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Investigation of Leptin Status and Some Biochemical Parameters for infertile Women in Gaza Palestine

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Signature: 
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Investigation of Leptin Status and Some Biochemical Parameters for Infertile Women in Gaza Palestine

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نتيجة الحكم على أطروحة ماجستير

بناءً على موافقة شئون البحث العلمي والدراسات العليا بالجامعة الإسلامية بغزة على تشكيل لجنة الحكم على أطروحة الباحثة/ د/ داجرة محمد إبراهيم أبو خاطر لتنيل درجة الماجستير في كلية العلوم قسم العلوم الحياتية - تحليل طبيبة وموضوعها:

تشخيص الانتان ومع بعض العوامل الكيميائية لدى النساء العقمات في قطاع غزة

Investigation of Leptin Status and Some Biochemical Parameters for infertile Women in Gaza Palestine

وبعد المناقشة التي تمت اليوم الثلاثاء 21 رجب 1435 هـ الموافق 20/05/2014م الساعة الواحدة ظهراً، اجتمعت لجنة الحكم على الأطروحة وتم إقرارها من:

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واللجنة تعتمد على هذه الدرجة فإنها توصي بتقديم الله ولزوم طاعته وأن تسخر علمها في خدمة دينها ووطنها.

واهود بالتوافق،

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Investigation of Leptin Status and Some Biochemical Parameters for Infertile Women in Gaza Palestine

Abstract

**Background:** The size of body fat stores is known to influence fertility, indicating a link between adipose tissue and the reproductive system. Thus adipocyte-derived hormone, leptin (Ob protein), is a possible mediator of this effect.

**Objective:** To investigate leptin hormone status among infertile women of unknown causes in Gaza Strip.

**Methodology:** The study is a case control and carried out in Specialized Medical Centers in Gaza Strip. A total of 40 women with primary infertility were interviewed to fill questionnaire and blood analysis was performed. Moreover, forty fertile women were served as controls and has been selected on the basis of being married, having children and matching the case in age and residence. SPSS system was used to analyze the data.

**Results:** The mean ages of controls and patients were 28.2 ± 5.2 and 28.7 ± 5.1 years old; the mean body mass index (BMI) were 24.7 and 26.9 kg/m2, respectively.

The mean serum leptin levels were increased significantly in the unexplained infertile group compared with the fertile one, 19.1 versus 14.0 ng/ml, respectively (P=0.04).

Moreover the serum FSH and LH were increased in patients compared to controls (8.90±5.03 µg 5.09±3.13 mIU/ml, P=0.000) and (7.72±4.77 v 6.53±4.55 mIU/ml, P=0.261), respectively.

Leptin was positively correlated with BMI (r=0.776 and P=0.000) among the study population. Its correlation with FSH and LH were not observed (P>0.05).

**Conclusion:** A significant difference in serum leptin levels between unexplained infertile and fertile women suggests that this cytokine hormone is involved in pathophysiology of unexplained infertility. In contrast, no significant correlation was observed between the leptin and gonadotrophic hormones which suggest the physiological concentration of serum leptin does not directly influence FSH and LH production at hypothalamic level or secretion at anterior pituitary level.

**Key words:** Infertile women, Leptin status, Gonadotrophic hormones, Gaza Strip.
حالة اللجتين وبعض العوامل الكيميائية لدى السيدات العقيمات في مدينة غزة / فلسطين

الملخص:

من المعروف أن حجم مخازن الدهون في الجسم تؤثر على نسبة الخصوبة، لذلك من المفروض وجود علاقة بين الأنسجة الدهنية والجهاز التناسلي. يوجد حاليًا هرمون ينتج من تلك الأنسجة الدهنية يسمى هرمون اللجتين وله صلة بالعقم لدى السيدات اللائي يجربن عدم إنجاب تديرهن.

الهدف:

تهدف الدراسة إلى معرفة دور اللجتين وبعض الهرمونات الأخرى لدى السيدات غير معروف سابع العقم لدى نما في مدينة غزة.

الألات الدراسة:

تتبع الدراسة منهجية اختيار عينة تجريبية وأخرى ضابطة، والتي تم اختيارها من المراكز الطبية المتخصصة في العقم في مدينة غزة.

أجريت هذه الدراسة على مجموعه مكونة من 40 سيدة مصابة بالعقم الأولي (لم يسبق لهن الإنجاب أبدا) بالمقارنة مع 40 سيدة لديهن قدرة منتظمة على الإنجاب (العينة الضابطة)، وتم إجراء مقابلة لعينة الاستبيان وأخذ عينات دم لإجراء الفحوصات الهرمونية المختلفة واستخدمت الحزمة الإحصائية SPSS للتحليلية البيانات والنتائج التي تم الحصول عليها.

النتائج:

أظهرت نتائج الدراسة أن متوسط عمر العينة التجريبية والعينة الضابطة هو 5.14±28.67 و 28.17±5.23 عاماً. وأن متوسط مؤشر كتلة الجسم كان 26.9 و 24.7 كيلوغرام/م2 على التوالي.


وكان هناك نقص فى ميلوحة عند المريض بالمقارنة مع العينة الضابطة．

كما بينت الدراسة وجود ارتباط في الثالث بين مستوى هرمون اللجتين ومؤشر كتلة الجسم.

الخلاصات:

الارتباط الواضح في مستويات اللجتين لدى النساء العقيمات يشير إلى أن الهرمون له دور ذو أهمية كبيرة في الفسيولوجيا المرضية للعقم غير المجري لدى السيدات، في مقابلة لدينا وجود ارتباط كبير بين اللجتين وكلا من هرمون FSH و LH، وذلك يشير إلى أن التركيز الفسيولوجي لأجر موفير اللجتين لا يؤثر مباشرة على إنتاجهما على مستوى الغدة الهرمونية الأمامية.

الكلمات المفتاحية:

النساء العقيمات، المتغيرات البيوكيميائية، اللجتين، غزة.
Dedication

This work is dedicated to: my beloved parents, my uncle; Engineer Nagy Eshaq and my husband Dr. Raft Qudiah.

The dearest to me; my daughter Juwan, brother and sisters.

Dedication is almost expressed to the Palestinian people who have suffered and struggled with the persistence to have a free Palestine.
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My thanks also to all members in The Islamic University of Gaza and Faculty of Science for giving me the opportunity to achieve this research.

