very prevalent with hyperprolactinemia, nevertheless there are different reasons of altered regulation of gondotropin secretion, and suggests that these conditions have independent origins. Recent investigators using serial serum sampling have excluded transient elevations of PRL and have shown a less frequent association of these two disorders. According to clinical guidelines PCOS patients with increased PRL levels must be investigated for other causes of hyperprolactinemia, because hyperprolactinemia may be due to a reason of concomitant disease, but not proved the cause-relationship to PCOS.

Treatment of infertility associated with PCOS has changed in the last decade due to the introduction of new medications such as insulin-sensitizing drugs, aromatase inhibitors, gonadotropin treatment etc. (Bracero 2001, Urman, 2006, Escobar-Morreale, 2004).

- In a study conducted in Brazil, among the 82 PCOS women, 13 (16%) presented high PRL levels (over 100 microg/l). There were several reasons of hyperprolactinemia: pituitary adenoma; drug-induced hyperprolactinemia, or macroprolactinemia. The nonhyperprolactinemic PCOS patients (over 80%) represented normal PRL levels. The authors concluded that hyperprolactinemia is not a clinical manifestation of PCOS (Filho, 2007).

1-4.5. Medications

Some medications can cause higher levels of prolactin to be produced. The most common medications that do this are known as anti-psychotic medications. The antipsychotics mostly act as dopaminergic neurotransmitters/ receptor blockers can also cause endocrine side effects, as hyperprolactinaemia and it is most common side effect of first-generation antipsychotics. The second- and third generation antipsychotics have a weaker affinity for D2 dopamine receptors, thus hyperprolactinemia is less common when such medication is used. (Uzun et al. 2005). The risk of side effects caused by antipsychotics is individual and it does not depend solely on the therapeutic dose and may have influence on some predisposing conditions. (Ružić 2011)Other medications which may increase prolactin levels:

- Some types of anti-depressants, serotonin reuptake inhibitors, SRIs (fluvoxamine; fluoxetine; paroxetine, duloxetine etc)
- Some types of sedatives
- Catecholamine depleter
- Dopamine synthesis inhibitor
- Neuropeptides
- Anticonvulsants

- Opiates and opiate antagonists
- Estrogen Oral contraceptives (birth control pills)
- Some types of blood pressure medications (methyldopa, verapamil)
- A medication for nausea (Reglan, metoclopramide)
- Antacids (cimetidine)

1-4.6. Stress

A high prolactin level can sometimes be related to physical stress. Even drawing blood can by itself cause someone to produce and immediate prolactin-release. PRL eleveation can also detected in response to strong or sudden external stimuli in general, such as stressful environmental conditions, or can be related to physchological reasons. This latter can be evaluated by stress profile or measured by experimental conditions, such as "Screamer Index", which is shown resulting in values to be parallel to levels of hyperprolactinemia in women (Harrison, 1988; Cepisky, 1992). On the other hand, anxiety and irritability maybe a result of hyperprolactinemia. In rat models PRL increased the stimulatory effect of ACTH inducedcorticosterone secretion (Jaroenporn, 2007).

- Endocrine abnormalities are frequently associated with a wide range of psychological symptoms. These symptoms may reach the level of psychiatric illness (mainly mood and anxiety disorders) or just being identified by the subclinical forms of assessment provided by the Diagnostic Criteria for Psychosomatic Research (DCPR). In a population study reported by Sonino *et al*, (2007) the majority of patients suffered from at least one of the three DCPR syndromes considered: irritable mood (over 45%), demoralization, persistent somatization. Long-standing endocrine disorders may imply a degree of irreversibility of the pathological process. Endocrine treatment may cause even the worsening of psychological symptoms. The methodology and assessment score provided by DCPR tests have been demonstrated to be a valuable tool for psychological assessment in endocrine disease from diagnostic to follow-up periods (Sonino 2007).
- In clinical environment the variability of PRL concentration in random estimationsunderline the need for special testing to rule out stress-related hyperprolactinemia and diagnostic pitfalls. It was recommended by the results, that two or three serial PRL determinations in resting conditions provide more reliable results (Muneyyirci-Delale, 1989).
- In experimental conditions, hyperprolactinemia and stress interact differentially according to the length of the stimuli and that is connected to the immune response modulated by PRL. Surgical or restraint stress induce marked (2x- 4x) increase of plasma PRL of control rats, but interestingly did not change the PRL levels of hyperprolactinemic rats. In both cases the plasma glucose levels reported elevated (Reis, 1996).
- It is suggested as a result of a retrospective observational study, that life events such as changes in subject's social or personal environment indicated that these stressful conditions may provoke hyperprolactinemia. Even an exposure during childhood to a stressful environment maybe associated with hyperprolactinemia and/or galactorrhea later in life as a response to specific environmental changes (Sobrinho, 1984). Patients with hyperprolactinemia reported significantly more life events, these events rated as being of „moderate", marked or severe „negative" impact compared with control subjects (Sonino, 2004).
- There is evidence that several external stress-factors may contribute to the occurrence of hyperprolactinemia. In theory, stress might have been involved in facilitation of a clonal proliferation of a single mutated cell and cause prolactinomas. Patients in functional hyperprolactinemic status, stress

