The Effect of Recurrent Use of Clomiphene Citrate in the Treatment of Infertility on the Level of Serum Total Protein, Total Cholesterol and Triglyceride

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Abstract

For the last forty years, the first line of treatment for anovulation in infertile women has been clomiphene citrate; the choice of clomiphene was appropriate because the drug was highly effective in inducing ovulation in selected patients with the advantage of being orally administered, relatively safe and inexpensive. In contrast alternative treatment usually involved parenteral gonadotropins that were significantly more complicated and uncomfortable to administer. However clomiphene was also found to have adverse effects, especially in the form of common antiestrogenic, endometrial and cervical mucous changes. The aim of this study is to identify whether there is a relation between recurrent administration of clomiphene (as ovulation induction) and serum concentration of total protein, total cholesterol and triglyceride.

Thirty women were involved in this study, twenty with infertility and ten as control group. The twenty infertile female patients undergo ovulation induction by clomiphene citrate as treatment to their condition.

The results show a significant difference in the serum level of total protein, total cholesterol and triglyceride between infertile patients (primary and secondary) and control group in the third month of clomiphene treatment. The total protein was significantly higher in the control group than infertile patients; this is the same for total cholesterol while for triglyceride it was significantly higher in infertile patients than in the control group.

Introduction

The ovarian cortex at puberty contains hundreds of thousands of primordial follicles[1]. In response to unknown signal, independent of gonadotropins, a hundreds of primordial follicles are recruited to grow[2]. During this early follicle development, the oocyte enlarges and the granulosa cells proliferate to form a preantral follicle. Over 3-6 months, the follicle develops follicles stimulating hormone receptors in the granulosa cells and luteinizing hormone receptors in the theca cells, and the follicle forms a fluid filled...
space called an antrum[1]. At this stage, antral follicles become acutely dependent on follicle stimulating hormone for further development [1]. In natural cycle just before menses, falling estrogen levels result in withdrawal of negative feedback centrally leading to increased gonadotropins levels [3]. Follicle stimulating hormone stimulates granulosa cell proliferation and differentiation, with the development of more follicle stimulating hormone receptors and production of aromatase [4]. Luteinizing hormone stimulates androstenedione production by theca cells that diffuses into the granulosa cell providing substrate for estrogen secretion[5]. Follicle stimulating hormone concentration must exceed a certain level (FSH threshold) before follicular development will proceed[6]. The duration of this period in which the threshold is exceeded (the FSH window) is limited in the normal cycle by a gradual decrease in follicle stimulating hormone (FSH) occurring in the early midfollicular phase as a response to negative feedback mechanism from rising estrogen levels produced by the larger follicles[7]. Smaller follicles, with fewer FSH receptors, are no longer stimulated to grow by FSH levels below the FSH threshold and undergo atresia. Therefore, generally only one follicle reaches the stage of ovulation each cycle[8].

Administration of exogenous FSH prolongs the time FSH levels are above the FSH threshold and extends the FSH window. This allows multiple ovulation by rescuing smaller follicles that would otherwise have undergone atresia[9]. This will lead to increasing levels of estradiol produced by the mature preovulatory follicle[10]. This sequence of events is orchestrated by the interaction of local ovarian factors and endocrine factors from the pituitary and hypothalamus. The World Health Organization (WHO) classify the anovulation in to three group(11). Women in WHO group II are not estrogen deficient, their FSH and prolactin levels are normal[11]. They typically experience oligomenorrhea, but they may have anovulatory cycles or amenorrhea with bleeding in response to progestin challenge. This is the most common type of anovulation and includes women with polycystic ovary syndrome (PCOS)(12). For this group, oral agents such as clomiphene citrate are useful for ovulation induction[13]. Chemically clomiphene citrate (CC) is nonsteroidal triphenylethylene derivative that exhibits both estrogen agonist and antagonist properties, that is to say selective estrogen receptor modulating activity[14]. The mechanism of action of CC binds to estrogen receptors (ERs) throughout the body due to it is structural similarity to estrogen. CC binding to ERs occur for an extended period of time, weeks rather than hours as with natural estrogen. Such extended binding ultimately depletes ER concentrations by interfering with the normal process of ER replenishment [15]. The antiestrogenic effect on the hypothalamus, and the pituitary, is believed to be the main mechanism of action for ovarian stimulation. Depletion of hypothalamic ERs prevents correct interpretation of circulating estrogen levels; estrogen concentrations are falsely perceived as low leading to reduced estrogen-negative feedback on gonadotropins releasing hormone (GnRH) production and subsequent increased gonadotropins follicle stimulating hormone (FSH) & luteinizing hormone (LH ) secretion[16]. The rise of FSH promotes growth of ovarian follicles and ovulation in anovulatory women. It is believed that the hypothalamus is the main site of action because in
normally ovulatory women, CC treatment was found to increase GnRH pulse frequency[17]. However, actions at the pituitary level may also be involved because CC treatment increased pulse amplitude but not frequency in anovulatory women with PCOS in whom the GnRH pulse frequency is already abnormally high[18]. During CC treatment, levels of both LH and FSH undergo a prolonged rise, compared with a natural cycle, because of the prolonged ER depletion in the brain[19].