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At the end, I am very grateful to those who participated and helped me to complete this study.
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### Abbreviations

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<tr>
<td>BMI</td>
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<tr>
<td>FSH</td>
<td>Follicular stimulating hormone</td>
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<td>GnRH</td>
<td>Gonadotropin- releasing hormone</td>
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<td>IVF</td>
<td>In vitro fertilization</td>
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<td>LH</td>
<td>Luteinizing hormone</td>
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<td>Ob</td>
<td>Obese</td>
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<td>PCOS</td>
<td>Polycystic ovary syndrome</td>
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Chapter 1: Introduction

1.1 Overview

Infertility means not being able to get pregnant after one year of trying (or six months if a woman is 35 or older). Women who can get pregnant but are unable to stay pregnant may also be infertile. Pregnancy is the result of a process that has many steps. To get pregnant: a woman's body must release an egg from one of her ovaries (ovulation), the egg must go through a fallopian tube toward the uterus (womb), a man's sperm must join with (fertilize) the egg along the way and the fertilized egg must attach to the inside of the uterus (implantation); Infertility can happen if there are problem with any of these steps. About one-third of infertility cases are caused by women's problems. Another one third of fertility problems are due to the man. The other cases are caused by a mixture of male and female problems or by unknown problems.

Most cases of female infertility are caused by problems with ovulation. Without ovulation, there are no eggs to be fertilized. Some signs that a woman is not ovulating normally include irregular or absent menstrual periods. This is due to imbalance in the hormones responsible for regulation work of the ovary [1].

The regulation of the ovaries function is mediated primary by two hormones; gonadotropin releasing hormone (GnRH) from hypothalamus and gonadotropin stimulating hormones (FSH and LH) released from anterior pituitary. Follicular stimulating hormone initiates follicular growth, specifically affecting granulosa cells with the concomitant rise in inhibin B. Follicular stimulating hormone levels then decline in the late of follicular phase. This seems to be critical in selecting only the most advanced follicle to proceed to ovulation. At the end of the
luteal phase, there is a slight rise in FSH that seems to be of importance to start the next ovulatory cycle [2].

Luteinizing hormone is necessary to maintain luteal function for the first two weeks. In case of a pregnancy, luteal function will be further maintained by the action of human chorionic gonadotropin (a hormone very similar to LH) from the newly established pregnancy. Luteinizing hormone supports theca cells in the ovary that provide androgens and hormonal precursors for estradiol production [3].

The causes of female infertility generally fall in one of these categories:

Abnormal FSH and LH secretion, polycystic ovary syndrome (PCOS), luteal phase defect, premature ovarian failure, tubal infertility, endometriosis, cervical narrowing or blockage, uterine causes and unexplained infertility [4].

Leptin hormone is the endocrine product of the LEP gene. It is the lookout hormone and the gatekeeper of fat metabolism, monitoring how much energy an organism takes in. It surveys and maintains the energy balance in the body and it regulates hunger via three pathways:

- By counteracting the effects of neuropeptide Y, a potent feeding stimulant secreted by the hypothalamus and certain gut cells.
- By counteracting the effects of anandamide, another feeding stimulant.
- By promoting the production of a-MSH, an appetite suppressant.

Leptin circulates in blood in free form and bound to proteins. Leptin levels vary exponentially, not linearly, with fat mass. The levels in blood are higher between midnight and early morning, perhaps suppressing appetite during the night. The diurnal rhythm of blood leptin levels can be modified by meal-timing. It is also directly tied to insulin levels.

Many people these days are Leptin resistant and there are many health problems tied to this problem. High leptin levels have been tied to high blood pressure, obesity, decrease fertility, heart disease and stroke, as well as blood sugar related problems [5].
Chapter 1: Introduction

There are many studies conducted in Gaza Strip about leptin hormone, for example: lifestyle, serum leptin and lipid profiles of obese adolescents in secondary schools by Samaher Younis [6], assessment of leptin hormone and some cardiac biomarkers among congestive heart failure patients by Mohammed El-elaham [7], leptin status and some biochemical parameters in germ cell aplasia among infertile men by Eihab Jaber [8], role of leptin, gerlin and insulin hormones among type II diabetes mellitus patients by Fatima snymah [9] But does not have any previous study reported the relationship of this hormone with female infertility.

Therefore, this will be the first study to assess leptin status and relate it to female reproductive hormones among unexplained infertile women in Gaza strip.

1.2 General Objective

The general objective of the present study is to investigate leptin hormone status among infertile women of unknown causes in Gaza Strip.

1.3 Specific objectives

1. To determine leptin level in patients and compare it with that of controls.
2. To measure FSH, LH levels in study population.
3. To assess BMI in patients versus controls.
4. To investigate the possible relationships between leptin and the previous studied parameters.

1.4 Significance

1. In Gaza Strip, this is the first study to assess leptin status among infertile women.
2. Understanding the possible role of leptin hormone in female infertility could be useful in treatment strategy.
Chapter 2

Literature review

2.1 Structure of ovaries

The primary female reproductive organs, or gonads, are the two ovaries. Each ovary is a solid, ovoid structure about the size and shapes of an almond, about 3.5 cm in length, 2 cm wide, and 1 cm thick. The ovaries are located in shallow depressions, called ovarian fossae, one on each side of the uterus, in the lateral walls of the pelvic cavity. They are held loosely in place by peritoneal ligaments [10].

The ovaries are covered on the outside by a layer of simple cuboidal epithelium called germinal (ovarian) epithelium. This is actually the visceral peritoneum that envelops the ovaries. Underneath this layer there is a dense connective tissue capsule, the tunica albuginea. The substance of the ovaries is distinctly divided into an outer cortex and an inner medulla. The cortex appears more dense and granular due to the presence of numerous ovarian follicles in various stages of development (Figure 2.1). Each of the follicles contains an oocyte, a female germ cell. The medulla is loose connective tissue with abundant blood vessels, lymphatic vessels, and nerve fibers [11].
Chapter 2: Literature review

2.2 Oogenesis/Meiosis

Figure 2.1 Cross section of the ovary [12].

Figure 2.2 Process of oogenesis [13].
Female sex cells, or gametes, develop in the ovaries by a form of the meiosis called oogenesis. The sequence of events in oogenesis is similar to the sequence in spermatogenesis, but the timing and final results are different. Early in fetal development, primitive germ cells in the ovaries differentiate into oogonia. These divide rapidly to form thousands of cells, still called oogonia, which have a full complement of 46 (23 pairs) chromosomes. Oogonia then enter a growth phase, enlarge, and become primary oocytes (Figure 2.2). The diploid (46 chromosomes) primary oocytes replicate their DNA and begin the first meiotic division, but the process stops in prophase and the cells remain in this suspended state until puberty [13]. Many of the primary oocytes degenerate before birth, but even with this decline, the two ovaries together contain approximately 700,000 oocytes at birth. This is the lifetime supply, and no more will develop. This is quite different than the male in which spermatogonia and primary spermatocytes continue to be produced throughout the reproductive lifetime. By puberty the number of primary oocytes has further declined to about 400,000 [14].