might trigger neuroendocrine changes involving DAand/or serotonin, which both can consequently affect PRL release. (Verhelst, 2003; Freeman, 2000; Fava, 1981.)

1-5. Hyperprolactinemia and infertility

Prolactin is a pituitary-derived hormone that plays an important role in a variety of reproductive functions. It is an essential factor for normal production of breast milk following childbirth. Additionally, prolactin negatively modulates the secretion of pituitary hormones responsible for gonadal function, including luteinizing hormone and follicle stimulatinghormone. Clinically significant hyperprolactinemia may result in hypogonadism, infertility, and galactorrhea, or in some cases it may remain asymptomatic for a long period (Klibanski 2010).

The most commonly cited indications for treatment of microprolactinomas is infertility and hypogonadism. Hypogonadism and infertility associated closely with the treatment: DA agonists can restore normal PRL levels and consequently the normal gonadal function. According to the date of a meta-analyis, patients treated with bromocriptine had normalization of prolactin levels and it was successful in 53% of patients with infertility. Studies with cabergoline showed similar results: cabergoline was shown more effective than bromocriptine reducing PRL levels, or in symptoms of amenorrhea /oligomenorrhea, or in some of the patient-important outcomes (Gillam, 2006; Wang, 2012).

Prolactin is under dual regulation by hypothalamic hormones delivered through the hypothalamic–pituitary portal circulation. The differential diagnosis and causes of pathological hyperprolactinemia are summarized in Figure 1.The predominant signal is inhibitory, preventing prolactin release, and is mediated by the neurotransmitter dopamine. The stimulatory signal is mediated by the hypothalamic TRH.

The balance between the two opposite signals determines the amount of prolactin released from the anterior pituitary gland (Verhelst; 2003).

1-6. Hyperprolactinemia management

- The first steps in cases of signs of hyperprolactinemia should be a critical diagnosis, as discussed above, may involve dynamic testings, assessment for macroprolactinemia and further laboratory tests to eliminate false positive or negative results.

2- Material and Methods

The Materials used for this study include: records of female patients that visited Hospital on account of infertility, including their chemical pathology Immuno Assay forms. From these records, data on the prolactin hormone levels of the infertile women, type of infertility suffered and bio data of the women were obtained.

2-1 METHODOLOGY FOR DATA COLLECTION

A Project proposal as well as an application for Ethical Approval of the study was drawn up and presented to the Hospital Ethical Committee. The Ethical approval was granted and a senior registrar in Hilla Obstetrics and Gynecology hospital was assigned to supervise the study. This study was carried out at the laboratory of Hormonal of Babylon, a maternity hospital and children / Advisory department infertility from November to January , 2015/2016. From a total of 45 women with age range 17-50 were studied,, the samples from 45 women .Blood samples (2.5 mL) were collected from a vein from each fasting woman in the morning, The collected blood was transferred to gel and clot activator tube, left to clot, and serum separated by centrifuged at 800 x g for 10 minutes at room temperature and freezing in -4C…and then transported the samples to the VIDUS.

With the assistance of the consulting physicians and the chemical pathology laboratory staff, the chemical pathology request forms of 44 randomly selected female infertility patients, were filled and their prolactin

hormonal assay carried out. The data obtained from the chemical pathology request form included the following: Age, Length, weight, cystic ovary, Irregular menstrual cycle, The proportion of prolactin, The number of abortions . However, the data relevant to our study from the above form were; Prolactin hormone level of the patients, the parity of the patients, the duration of the infertility problem.