Supraphysiological levels of estrogen can occur without central suppression of FSH because the normal ER mediated feedback mechanism is blocked as a result of prolonged ER depletion, this perturbation of normal negative feedback is reflected in the FSH window being extended, leading to multiple follicle growth and a higher levels of estrogen hormone [20,21].

Subjects and Methods
Thirty women were involved in this study. Their age ranged from 23 to 33 years, their weight range between 70-75 Kg. 10 female from the thirty were with primary infertility, the other 10 were with secondary infertility and the last 10 were control group.

Two blood samples were taken each month for three months the first blood sample was taken in the second day of the cycle to measure the concentration of estrogen hormone, the second blood sample were taken in the tenth day of the cycle to measure estrogen concentration, serum total protein, serum total cholesterol and triglyceride.

Enzyme linked fluorescent assay techniques were used to determine serum concentration of estrogen hormone(Biomerieux kit,France). In this study the minividas instrument was used in the assay. it is principle is to combines an enzyme immunoassay sandwich method with a final fluorescent detection.

Serum total cholesterol concentration was measured by enzyme colorimetric testing (Biomerieux Kit, France) and serum triglyceride (TGL) was measured by enzyme colorimetric testing Kit (Biomerieux Kit, France). Serum total protein was also measured by colorimetric biuret method (Spinreact Girona Spain Kit).

Ovarian stimulation was induced by clomiphene citrate (clomid, Merrel company, England) 100 mg/day starting from cycle day 2 and for 5 days given to the infertile group (primary and secondary). Comparisons between groups are assessed using student (t-test), the results were considered significant when (P<0.05).

Results
The serum concentration of estrogen hormone in the first month in infertile women was not significantly different from the control group when measured in the second day of the cycle as shown in fig (1). This is true also for the second month of treatment fig (2), while in the third month the control group shows a significantly higher (P<0.05) estrogen hormone level than women with primary and secondary infertility fig(3).

The serum concentration of estrogen hormone in the tenth day of the cycle shows a significant rise (P<0.05) in the infertility groups than control group as expected due to the induction treatment by clomiphene citrate in the first, second and third month as shown in table (1).

The measurement of the serum concentration of total protein in the first month of treatment shows no significant differ between infertile groups and control group fig(4), similar results in the second month fig
(5), while in the third month there was a significant higher level of total protein in the control group (P<0.05) than infertile groups fig (6) as shown in table (2).

Serum total cholesterol concentration was not significantly differ between control and infertile groups (primary and secondary) in first and second month of treatment fig (7) and fig (8). While in the third month it was significantly higher in the control group (P<0.05)fig (9) also shown in table (2).

Serum concentration of triglyceride again showed no significant difference between infertile and control group in the first and second month fig (10) and (11) respectively, while in the third month it was significantly higher in the infertile patients more than control group fig (12) as shown also in table (2).

Table 1 Shows the (mean ± SD) of estrogen hormone concentration in the three month after treatment for the three groups

<table>
<thead>
<tr>
<th></th>
<th>Serum estrogen level in control group</th>
<th>Serum estrogen level in primary infertility group</th>
<th>Serum estrogen level in secondary infertility group</th>
<th>P &lt;0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First month of treatment</strong></td>
<td>241.7 ± 40.7</td>
<td>341.9± 14.5</td>
<td>342.5 ± 9.9</td>
<td>S</td>
</tr>
<tr>
<td><strong>Second month of treatment</strong></td>
<td>226.6 ± 10.7</td>
<td>368 ± 6.7</td>
<td>369 ± 4.1</td>
<td>S</td>
</tr>
<tr>
<td><strong>Third month of treatment</strong></td>
<td>224.4 ± 12.7</td>
<td>446.6± 20.2</td>
<td>437.9 ± 17.4</td>
<td>S</td>
</tr>
</tbody>
</table>

Table 2 Shows the (mean ±SD) of serum total protein, triglyceride and total cholesterol in the three month of treatment in the Three groups