Beginning at puberty, under the influence of FSH, several primary oocytes start to grow again each month. One of the primary oocytes seems to outgrow the others and it resumes meiosis I. The other cells degenerate. The large cell undergoes an unequal division so that nearly all the cytoplasm, organelles, and half the chromosomes go to one cell, which becomes a secondary oocyte. The remaining half of the chromosomes go to a smaller cell called the first polar body. The secondary oocyte begins the second meiotic division, but the process stops in metaphase. At this point ovulation occurs. If fertilization occurs, meiosis II continues. Again this is an unequal division with all of the cytoplasm going to the ovum, which has 23 single-stranded chromosomes. The smaller cell from this division is a second polar body. The first polar body also usually divides in meiosis I to produce two even smaller polar bodies. If fertilization does not occur, the second meiotic division is never completed and the secondary oocyte degenerates. Here again there are obvious differences between the male and female. In
spermatogenesis, four functional sperm develop from each primary spermatocyte. In oogenesis, only one functional fertilizable cell develops from a primary oocyte. The other three cells are polar bodies and they degenerate [15].

2.3 Hormonal control of oogenesis

Early hormone control helps the follicle to develop and forces oogenesis to occur in a cycle in a certain time period. The control begins in the hypothalamus which produces GnRH. GnRH is received by receptors in the anterior pituitary gland, which responds by releasing FSH and LH.

At the beginning of development, the granulosa cells express FSH receptors, which stimulate growth of the follicle (Figure 2.3). Theca cells express receptors for LH, which stimulates growth of the corpus luteum. Theca cells also produce androgens, which the granulosa cells convert to estrogen. Estrogen act back on the anterior pituitary gland to further FSH and LH surges, and also supports the growth of the endometrium. At some point, the dominate follicle begins to secrete inhibin B, which acts back on the anterior pituitary gland to stop producing FSH. Only the dominant follicle, which is now FSH independent, will continue to grow [16].

During further development, the granulosa cells increase their FSH receptors and express LH receptors, while the Theca cells increase their LH receptors. This surge in hormone reception results in ovulation. After ovulation, if fertilization occurs, the corpus luteum secretes progesterone that supports the further growth of the endometrium. If, however, fertilization does not take place, then the hormone levels drop, the corpus luteum breaks down, no longer secreting progesterone, so that the endometrium sloughs off producing menstruation [16].
Chapter 2: Literature review

2.4 Ovulation

Ovulation, prompted by LH from the anterior pituitary gland, occurs when the mature follicle at the surface of the ovary ruptures and releases the secondary oocyte into the peritoneal cavity. The ovulated secondary oocyte, ready for fertilization is still surrounded by the zona pellucida and a few layers of cells called the corona radiata. If it is not fertilized, the secondary oocyte degenerates in a couple of days. If a sperm passes through the corona radiata and zona pellucida and enters the cytoplasm of the secondary oocyte, the second meiotic division resumes to form a polar body and a mature ovum [18].

After ovulation and in response to LH (Figure 2.3), the portion of the follicle that remains in the ovary enlarges and is transformed into a corpus luteum. The corpus luteum is a glandular structure that secretes progesterone and some estrogens. Its fate depends on whether fertilization occurs. If fertilization does not take place, the corpus luteum remains functional for about 10 days then it begins to degenerate into a corpus albicans, which is primarily scar tissue, and its hormone output ceases. If fertilization occurs, the corpus luteum persists and

Figure 2.3: Hormone levels during oogenesis [17].
continues its hormone functions until the placenta develops sufficiently to secrete the necessary hormones. Again, the corpus luteum ultimately degenerates into corpus albicans, but it remains functional for a longer period of time [19].

2.4.1 Hormonal imbalance and infertility

Female infertility is often caused by a woman’s inability to ovulate, or release an egg [20]. When a woman isn’t ovulating, the problem is usually an imbalance in her hormones. In other words, she may be producing too little of one hormone or too much of another. Often, a woman will experience hormonal symptoms that can give clues about an underlying hormonal imbalance. Fortunately, hormonal imbalances are not hard to detect and treatments are straightforward and relatively effective [21].

Hormonal imbalances can be a result of:

1- Glandular problem.
2- Ovulatory disorders.
3- Polycystic Ovary Syndrome (PCOS).
4- Premature menopause.
5- Abnormal cervical mucus.
6- Stress.

2.4.2 Measurement of FSH and LH Levels in female

The correct time to measure the FSH and LH levels in the blood is on the third day of the menstruation cycle. On day 3, normal FSH levels would be 3-20 mIU/ml while normal LH levels would be less than 7 mIU/ml. FSH and LH levels in PCOS (ovarian cyst) are found to be abnormal. LH levels higher than FSH levels indicate that the woman has ovarian cyst or PCOS [22].
Low FSH levels and low LH levels in women indicate secondary ovarian failure due to a pituitary or hypothalamic problem while low FSH serum levels are associated with increased risk of ovarian cancer. High LH levels and high FSH levels are consistent with primary ovarian failure [23-24].

2.5 Leptin hormone

2.5.1 Definition and site of secretion

Leptin was identified through positional cloning of the obese (ob) gene, which is mutated in the massively obese ob/ob mouse, and it has a vital role in regulating food intake and energy expenditure [25]. Leptin is a protein that consists of 167 amino acids and has a molecular weight of about 16 kD or 16,000 g/mol (Figure 2.4). The DNA sequence for the protein was determined by Friedman in 1994 [26].

![Structure of leptin](image)

**Figure 2.4** Structure of leptin [27].
2.5.2 Mechanism of leptin action

Leptin binding induces dimerization of the leptin receptor, activating a kinase molecule (JAK-2) which phosphorylates tyrosine (Y) residues on the receptor. This creates phosphotyrosine docking sites on for signal transducers and activators transcription (STAT) proteins (Figure 2.5). These STAT proteins then dimerize and return to the nucleus to control expression of response genes [28].

![Figure 2.5 Mechanism of leptin action](image)

Genes expression in response to leptin action causes an increase in a prohormone called proopiomelanocortin (POMC). This protein is processed into smaller pieces which each act as individual hormones. One of products of POMC is alfa-melanocyte stimulating hormone (alpha-MSH) which decreases hunger. The enzyme that processes POMC into a-MSH is called proenzyme convertase1 (PC-1).
a-MSH activates a receptor on cell surfaces in hypothalamus called type 4 melanocortin receptor (MC4R). Activation of this receptor is like flipping a switch, telling the cell to inhibit appetite [30].

![Diagram of the hypothalamic leptin-melanocortin system](image)

**Figure 2.6** The Hypothalamic Leptin-Melanocortin System [30].

### 2.5.3 Action of leptin on ovary

Leptin from adipose tissue may signal the reproductive system to indicate whether adequate energy stores are available for normal reproduction [31]. Research along these lines indicates leptin may be important in controlling the hypothalamic-pituitary-gonadal axis and thus influence states of reproductive transitions, such as puberty [32].