2-2 GROUPING PROTOCOL

The 45 Patients were grouped into three based on the type of infertility they suffered; the three groups were those suffering infertility. Group 1- Women suffering from Infertility in age 18-26 , while Group 2 – Women suffering Infertility in age 27-35 years, and finally group the third group in age 36-44 years.

2-3 STATISTICAL ANALYSIS

Statistical analysis was carried out on the data obtained using the student T- test method and the results were presented in the form of tables and Bar Charts.

2-4 SPECIMEN COLLECTION AND PREPARATION

1. Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only. Avoid grossly hemolytic, lipemic, or turbid samples.

2. Specimens should be capped and may be stored for up to 48 hours at 2-8°C prior to assaying. Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

MATERIALS REQUIRED

1. Distilled or deionized water.

2. Precision pipettes: 0.05, 0.1, 0.2, and 1 mL.

3. Disposable pipette tips.

4. Microtiter well reader capable of reading absorbance at 450nm.

5. Vortex mixer or equivalent.

6. Absorbent paper.

7. Linear graph paper.

8. Quality control material.

STORAGE CONDITIONS

Store the unopened kit at 2-8°C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date.

Mini VIDAS

The VIDAS Prolactin assay aids in diagnosing hyperprolactinemia. This test preferentially recognizes monomeric prolactin (‘‘little prolactin’’)

PRINCIPLE

The assay principle combines an enzyme immunoassay sandwich method with a final fluorescent detection (ELFA).

The Solid Phase Receptacle (SPR). serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready-to-use and pro-dispensed in the sealed reagent stripe.

All sample is taken and transferred into the well containing alkaline-phosphatase-labeled anti-prolactin (conjugate). The sample/conjugate mixture is cycled in and out of the SPR several times to increase the reaction speed. The antigen binds to antibodies coated on the SPR and to the conjugate forming a "sandwich".

Unbound components are eliminated during the washing steps .during the final detection step, the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-methyl-umbelliferone), the fluorescence of which is measured at the concentration of prolactin present in the sample.

AT the end of the assay, results are automatically calculated by the instrument in relation to the calibration curve stored in memory, and then printed out.

The SPR

The interior of the SPR is coated during production with monoclonal anti-prolactin immunoglobulins (mouse). Each SPR is identified by the "PRL" CODE. Only remove the required number of SPRs from the pouch and carefully reseal the pouch after opening.

The strip

The strip consists of 10 wells covered with a labeled, foil seal. The label comprises a bar code which mainly indicates the assay cods, kit lot number and expiration date. The foil of the first well is perforated to facilitate the introduction of the sample. The last well of each strip is a cuvette in which the fluorometric reading is performed . The wells in the center section of the strip contain the various reagents required for the assay.

Mini VIDAS PROCEDURE

1. only remove the required reagents from the refrigerator and allow them to come to room temperature for at least 30 minutes .
2. use one "PRL" strip and one "PRL" SPR for each sample ,control or calibrator to be tested .Make sure the storage pouch has been carefully resealed after the required SPRs have been removed .
3. the test is identified by the "PRL" code on the instrument .the calibrator must be identified by "S1" and tested in duplicate .if the control is to be tested .it should be identified by "C1" .
4. if necessary, clarify samples by centrifugation .
5. mix the calibrator ,control and samples using a vortex – type mixer (for serum or plasma separated from the pellet).
6. for this test , the calibrator, control, and sample test protein is 200 µl .
7. insert the "PRL" SPRS and "PRL" strips into the instrument . Check to make sure the color labels with the assay code on the SPRs and reagent strips match.
8. initiate the assay as directed in the user´s manual. All the assay steps are performed automatically by the instrument .
9. Reclose the vials and return them to the required temperature after pipetting .
10. the assay will be completed within approximately 40 minutes. After the assay is completed, remove the SPRs and strips from the instrument .
11. dispose of the used SPRs and strips in to and appropriate recipient.

RESULTS and INTERPRETAYION

Once the assay is completed , results are analyzed automatically by the computer. Fluorescence is measured twice in the reagent strip´s reading cuvette for each sample tested . the first reading is a background reading of the substrate cuvette for each sample tested. The first reading is a background reading of the substrate cuvette before the SPR is introduced into the substrate. The second reading is taken after incubating the substrate with the enzyme remaining on the interior of the SPR . the RFV is calculated by subtracting the background reading from the final result. This calculation appears on the result sheet.