<table>
<thead>
<tr>
<th></th>
<th><strong>Primary infertility</strong></th>
<th><strong>Secondary infertility</strong></th>
<th><strong>Control group</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total protein</td>
<td>triglyceride</td>
<td>total cholesterol</td>
</tr>
<tr>
<td><strong>First month</strong></td>
<td>7.34 ± 0.5</td>
<td>117.9± 5.3</td>
<td>181.1± 6.5</td>
</tr>
<tr>
<td><strong>Second month</strong></td>
<td>6.77 ± 0.37</td>
<td>169.3± 7.2</td>
<td>169.3± 7.2</td>
</tr>
<tr>
<td><strong>Third month</strong></td>
<td>4.87 ± 0.34</td>
<td>117.5± 10.2</td>
<td>117.5± 10.2</td>
</tr>
</tbody>
</table>
Figure 1: Show the level of serum estrogen in the first month of treatment second day of cycle no significant difference between control and infertility group.

Figure 2: Show the level of serum estrogen in the second month of treatment second day of cycle no significant difference between control and infertility groups.
Figure 3: Show the level of serum estrogen in the third month of treatment second day of the cycle it was significantly higher in control group than infertility groups.

Figure 4: Show the level of serum total protein in the first month of treatment no significant difference between control and infertility groups.
Figure 5 Show the level of serum total protein in the second month of treatment no significant difference between control and infertility groups.

Figure 6 Show the level of serum total protein in the third month of treatment it was significantly higher in control group than infertility groups.
**Figure 7** Show the level of serum total cholesterol in first month of treatment no significant difference between control and infertility groups.

**Figure 8** Show the level of serum total cholesterol in second month of treatment no significant difference between control and infertility groups.
Figure 9 Show the level of serum total cholesterol in third month of treatment it was significantly higher in control group.

Figure 10 Show the level of serum triglyceride in the first month of treatment no significant difference between control and infertility groups.
Figure 11  Show the level of serum triglyceride in the second month of treatment no significant difference between control and infertility groups.

Figure 12  Show the level of triglyceride in the third month of treatment it was significantly higher in infertility groups than control group.
**Discussion**

In this study the recurrent increase in the levels of gonadotropins more than normal concentration due to the induction programs by clomiphene citrate is found to have a serious effect on the protein causing a low level of serum protein specially after the third month of treatment. The reason for these changes could be related to the general effect of gonadotropins on metabolic pathways either directly or through the effect on other hormones specially estrogen.

Many studies confirm that regulation of the proliferation, cytodifferentiation and atresia associated with folliculogenesis involves complex interactions between a host of factors originating from within the follicle itself and gonadotropins from the pituitary [22,23].

Some studies established the presence of a functional bone morphogenetic protein (BMP) system in the ovary; these BMP enhanced and attenuated the stimulatory action of follicle stimulating hormone on estradiol and progesterone production respectively [24,25].

The increase in estrogen levels (as a result to the increase in number of mature follicle) is possible to cause the increment in the triglyceride serum concentration that could lead to hypertriglyceridemia that may cause later the development of cardiovascular disease in women as confirmed by some studies [26,27].

In this study the use of induction leads to more mature follicle that means extraordinary levels of estrogen the use of induction for three month lead to increase of triglyceride levels specially in the third month.

Several studies have shown an association between the increase in estrogen level and hypocholesterolemia [28,29]. However some other studies confirm no relation between estrogen increase and cholesterol serum level [30,31].

In this study cholesterol was found to be decreased with increasing level of estrogen hormone specially after the use of clomiphene citrate for the third month of clomid treatment.

Some studies show that estrogen increase the rate of LDL catabolism suggesting the importance of estrogen in regulation of cholesterol. Studies on rats shows that pharmacological doses of estrogen mediated uptake of LDL and decreased plasma LDL concentration [32-34].

In this study the use of induction program by clomiphene citrate result in endogenous increase of estrogen level to an abnormal concentration this was associated with decreased serum level of cholesterol.

So the use of clomid is useful to stimulate follicular maturation but the recurrent administration of induction program could lead to a very serious hormonal changes that might effect the general metabolic pathways it need further studies to identify the possible ways to avoid those complication.

**References**


4-Marsters P, Kendall NR, Campbell BK 2003 Temporal relationships between FSH induced differentiation of bovine granulosa cells maintained in
serum-free culture. Mol cell Endocrinol 203:117-127.
22-Hirshfield AN 2001: Comparision of granulosa cell proliferation in small follicles of hypophysectomized,