Evidence has also emerged indicating a potential direct role for leptin in regulation of mammalian ovarian function as well as oocyte and preimplantation embryo development [33].
Leptin receptors have been identified in theca and granulose cells, cumulus cells [33], oocytes and embryos [34]. In addition, the ovary may be a site of leptin synthesis. Leptin and/or its mRNA have been identified in ovaries, oocytes, and preimplantation embryos. Last, follicular and uterine fluids contain leptin suggesting its availability to act on follicle, oocyte, and preimplantation embryo leptin receptors [35].

Direct effects of leptin on regulation of ovarian steroidogenesis have been investigated in numerous cell culture systems and species. Many of these studies indicate leptin attenuates gonadotropin or growth factor-stimulated steroidogenesis in isolated theca or granulosa cells [36].

Additionally, a biphasic effect of leptin was observed in pig granulosa cells, where physiological level of leptin were stimulatory to activity of steroidogenic acute regulatory protein (StAR) and steroidogenesis, while higher levels resulted in inhibition of StAR expression and estrogen production [37]. Leptin also increases granulosa cell aromatase activity [38]. Because oocytes and embryos contain leptin receptors, recent studies have focused on potential direct effects of leptin on oocyte maturation and early embryo development.

Culturing preovulatory mouse follicles in the presence of leptin increased subsequent rates of oocyte germinal vesicle breakdown and reduces cumulus cell coupling in oocytes [39].

Interestingly, reports on leptin’s direct effects on embryo development are contradictory. Leptin exposure during culture of two cell mouse embryos compromised blastocyst development and hatching [40].
Conversely, treatment of two-cell embryos with increasing concentrations of leptin increased development to the blastocyst, expanded blastocyst and hatched blastocyst stages, while increasing the total blastocyst cell number [41].

The research also observed a significant positive correlation between elevated leptin : BMI ratios and inferior quality of human embryos, resulting in lower implantation and live birth rate [42].

2.5.4 Degradation of leptin

The half-life of leptin is 9.4 ± 3.0 min, and the leptin production rate was 3.6 ± 1.2 ng /100 g fat/min. The human kidney plays a substantial role in leptin removal from plasma by taking up and degrading the peptide. Renal leptin uptake could account for 80% of all leptin removal from plasma [43].

2.5.5 Leptin resistant

Leptin become resistant by the same general mechanism that insulin become resistant by continuous overexposure to high levels of the hormone. If you eat a diet that is high in sugar particularly fructose, grains, and processed foods, the sugar gets metabolized in your fat cells and the fat releases surges in leptin. Over time, if your body is exposed to too much leptin, it will become resistant [44].

The only known way to re-establish proper leptin signaling is to prevent those surges, and the only known way to do that is via diet. As such, diet can have a more profound effect on your health than any other known modality of medical treatment.

A strategic whole food diet that emphasizes good fats and avoids blood sugar spikes coupled with targeted supplements will enhance leptin sensitivity so that your brain can once again hear the feedback signals from these hormones [44].
2.6 Previous studies:-

In 2005 Shafi, et al., assessed the serum leptin levels in females with primary infertility compared to fertile subject. The results revealed that serum leptin levels were significantly raised in infertile women (69.7±40.2ng/ml) as compared to fertile controls (41.1±27.3ng/ml) with p=0.000. Mean body mass index (BMI) was also found to be significantly higher in infertile women (27.2±6.8kg/m2) as compared to fertile females (24.1±5.2kg/m2) with p-value of 0.019. Moreover, a strong positive correlation was found between BMI and leptin levels as leptin levels increased with increase in BMI. Mean leptin levels in overweight women were significantly higher (81.4±32.4ng/ml) as compared to normal weight women (30.6±20.6ng/ml) with p=0.000 [45].

In 2007 Zabut, et al., studied the relationship between leptin hormone and BMI among adult individuals in Gaza Strip. The results revealed that serum leptin levels were significantly higher (p=0.00) for the females (mean=72.40 ng/ml) than for the males (mean= 44.05 ng/ml). They concluded serum leptin levels were increased with increasing BMI [46].

In 2007 Demir, et al., compared serum levels of leptin in women with unexplained infertility with fertile subjects. Considering normal weight subjects, mean serum leptin levels were increased significantly in the unexplained infertile group compared with the fertile group 7.2 (range, 4.3-10.4) versus 3.5 (range, 1.9-6.2)ng/ml, respectively; p<0.0001. The significant increase in serum leptin levels was observed also in overweight patients 6.8 (range, 1.3-5.2) versus 3.3 (range, 4.2-8.9)ng/ml, respectively; p<0.0001. They suggested that this cytokine hormone may be involved in pathophysiology of unexplained infertility [47].
In 2008 Mukhtiar Baig studied role of serum leptin in primary infertile females and he found serum leptin was significantly correlated with BMI in all infertile and fertile groups (except in underweight fertile one), in preovulatory and luteal phase of the menstrual cycle. He also found that serum leptin levels were different in all four infertile groups (underweight, normal, overweight and obese ones) as compared to respective fertile groups having similar BMI. He concluded that serum leptin levels might be related in the process of fertility. Researcher also studied the level of FSH and LH and their relationship to the leptin hormone at preovulatory and luteal phase of the menstrual cycle, he found that serum leptin level does not influence LH and FSH level in fertile control group as well as infertile subjects. Therefore the alternation in gonadotrophin level during menstrual cycle are independent of serum leptin [48].

In 2013 Reshma, et al., studied the relationship between serum leptin and sex hormones (FSH and LH) in primary infertile women compared to fertile subject. The results revealed that serum leptin levels in fertile and infertile female with (BMI>24) were significantly (p<0.01) higher than normal with (BMI<24). The values of LH and FSH were significantly (p<0.01) higher in obese (BMI>24) infertile women than normal (BMI<24) subjects. This study has concluded that hyperleptinemia is associated with infertility in women. Sex hormonal imbalance may also be associated with BMI and serum leptin in infertility [49].
Chapter 3

Materials and Methods

3.1 Study design

The present study is a case control one.

3.2 Target population

The target population was infertile women with unknown reasons.

3.3 Sampling and sample size

The sample was collected from women who visiting the Specializing Medical Centers in Gaza with at least 3 years duration of infertility. The number of patients (sample size) was 40 women with primary infertility. A total number of 40 controls was selected at the basis of being married, having children and matching the case in age and residence.

3.4 Inclusion criteria

- Infertile women aged 18-40 years.
- Having regular menstrual cycle.
- Their husbands having normal sperm parameters (sperm motility equal or more than 40%).
Chapter 3: Materials and Methods

Exclusion criteria

- Fertile women.
- Infertile women aged less than 18 and more than 40 years.
- Those females whom semen analysis of their husbands' gave abnormal results.
- Female reproductive system diseased.