3-Results:

The result obtained from the study carried out in Hilla Obstetrics and Gynaecologyhospital was arranged in tables and interpreted using the T-test analytical tool. The data obtained consisted of Age, Length, weight, cystic ovary, Irregular menstrual cycle, The proportion of prolactin , The number of abortions.

The study was designed to study the effect of age on the levels of Prolactin hormone in infertility woman fourty five women were used in this study from AL-Hilla city. There are divided into three groups, the first group in age (18-26 years), the second group (27-35) and the third group from 36 to 44 years.

The result of the present study showed significant increase (0<0.05) in the level of prolactine hormone in group 1.Tab 1 of fig 1.

Tab-1- level of Prolactin in G1 (18-26y)

level of prolactin	Number of abortions	Irregular menstrual cycle	Cystic Ovary	Weight	Length	Age	Age 18-26
-.092	.149	-.150	-.168	-.187	-.347	1	**Age**
.190	-.536-*	-.250	.281	.681**	1		**Length**
.057	-.481-*	-.180	.140	1			**Weight**
-.052	-.415	-.601-**	1				Cystic ovary
.012	.169	1					Irregular menstrual cycle
.071	1						Number of abortions
1							level of prolactin

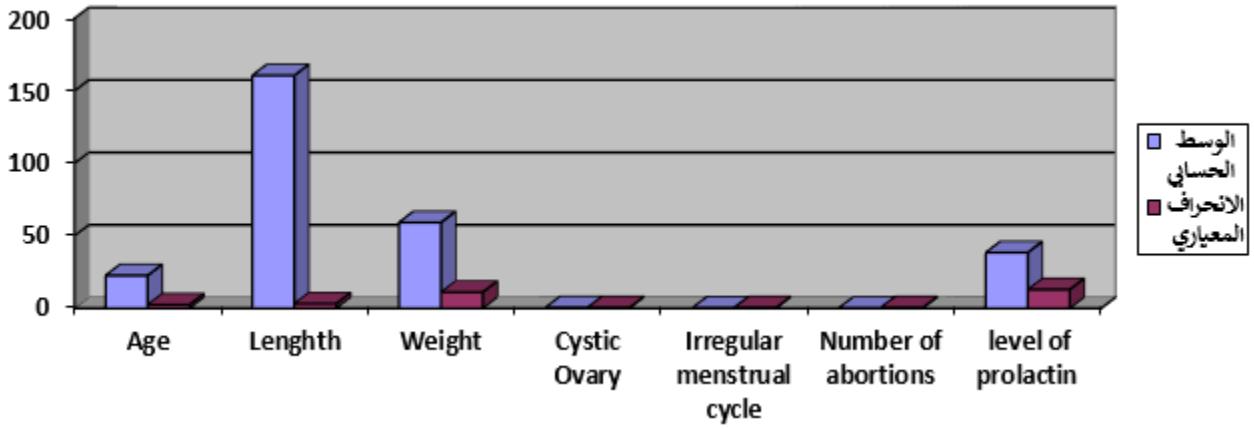

Fig-1-level of prolactin in G1 (18-26y)

Tap 2 of fig 2 should that the results in the second group showed significant increase too .

Tab-2- level of Prolactin in G2 (27-35y)

level of prolactin	Number of abortions	Irregular menstrual cycle	Cystic Ovary	Weight	Length	Age	Age 27-35
.022	.163	.035	.188	.080	.020	1	**Age**
.447*	-.275	.077	.240	.751**	1		**Length**
.292	-.461-*	.066	.228	1			**Weight**
.268	-.154	-.314	1				Cystic Ovary
-.161	-.199	1					Irregular menstrual cycle
-.080	1						Number of abortions
1							level of prolactin

