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Effect of zinc and clomiphene citrate on seminal fluid parameter in subfertile male

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Abstract---Subfertility is a universal, general problem of male and females factor. Zinc is one of the most important trace mineral, that plays an essential and necessary role in sperm generation and normal, physiological male reproduction. Pituitary Gonadotropins (FSH) and (LH) are basic for reproduction, FSH is a crucial role for sperm production and LH energize synthesis and production of testosterone Hormone. Currently, the most common therapy used in case of male subfertility clomiphene citrate, the study show significant increase in semen parameter and hormonal status after treatment with zinc 50 mg once/day and clomiphene citrate 50 mg twice/day for at least 2- 3 months. The aim of this research is. To evaluate the role of the zinc and clomiphene citrate on male subfertility case.

Keywords---Zinc, clomiphene citrate, testosterone, LH, FSH, spermatogenesis.

Introduction

According to World Health Organization (WHO) and Assisted Reproductive Technology, subfertility is defined as inability reaching clinical pregnancy after 1 year of non- contraceptive regular sexual intercourse[1] sperm dysfunction is consider one of the most common causes of male subfertility [2]This dysfunction can be due to different factors effect on spermatogenesis such as environmental factors, trauma, obstructive cause, Varicocele, cystic fibrosis disease,

cryptorchidism, sexual transmitted disease, urinary infection, and dietary unbalance of trace elements particularly magnesium(mg), zinc, selenium, calcium, copper, vitamins and oxidative stress[3],[4]

The Zinc is a natural element, It is found in the earth, Zinc is the 2nd to iron as the most an essential trace element in the body of all living cells [5]important in different life processes involve; cell proliferation, defense mechanism against free radicals, reproduction and plays a significant function in homeostasis of hormones that enter in spermatogenesis [6],[7]

Role of Zinc and Clomiphene citrate in healthy Male Reproduction

Zinc is an important and crucial trace elements mineral that actively influence the male reproductive system function and enter in more than > 300 enzymes in different biochemical processes like signal transcription, replication and transduction [8] . Also the zinc found in high concentration level the sex organs of the male like testicles, prostate gland [9] and in sperm itself, it's also, enhance spermatogenesis process by an increase in sperm development and maturation, protect of germinal epithelium [10]. And plays a role in the testosterone secretion from the Leydig cells under effect Follicle stimulating hormone (FSH). It's play a key role in regulating sperm production process [11] Several research report a positive correlation between level of the zinc and sperm motility, number, and levels of testosterone in the serum [12],[13].the most significant influence of zinc is on spermatozoa motility , It supports and tighten the surface dense fibers by the organization of di-sulfide bridges throughout the maturation stage of the sperm in the epididymus, this important step for propagation of sperm motility, chiefly progressive motility[14].

Clomid (clomiphene citrate) is a non-steroidal medical drug used in case of male subfertility, It acts as an antagonist estrogen receptor on the hypothalamus gland therefore lead to inhibiting normal estrogen level and lead to negative feedback mechanism on the both (pituitary and hypothalamus) glands, by this mechanism lead to raise of LH and FSH hormone secretion, which motivate Leydig cells to intensification testosterone hormone production [15][16] ,Outcome lead to rise in sperm number and production of testosterone in the testes [17].

Study design

This is a planned as a prospective cohort study, members were recorded (N=51) subfertile male undergoing seminal fluid analysis who are referred from different Iraq cities to the fertility center in Al -Sadder Medical City / AL- Najaf /Iraq. Under management of Urology department in AL- Kufa medical college.

Materials and Methods

In this study, we assess 51 subfertile men seen in fertility center at Al -Sadder Medical City. Most of these patients were healthy individuals investigates for subfertility. The medium age of these individuals was 29 -44 years had primary and secondary subfertility. All patients were examined for the cause of subfertility

by examining the seminal fluid and a blood sample to detect baseline of FSH, LH and testosterone level, All patients were started on zinc 50 mg once daily and clomiphene citrate 50 mg twice daily for 2- 3 months. All males were followed prospectively and observed for assessment of management response by repeat the seminal fluid and blood sample.

Result

Table (1) Demographic characteristics of all study sample

| Variable | Minimum | Maximum | Mean± SD |
|----------------------------|----------------|-----------|--------------|
| Age(years) | 29 | 44 | 35.82 ± 3.52 |
| BMI (kg / m ²) | 21.50 | 38.70 | 29.61 ± 4.02 |
| Duration (years) | 2.00 | 11.00 | 5.17 ± 2.59 |
| Infertility cause | Male, n(%) | 29 (56.9) | |
| | Combined, n(%) | 22 (43.1) | |
| Type of infertility | Primary | 28 (54.9) | |
| | Secondary | 23 (45.1) | |

n: number of cases; SD: standard deviation; BMI: body mass index

Table (2) show the mean ± SD of semen parameter both before and after treatment with clomiphene citrate and zinc sulphate in subfertile males

| Variable | Before treatment | After treatment | % of change |
|--------------------------------|------------------|-------------------|-------------|
| Volume (ml) | 2.17 ± 0.6 | ** 2.81 ± 0.73 | 22.7%↑ |
| Concentration | 7.74 ± 3.98 | ** 11.62 ± 4.37 | 33.3%↑ |
| Total count 10 ⁶ /m | 17.29 ± 10.89 | ** 32.86 ± 16.004 | 47.3%↑ |
| Motility % | 14.74 ± 10.69 | ** 20.23 ± 11.01 | 27.1%↑ |
| Progressive motility% | 7.64 ± 7.61 | ** 13.03 ± 9.2 | 41.3%↑ |
| Morphology% | 1.56 ± 1.08 | ** 2.29 ± 1.23 | 31.8%↑ |