3.5 Ethical consideration

The necessary approval to conduct this study was obtained from local ethical committee in Gaza Strip.

3.6 Data collection

3.6.1 Questionnaire interview

A face to face interview was used for filling questionnaire which is designated for matching the study need. The questionnaire (Annex 1) was based on female infertility patient questionnaire with some modification related to medical history [50]. During the study the interviewer was explained to the patients any of the confuse question that were not clear to them. Most questions was yes/no ones, which offer a dichotomous choice. The questionnaire was piloted with 5 patients, and was modified as necessary to improve reliability and validity.

3.6.2 Body mass index

Body mass index was calculated as the ratio of body weight in Kg/height in meter square. Women with BMI=18.5-24.9 was considered to have normal weight. Women with BMI=25-29.9 was classified overweight, women with BMI more than or equal 30 was considered obese [51].
3.6.3 Blood sampling and processing

Venous blood sample (5 ml) was drawn by the researcher herself into vacutainer tube from each patient. Blood left for a while without anticoagulant to allow blood to clot. Then serum samples was obtained by centrifugation at room temperature at 300 rpm/10 min for hormonal analysis.

3.6.4 Hormonal analysis

3.6.4.1 Determination of serum leptin

Determination of human serum leptin level was carried out by competitive enzyme immunoassay Asbach Medical Products [52].

Principle

The AMP 40-E22900 Human Leptin ELISA Kit is based on the sandwich-type immunoassay. In the assay, Standards, Controls and unknown serum or plasma samples were incubated in microtiter wells which have been coated with anti-human leptin antibody. After incubation and washing, the wells were treated with another anti-human leptin detection antibody labeled with the enzyme horseradish peroxidase (HRP). After a second incubation and washing step, the wells were incubated with the substrate tetramethylbenzidine (TMB). An acidic stopping solution 0.2 M sulphuric acid was then added and the degree of enzymatic turnover of the substrate was determined by dual wavelength absorbance measurement at 450 and 620 nm. The absorbance measured was directly proportion to the concentration of human leptin present. A set of human leptin standards was used to plot a standard curve of absorbance.
versus human leptin concentration from which the human leptin concentration in the sample can be calculated.

**Assay procedure**

Annabel all specimens and reagents to reach room temperature (25C) and mix thoroughly by gentle inversion before use. Standards, Controls and samples should be assayed in duplicate.

1. Secure the desired number of Microtiter wells in the holder.
2. Dispense 15 micro liter of each Standard, controls and samples with new disposable tips into appropriate wells.
3. Dispense 100 micro liter assay buffer into each well.

Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.

4. Incubate for 120 min at room temperature (without covering the plate).
5. Briskly shake out the contents of the wells.

Rinse the wells 3 times with diluted Wash Solution.

6. Add 100 micro liter antiserum to each well.
7. Incubate for 30 min at room temperature.
8. Briskly shake out the contents of the wells.

Rinse the wells 3 times with diluted Wash Solution.

9. Dispense 100 micro liter enzyme complex into each well.
10. Incubate for 30 min at room temperature.
11. Briskly shake out the contents of the wells.
Rinse the wells 3 times with diluted Wash Solution.

12. Add 100 micro liter of substrate solution to each well.

13. Incubate for 15 min at room temperature.

14. Add 50 micro liter of stop solution to each well.

15. Determine the absorbance of each well at 450 with micro liter plate reader. It is recommended that the wells be read within 10 min after adding the Stop Solution.

Calculation

1- The mean absorbance for each standard, control and samples were calculated.

2- Plot the log of human leptin concentration in ng/ml along the x-axis versus the mean absorbance reading for each of the standards along the y-axis versus, using a linear curve-fit. Alternatively, the data can be plotted linear vs. linear and a smoothed spine curve-fit can be used.

3- Determine the human leptin concentration of controls and samples from the standard curve by matching their mean absorbance readings with the corresponding human leptin concentration.

Normal reference value of leptin in follicular and luteal phase for adult female are

3.7-11.5 ng/ml [52].
3.5.6.2 Determination of serum luteinizing hormone

Luteinizing hormone level was determined by using ELISA DRG kit for LH [53].

**Principle**

The DRG LH ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The microtiter wells are coated with a monoclonal [mouse] antibody directed towards a unique antigenic site on a LH molecule. An aliquot of patient sample containing endogenous LH is incubated in the coated well with enzyme conjugate, which is an anti-LH monoclonal antibody conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off. The amount of bound peroxidase is proportional to the concentration of LH in the sample. Having added the substrate solution, the intensity of color developed is proportional to the concentration of LH in the patient sample.

**Kit component**

One strip holder containing 96 microtitration wells coated with anti-LH antibody, six LH reference standards with concentration of approximately (0, 10, 20, 40, 100 and 200 mIU/mL), enzyme conjugate, TMB chromogenic solution, stop solution and wash solution concentrate.

**Assay procedure**

All specimens and reagents to reach room temperature (25°C) and mix thoroughly gentle inversion before use. Standards, controls and samples should be assayed in duplicate.

1. Microtitration strip was marked to be used.
2. Dispense 25 µL of each Standard, controls and samples with new disposable tips into appropriate wells.

3. Dispense 100 µL Enzyme Conjugate into each well.

Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.

4. Incubate for 30 minutes at room temperature.

5. Briskly shake out the contents of the wells. Rinse the wells 5 times with washed solution (400 µL per well).

6. Add 100 µL of Substrate Solution to each well.

7. Incubate for 10 minutes at room temperature.

8. Stop the enzymatic reaction by adding 50 µL of Stop Solution to each well.

9. Determine the absorbance (OD) of each well at 450±10 nm with a microtiter plate reader.

It is recommended that the wells be read within 10 minutes after adding the stop solution.

Calculation:

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal in mlU/ml along (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration of LH in mIU/ml from the standard curve.

**Normal reference value of LH in follicular and luteal phase for adult female are**

1.0 – 20 mIU/ml [53].

### 3.6.4.3 Determination of follicle stimulating hormone

Follicle stimulating hormone level was determined by using ELISA DRG kit for FSH [54].

**Principle**

The DRG FSH ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site on a FSH molecule. An aliquot of patient sample containing endogenous FSH is incubated in the coated well with enzyme conjugate, which is an anti-FSH monoclonal antibody conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off. The amount of bound peroxidase is proportional to the concentration of FSH in the sample. Having added the substrate solution, the intensity of color developed is proportional to the concentration of FSH in the patient sample.

**Assay procedure**

All specimens and reagents to reach room temperature (25°C) and mix thoroughly gentle inversion before use. Standards, controls and samples should be assayed in duplicate.

1. Microtitration strip was marked to be used.
2. Dispense 25 µL of each Standard, controls and samples with new disposable tips into appropriate wells.