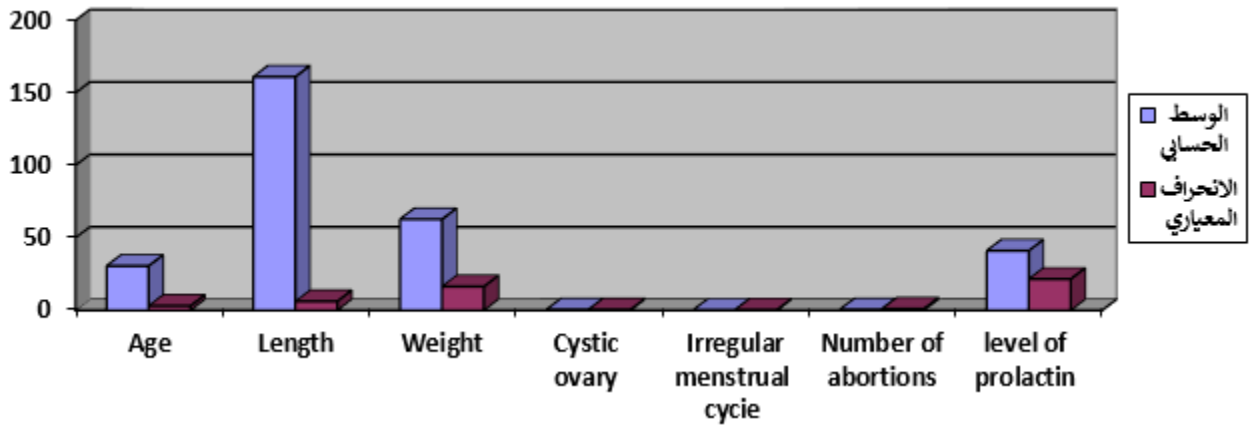

Fig-2-level of prolactin in G2 (27-35y)

finally the results of the present stage in Tab 3of fig 3 should should signification increase in third group which is age from 36 to 44 years old.

Tab-3- level of Prolactin in G3 (36-44y)

level of prolactin	Number of abortions	Irregular menstrual cycle	Cystic Ovary	Weight	Length	Age	
.409	.512	-.748-*	.354	-.082	.430	1	**Age**
.557	.224	-.200	-.153	.344	1		**Length**
-.527	.284	.095	-.663	1			**Weight**
.331	.333	-.333	1				Cystic Ovary
-.291	-.333	1					Irregular menstrual

							cycle
-.225	1						Number of abortions
1							level of prolactin

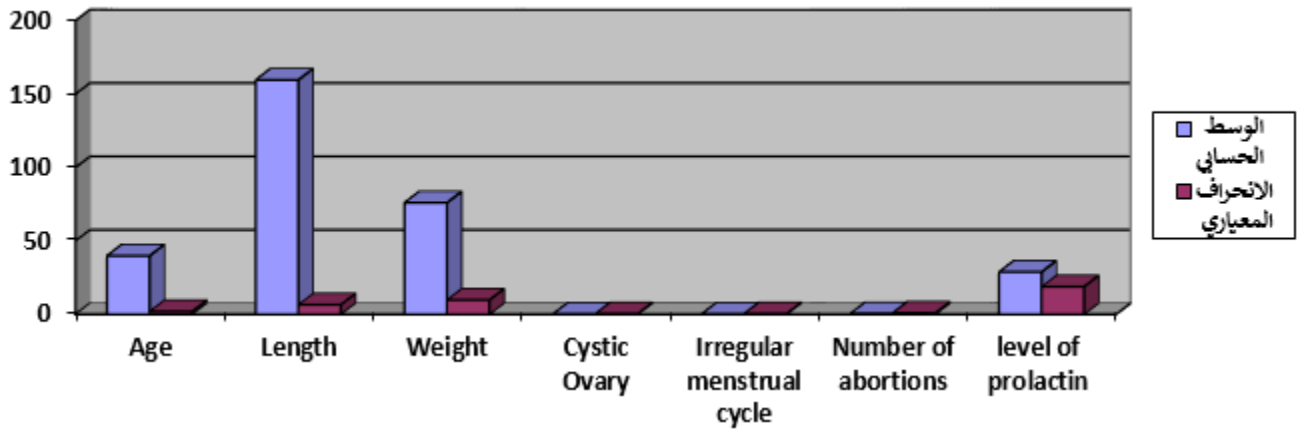

Fig-3-level of prolactin in G3 (36-44y)

Note :

In our present study, we cannot showed any effect for the weight of women on the Prolactin level , and in other hand not showed significant effect between the short and long women in the level of prolactin.