SD: Standard deviation* : p ≤ 0.05 / ** : p ≤ 0.01

Table (2) show the difference in semen parameter (mean ± standard deviation) before and after the treatment with clomiphene citrate and zinc sulphate which is highly significant. The ejaculatory volume (ml) is significantly increase after the treatment (2.81 ± 0.73) in compare with before the treatment (2.17 ± 0.6) with increment percentage up to 22.7% . also, total sperm count, sperm concentration, motility (specifically progressive motility) , and normal sperm morphology of subfertile males were significantly rise after the treatment in contrast with before (11.62 ± 4.37) vs. (7.74 ± 3.98) ,(32.86 ± 16.004) vs. (17.29 ± 10.89) ,(20.23 ± 11.01) vs. (14.74 ± 10.69) ,(13.03 ± 9.2) vs. (7.64 ± 7.61) , and (2.29 ± 1.23) vs. (1.56 ± 1.08) respectively with increase in sperm concentration, total sperm count, motility , progressive motility, and normal sperm morphology percentage up to, 33.3%, 47.3%, 27.1% ,41.3% , and 31.8% respectively.

Table (3) show the mean \pm SD of serum level of hormone both before and after treatment with clomiphene citrate and zinc sulphate in subfertile males

| Variable | Before treatment | After treatment | % of change |
|-------------------|------------------|-------------------|-------------------|
| testosterone Ng/m | 7.63 \pm 0.81 | ** 9.2 \pm 1.13 | 17.06% \uparrow |
| FSH mIU/m | 5.32 \pm 0.82 | ** 8.5 \pm 0.8 | 37.4% \uparrow |
| LH mIU/m | 4.61 \pm 0.69 | ** 7.4 \pm 0.76 | 37.7% \uparrow |

FSH: follicular stimulating hormone; LH: luteinizing hormone; **: $p \leq 0.01$ *: $p \leq 0.05$

Table (3) display the mean and stander deviation of hormonal status of the subfertile males before and after the treatment .there was highly significant increase (>0.01) in serum level of testosterone (7.63 \pm 0.81) vs. (9.2 \pm 1.13) before in contrast with after the treatment. With increment rate up to 17.06%. furthermore, the serum level of follicular stimulating hormone(FSH) and luteinizing hormone(LH) also show significant increase after the three months treatment with clomiphene citrate and zinc sulphate with percentage of changes up to 37.4%, and 37.7% for FSH and LH respectively.

Discussion

Initial results of this study shows significant improvement of semen parameters and serum hormonal levels .Our study support the conclusion of meta-analysis done in 2014 about the role of antioxidant in subfertile male which establish that supplementary oral antioxidants is effective . and the zinc is one of the most antioxidant associated with increased the rate of pregnancy (18) in another study the zinc and folic acid appear to have a positive influence on semen parameters ,the study revealed that sperm morphology significantly increases in Patients receiving zinc sulfate while those receiving folic acid shows significant increases in sperm concentration. Furthermore the combination of both therapy demonstrated substantial improvement in sperm morphology, concentration, and motility(19). So, Estrogen receptor modulators (clomiphene) and other antioxidant (such as zinc) potentially enhance semen parameters(20).

Spermatogenesis depends on sufficient secretion of gonadotropin with consequent raise in testosterone concentrations. Testosterone reversibly inhibits pituitary LH and FSH secretion, which suppresses spermatogenesis,(21) the role of CC are reached by suppressing the negative feedback of estradiol(E2) on the hypothalamic–pituitary–gonadal axis at the hypothalamus level . This cause increment in LH and FSH release from the anterior pituitary gland, which stimulates Leydig cells that produce testosterone.(22)

Another study made by Taylor and Levine shows that CC contributed to significant rises in level of testosterone from baseline values (23) in contrast to other double-blind trial of CC, which found no enhancements in semen parameter in men with unexplained subfertility that is in consist with a different randomized trial done in 2015 which shows no significant improvements in semen parameter of patient receiving CC and anastrozole AZ compared with placebo (24.25)

CC seemed to efficiently enhance the hormonal profile, this treatment can elevate endogenous testosterone hormone level and better the testosterone/estrogen (T/E) ratio that effect on the spermatogenesis (26) Zinc enhances spermatogenesis by participating actively in sperm growth and maturation and germinal epithelium preservation (27)

Furthermore, its shows a significant role in production and secretion of testosterone from Leydig cells, which, is a crucial spermatogenesis regulator (28). Additionally, zinc is also protective function against bacteria.(29). research revealed that concentration of zinc in affects spermatogenesis closely,as zinc deficit leads to diminution in testicular bulk, seminiferous tubules shrinkage, and gonadal dysfunction. (30)

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