3. Dispense 100 µL Enzyme Conjugate into each well.

Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.

4. Incubate for 30 minutes at room temperature.

5. Briskly shake out the contents of the wells.

6. Rinse the wells 5 times with washed solution (400 µL per well).

7. Add 100 µL of Substrate Solution to each well.

8. Incubate for 10 minutes at room temperature.

9. Stop the enzymatic reaction by adding 50 µL of Stop Solution to each well.

10. Determine the absorbance (OD) of each well at 450±10 nm with a microtiter plate reader.

It is recommended that the wells be read within 10 minutes after adding the Stop Solution.

**Calculation**

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical(Y) axis and concentration on the horizontal in mlU/ml along (X) axis.
Chapter 3: Materials and Methods

3. Using the mean absorbance value for each sample determine the corresponding concentration of FSH in mlU/ml from the standard curve.

**Normal reference value of FSH in follicular and luteal phase for adult female are**

2.0 – 10 mlU/ml [54].

### 3.7 Statistical analysis

Data were computer analyzed using SPSS version 18.0. Simple distribution of the study variables and the cross tabulation were applied. Chi-square ($X^2$) was used to identify the significance of the relations, associations and interactions among various nominal variables. The independent sample t-test procedure was used to compare means of quantitative variables by the separated cases into two qualitative groups such as the relationship between patients and controls hormones. Person's correlation test between leptin and other studied variables was applied.

Ranges as minimum and maximum values were used.

The percentage difference was calculated according to the formula:

\[
\% \text{ difference} = \left( \frac{|V1-V2|}{(V1+V2)/2} \right) \times 100
\]

Microsoft Excel program version 11.0 for correlation graph plotting.

The results were accepted as statistical significant when the p-value was less than 5\% (p<0.05).
Chapter 4

Results

4.1 Demographic characters of the study population

Table 1 illustrates demographic data of the study population. Age classification showed that 23 (57.5%) controls and 24 (60%) cases were ≤29 years old. Age group 30-34 years comprised 13 (32.5%) controls and 9 (22.5%) cases. Controls and patients aged >34 years old were 4 (10%) and 7 (17.5%), respectively. The difference between controls and patients in term of age distribution was not significant ($X^2=1.56$, P=0.45). The mean ages of controls and patients were 28.2±5.2 and 28.7±5.1 years old with range of 19-40 for both of them. The independent sample t-test also showed no difference between mean ages of controls and patients (P=0.668).

Analysis of educational status of the study population showed that 11 (27.5%) controls and 22 (55%) patients had a university degree, 21 (52.5%) and 15 (37.5%) finished secondary school and 8 (20%), 3 (7.5%) had passed primary school. The difference between various education levels of controls and patients was significant ($X^2=6.94$, P=0.029). Regarding family history, 0 (0%) controls and 5 (12.5%) patients reported that they have a family history of female infertility ($X^2=5.33$, P=0.027). Moreover, 23 (57.5%) patients reported drugs consumption for activation of hormones (P=0.000).
Table 4.1 Demographic data of the study population

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Control (n=40)</th>
<th>Patients (n=40)</th>
<th>Chi-Square test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>≤29</td>
<td>23</td>
<td>57.5%</td>
<td>24</td>
<td>60.0%</td>
</tr>
<tr>
<td>30-34</td>
<td>13</td>
<td>32.5%</td>
<td>9</td>
<td>22.5%</td>
</tr>
<tr>
<td>&gt;34</td>
<td>4</td>
<td>10.0%</td>
<td>7</td>
<td>17.5%</td>
</tr>
<tr>
<td>Mean±SD (years)</td>
<td>28.2±5.2</td>
<td>28.7±5.1</td>
<td>t=0.431</td>
<td>0.668</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Education</th>
<th>university</th>
<th>11</th>
<th>27.5%</th>
<th>22</th>
<th>55.0%</th>
<th>6.94</th>
<th>0.029</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>secondary</td>
<td>21</td>
<td>52.5%</td>
<td>15</td>
<td>37.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>primary</td>
<td>8</td>
<td>20.0%</td>
<td>3</td>
<td>7.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family history</td>
<td>yes</td>
<td>0</td>
<td>0%</td>
<td>5</td>
<td>12.5%</td>
<td>5.33</td>
<td>0.027</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>40</td>
<td>100%</td>
<td>35</td>
<td>87.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug consumption</td>
<td>yes</td>
<td>0</td>
<td>0%</td>
<td>23</td>
<td>57.5%</td>
<td>32.28</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>40</td>
<td>100%</td>
<td>17</td>
<td>42.5%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.2 Distribution of BMI among the study population

Table 4.2 shows the distribution of various classes of BMI among the study population. The numbers of normal, overweight and obese patients were 9 (22.5%), 23 (57.5%) and 8 (20%) whereas in controls the numbers were 22 (55%), 18 (45%) and 0 (0%), respectively (P=0.001).
**Table 4.2** Distribution of BMI among the study population.

<table>
<thead>
<tr>
<th>BMI*</th>
<th>Control (n=40)</th>
<th>Patient (n=40)</th>
<th>Chi-Square test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>22</td>
<td>9</td>
<td>14.06</td>
<td>0.001</td>
</tr>
<tr>
<td>Overweight</td>
<td>18</td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obese</td>
<td>0</td>
<td>8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*People with BMI=18.5-24.9 were considered to have normal weight, people with BMI=25.0-29.9 were classified overweight; people with BMI ≥ 30.0 were considered obese [55].

**4.3 Serum leptin of the study population**

Mean levels of serum leptin among study population is presented in table 4.3 and figure 4.1.

The mean levels of leptin were significantly increased in patients compared to controls with percentage difference of 31% (19.1±15.1 v 14.0±8.1 ng/ml, P=0.04).

**Table 4.3** Leptin levels of the study population

<table>
<thead>
<tr>
<th>Serum leptin (ng/ml)</th>
<th>Controls (n=40)</th>
<th>Patients (n=40)</th>
<th>t-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group Statistics</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>14.0±8.11</td>
<td>19.1±15.1</td>
<td>5.112</td>
<td>0.041</td>
</tr>
<tr>
<td>Range</td>
<td>(3.1-48.2)</td>
<td>(1.2-82.6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Reference range: 3.7-11.5 ng/ml**

All values are expressed as Mean±SD.

P<0.05: Significant
4.4 Luteinizing hormone and follicle stimulating hormone of the study population

Tables 4.4 & 4.5 and figures 4.2 & 4.3 illustrate LH and FSH levels of the study population. The mean levels of LH and FSH were increased in patients compared to controls showing percentage difference 17% and 54%, respectively (7.72 ± 4.78 v 6.53 ± 4.56 mLU/ml, P=0.261 and 8.90 ± 5.04 v 5.09±3.13 mLU/ml, P=0.000, respectively).