4-Discussion

All the results of our present study in all gropes (1, 2 and grope 3) about the effect of prolactin hormone level on infertility of women which are description in tab 1 ,2 &3 and figures 1,2 and3 conformity with another studies in this side, the infertility return to the increase of prolactin level ,this result conformity with Horseman, 1997 the lactogenic hormones play role also in regulation of reproductive function. On one hand, PRL is essential to maintain regular estruos cycles. PRL knockout mice are completely infertile , One of the other actions of PRL is to stimulate ovarian production of progesterone. That is required in the process of preparation for embryo implantation and it is dependent on a continued estrogen and progesterone secretion by the corpus luteum, which is supported by a functional pituitary during the first half of pregnancy in rodents (Binart 2000). On the other hand, high prolactin levels are associated with anovulation or may cause directly or indirectly infertility. In young women, hyperprolactinemia is probably one of the most common endocrine disorders related to pituitary function. If a non-pregnant woman has abnormally high levels of PRL, it may cause her difficulty in becoming pregnant. It is considered as the most frequent cause of anovulatory sterility, although spontaneous pregnancy may occur occasionally.

The infertility of women may be return to the suppression of pituitary hormones by PRL, similar that described during lactation has an indirect anovulatory effect. PRL however, acts also directly on the ovary to inhibit the hCG-induced follicle rupture, resulting in the inhibition of ovulation (Yoshimura 1989).

Clinically significant elevation of PRL levels may cause infertility in several different ways.

First, prolactin may stop a woman from ovulating. If this occurs, a woman’s menstrual cycles will stop. In less severe cases, high prolactin levels may only disrupt ovulation once in a while. This would result in intermittent ovulation or ovulation that takes a long time to occur. Women in this category may experience

infrequent or irregular periods. Women with the mildest cases involving high prolactin levels may ovulate regularly but not produce enough of the hormone progesterone after ovulation. This is known as a luteal phase defect.

Deficiency in the amount of progesterone produced after ovulation may result in a uterine lining that is less able to have an embryo implant. Some women with this problem may see their period come a short time after ovulation (Shibli-Rahhal,2011)Hyperprolactinemia is commonly found in both female and male patients with abnormal sexual and/or reproductive function or with galactorrhea. If serum prolactin levels are above 200 μg/L, a prolactin-secreting pituitary adenoma (prolactinoma) is the underlying cause,but if levels are lower, differential diagnoses include the intake of various drugs,compression of the pituitary stalk by other pathology, hypothyroidism, renal failure,cirrhosis, chest wall lesions, or idiopathic hyperprolactinemia. When a pituitary tumour is present, patients often have pressure symptoms in addition to endocrine dysfunction, such as headaches, visual field defects, or cranial nerve deficits (Wang, 2012).

The hyperprolactinemia of hypothyroidism is related to several mechanisms. In response to the hypothyroid state, a compensatory increase in the discharge of central hypothalamic thyrotropin releasing hormone (TRH) results in increased stimulation of prolactin secretion. Asymptomatic patients with intact gonadal and reproductive function and moderately elevated prolactin levels may have macroprolactinemia (Vallette-Kasic, 2002).

PCOS (polycystic ovary syndrome) is a common problem that can cause infertility by inhibiting ovulation, affecting 3.5-10% of the reproductive age of women. For unknown reasons, some women with PCOS may have slightly high PRL levels. PCOS similar to hypoprolactinemic are both common causes of secondary amenorrhea in women. The relationship between PCOS and hyperprolactinemia so far has been reported still with controversial results: it seems that PCOS is very prevalent with hyperprolactinemia, nevertheless there are different reasons of altered regulation of gondotropin secretion, and suggests that these conditions have independent origins. Recent investigators using serial serum sampling have excluded transient elevations of PRL and have shown a less frequent association of these two disorders. According to clinical guidelines PCOS patients with increased PRL levels must be investigated for other causes of hyperprolactinemia, because hyperprolactinemia may be due to a reason of concomitant disease, but not proved the cause-relationship to PCOS.

Treatment of infertility associated with PCOS has changed in the last decade due to the introduction of new medications such as insulin-sensitizing drugs, aromatase inhibitors,gonadotropin treatment etc. (Bracero 2001, Urman, 2006, Escobar-Morreale, 2004).

A high prolactin level can sometimes be related to physical stress. Even drawing blood can by itself cause someone to produce and immediate prolactin-release. PRL eleveation can also detected in response to strong or sudden external stimuli in general, such as stressful environmental conditions, or can be related to physchological reasons. This latter can be evaluated by stress profile or measured by experimental conditions, such as “Screamer Index”, which is shown resulting in values to be parallel to levels of hyperprolactinemia in women. (Harrison, 1988; Cepisky, 1992). On the other hand, anxiety and irritability maybe a result of hyperprolactinemia. In rat models PRL increased the stimulatory effect of ACTHinduced corticosterone secretion (Jaroenporn, 2007).

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