Table 4.4 LH levels of study population

<table>
<thead>
<tr>
<th>Serum LH (mLU/ml)*</th>
<th>Group Statistics</th>
<th>Controls n=40</th>
<th>Patients n=40</th>
<th>t-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.10-22.4</td>
<td>6.53±4.56</td>
<td>7.72±4.78</td>
<td>1.131</td>
<td>0.261</td>
</tr>
<tr>
<td></td>
<td>(min -max)</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*LH: Luteinizing hormone

Reference value: 1.0 – 20 mLU/ml

All values are expressed as Mean±SD, P>0.05: not significant.
Figure 4.2 Mean serum LH levels of the study population

Table 4.5 FSH levels of study population

<table>
<thead>
<tr>
<th>Serum FSH (mlU/ml)**</th>
<th>Group Statistics</th>
<th>Controls n=40</th>
<th>Patients n=40</th>
<th>t-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td>5.09±3.13</td>
<td>8.90±5.04</td>
<td>4.011</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Range (min - max)</td>
<td>(1.40 - 15.3)</td>
<td>(2.3 - 22.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**FSH: follicle stimulating hormone;

Reference range: 2.0 – 10 mlU/ml.

All values are expressed as Mean±SD, P<0.05: Significant.
Figure 4.3 Mean serum FSH levels of the study population.

4.5 Leptin correlations

4.5.1 Leptin levels in relation to gonadotropin hormones of the study population

Table 4.6 illustrates the results of parson correlation between serum leptin and LH or FSH levels. Leptin level showed no significant positive correlation with LH or FSH ($r=0.156$, $P=0.166$ and $r=0.112$, $P=0.324$, respectively).

Table 4.6 Leptin levels in relation to LH and FSH of the study population

<table>
<thead>
<tr>
<th>Result LH</th>
<th>Pearson Correlation($r$)</th>
<th>Sig. (2-tailed)*</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.156</td>
<td>0.166</td>
<td>80</td>
</tr>
<tr>
<td>Result FSH</td>
<td>Pearson Correlation</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.112</td>
<td>0.324</td>
<td>80</td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.05 level (2-tailed).
4.5.2 Leptin level in relation to body mass index of the study population

Table 4.7 and Figure 4.4 points out the correlation between leptin level and BMI. The person correlation test showed significant positive correlation between leptin level and BMI ($r=0.766$, $P=0.000$).

**Table 4.7** The correlation between leptin level and BMI of the study population

<table>
<thead>
<tr>
<th>Body mass index</th>
<th>Pearson Correlation</th>
<th>Result leptin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.766</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>N</td>
<td></td>
<td>80</td>
</tr>
</tbody>
</table>

**Figure 4.4** Leptin in relation to BMI of the study population
Chapter 4: Results

4.5.3 Leptin level in relation to LH and FSH of the cases

Table 4.8 illustrates the results of parson correlation between serum leptin and LH or FSH levels among cases only. Leptin level showed not significant positive correlation with LH or FSH (r=0.218, P=0.177 and r=0.018, P=0.913, respectively).

**Table 4.8 Leptin levels in relation to LH and FSH of the cases**

<table>
<thead>
<tr>
<th></th>
<th>result leptin</th>
<th>result LH</th>
<th>Pearson Correlation(r)</th>
<th>0.218</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sig. (2-tailed)</td>
<td></td>
<td>0.177</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
<td></td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>result FSH</td>
<td>Pearson Correlation</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sig. (2-tailed)</td>
<td></td>
<td>0.913</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
<td></td>
<td>40</td>
</tr>
</tbody>
</table>

4.5.4 Leptin level in relation to body mass index of the cases

Table 4.9 and **Figure 4.5** point out the correlation between leptin level and BMI among the cases. The person correlation test showed significant positive correlation between leptin level and BMI (r=0.739, P=0.000).

**Table 4.9 The correlation between leptin level and BMI of the cases**

<table>
<thead>
<tr>
<th>Body mass index</th>
<th>Pearson Correlation</th>
<th>Result leptin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pearson Correlation</td>
<td>0.739</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>40</td>
</tr>
</tbody>
</table>
Figure 4.5 Leptin in relation to BMI among the cases
Chapter 5

Discussion

Fertility is the natural capability to giving life. As a measure, "fertility rate" is the number of children born per couple, person or population. Worldwide, According to the Centers for Disease Control, 1/3 of the diagnosed cases is due to female infertility, 1/3 of diagnosed cases is linked to male infertility, and the remaining cases of infertility are due to a combination of factors from both partners. For approximately 20% of couples, the cause cannot be determined. Women are born with a finite number of eggs. Thus, as the reproductive years progress, the number and quality of the eggs diminish. The chances of having a baby decrease by 3% to 5% per year after the age of 30. This reduction in fertility is noted to a much greater extent after age 40 [56].

The researchers found that the levels of female infertility were similar in 1990 and 2010, with only a slight overall decrease in primary infertility and a modest overall increase in secondary infertility (0.4%).

The primary infertility rates among women wanting children also varied by region, from 1.5% in Latin America and the Caribbean in 2010, to 2.6% in North Africa and the Middle East. With a few exceptions, global and country patterns of secondary infertility were similar to those of primary infertility [57].

However, in Gaza strip there are under-reporting or even no real figures on female infertility. Therefore, this is the first study to assess leptin status and it is relation to female reproductive hormones among unexplained infertile women in Gaza strip.
5.1 Sociodemographic characters of the study population

The present study was carried out on 40 patients with unexplained infertility. The mean age of patients (28.7 years) was lower than reported from west bank (30.1 years) study [58]. The younger age of our patient samples could be explained on the basis that the most women seeking out to have children at young age immediately after marriage.

Female age is a dependent factor because the fertility clearly declines with advancing age, especially after the mid-30s, and women who conceive are at greater risk of pregnancy complications [59].

It was found that 55% of patients and 27.5% of controls were university stage do reflect educated community. There was significant difference between controls and patients regarding family history and drug consumption. They are considered a risk factor for infertility. In addition, most of these patients undergone to in vitro fertilization; this require take many of drugs for stimulating hormones.

5.2 BMI of the study population

Data presented here showed that 8 (20.0 %) of patients were obese compared to 0 (0%) controls. Moreover 23 (57.5%) of patients were overweight compared to 18 (45.0%) controls. This finding indicates the increase in weight than normal range considered a risk factor for female infertility. Female infertility was reported to be associated with higher incidence of obesity [60]. The scientists explained the relationship between infertility and excess weight that estrogen is found in two sources in the body: the ovary and the adrenal gland. The ovary makes estrogen in quantities depending on the phase of the menstrual cycle. The adrenal glands make something called 'androstenedione'. These hormones are connected to cholesterol
and in the case of androstenedione; fat cells convert it into an estrogen called estrone. If someone is significantly overweight, the oversupply of estrogen from this conversion will affect how the ovary's function. This causes disruption to the natural ovulation cycle and often leads to infertility [61].

Obesity is also often associated with increased insulin production and insulin resistance. These two factors are believed to contribute to hyperandrogenism in obesity because insulin is important for the regulation of sex hormone production. This can be seen in obese women with PCOS, where excess weight gain leads to excessive insulin production, promoting increased androgen secretion and abnormal follicles in the ovary. This results in an overall disruption in ovarian and menstrual activity, causing fertility problems [62]. High BMI also found to be lead failure rates of successful pregnancy even after employing artificial methods including stimulation of ovulation and assisted conception [63].

### 5.3 Hormonal profile of the study population

Hormonal profile of the study population showed that the mean level of leptin was significantly increased in patients compared to controls. This finding result is in agreement with that declared by other authors [45,47,48].

During an infertility evaluation, exams and tests are carried out to find the cause of infertility. If a cause is found, treatment may be possible. An infertility evaluation is usually initiated after one year of regular unprotected intercourse in women under age 35 and after six months of unprotected intercourse in women age 35 and older. However, the evaluation may be initiated sooner in women with irregular menstrual cycles or known risk factors for infertility, such as endometriosis, a history of pelvic inflammatory disease, or reproductive tract
malformations [64]. Hormonal evaluation is considered one of the most important test to female infertility diagnosis.

Studies interpreted the relationship between infertility and increase leptin hormone, including the so-called "Leptin - a link between obesity and infertility". Since the body weight increases when food intake exceeds energy expenditure, the role of leptin to detect energy store levels in the body and to relay this information to the central nervous system (CNS). When fat stores and hence leptin levels are high, the brain acts to decrease food intake and to increase energy expenditure. Studies have found that laboratory mice without circulating leptin develop both obesity and infertility, and that administration of leptin action on the restoration of normal body weight and fertility. Although leptin is present in large amounts in obesity, excessive fatty tissue is maintained, along with infertility.

It is believed that obese individuals have some sort of resistance to leptin that cannot be overcome by high leptin levels, making it difficult for obese individuals to lose weight [65].

Studies also demonstrated the leptin:BMI ratio appears to be highly predictive of IVF success. Elevated leptin concentrations, particularly relative to BMI, may negatively impact fertility by assisted reproduction, possibly through direct ovarian actions resulting in impaired oocyte quality and/or early embryo development [66].

The mean levels of FSH obtained in the present study was significantly elevated in patients compared to controls. However LH hormone gave the results not significantly elevated in patients compared to controls. These results are in accordance with that documented in other study with respecting to FSH hormone only [49]. High levels of FSH and LH indicate that the normal restricting feedback from the gonad is absent. If these occurred during the reproductive years, it is abnormal and is accompany with irregular periods, premature ovarian
failure, premature ovarian aging and gonadal dysgenesis; Leads to female subfertility and/or infertility [67].

5.4 Leptin, body mass index and gonadotropic hormones

Serum leptin level was strongly positively correlated with overall adiposity as determined by BMI in infertile women. This finding is consistent with previous studies in predominantly overweight and obese populations [68-70]. Thus, overweight and obese people have large amounts of leptin, but their brains aren't getting the important signal to stop eating (leptin resistance). The leptin is being made by the fat cells, the fat cells are trying to tell the brain to stop eating but the brain can't get the signal. The patient feel hungrier, doesn't stop eating, becomes obese and there will be no fertility [71].

As indicated in the present data, there was no correlation between leptin with LH and FSH hormones. Similar finding was declared by other studies [48,72].

The scientists explained that leptin as a signal of plenty from fat tissues, provides an assessment of energy stockpiles to aid in the accounting of reproductive readiness. The response to this accounting may be coordinated by release or "lack of release" of GnRH [73]. The connection between leptin and reproductive function as well as the known interaction of leptin with hypothalamus, directs attention to a possible central action of leptin in controlling GnRH levels [74]. But it appears that serum leptin level does not influence LH and FSH level in fertile control group as well as infertile subjects as shown in this study. Therefore, it may be hypothesized that in physiological concentration serum leptin does not directly influence FSH and LH production at hypothalamic level or secretion at anterior pituitary level [75].
6.1 Conclusion

- The mean age of controls was 28.2±5.2 years old whereas that of patients was 28.7±5.1 years (P>0.05).

- Medical history showed that more than half of patients reported administration drugs for activation of hormones.

- Family history is associated with female infertility.

- Obesity was more frequent among cases than controls, (P=0.001).

- Serum leptin was significantly increased in patients compared to controls.

- Serum FSH was significantly elevated in patients compared to controls. However, serum LH showed no significant difference between patients and controls.

- Serum leptin was significantly correlated with BMI.

- Leptin was not significantly correlation with LH and FSH.
6.2 Recommendations

1- It appears that optimal leptin secretion is necessary for normal reproductive function. Therefore, married women must try to have a normal threshold level of serum leptin.

2- The overweight and obese women may improve their fertility through dietary and lifestyle modifications that lower leptin concentrations. Therefore, weight management should be offered as a first line treatment option.

3- Avoid eating foods containing a high energy because it contributes significantly to leptin resistance pathway.

4- Understanding the role of leptin in reproduction might be useful for new treatment in reproductive pathologies.

5- Further studies are recommended to find out clear picture of role of serum leptin for primary infertility among married females.
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Annex

Annex I

Female infertility Questionnaire

 أنا الباحثة / د.جذيرة أبو خاطر (طالبة ماجستير بالجامعة الإسلامية). أرجو المساعدة في تعبئة هذا الاستبيان من أجل عمل بحث حول دور هرمون اللبني لدى السيدات اللاتي يعانيين من العقم الغير معروف سببها في منطقة غزة.

<table>
<thead>
<tr>
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<td>العمر</td>
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<table>
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<td>□ جامعة أو دبلوم □ ثانوية عامة</td>
</tr>
<tr>
<td>□ إعدادي □ ابتدائي □ أمي</td>
</tr>
</tbody>
</table>

هل يعاني أحد أفراد عائلتك من العقم؟

| الوزن: |
| هنالك
| يعاني من الدوارة الشهرية متقطعة |
| هل تم حصول هرمونات سابقا؟
| إذا كانت الإجابة نعم ما هي?
| هل تتناولين أحد الأدوية المنتشرة للهرمون؟
| هل تعانيين من أحد الأمراض المزمنة؟ |
هل يعاني الزوج من أي مشكلة تمنع الإنجاب؟

<table>
<thead>
<tr>
<th>نعم</th>
<th>لا</th>
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النتائج

- Leptin*
- FSH*
- LH*

شكراً لكم لتعاونكم مع الباحثة / د. جانة أبو خاطر.