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Effect of Clomiphene Citrate on Insulin/IGF-1 and GH Levels in Mice Offspring

Wejdan M Henawi* **and Mohammed O Aljahdali**

Biological Sciences Department, King Abdulaziz University, Jeddah, Saudi Arabia

***Corresponding author:** Henawi WM, Biological Sciences Department, Faculty of Sciences, King Abdulaziz University, Jeddah, Saudi Arabia, Tel: +9660594470722; E-mail: wmhenawi@gmail.com

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Abstract

Infertility can cause considerable social, emotional and psychological stress. Ovulatory dysfunction is one of the most common causes of reproductive failure in sub-fertile and infertile women. There are several approaches to ovulation induction therapy for the management of women with ovulatory disorders. Fertility drugs are spreading worldwide fast and therefore many studies have reviewed the association between the use of these drugs and physiological, biochemical and histopathological alterations. The results of present study showed that there were observed effects of Clomiphene citrate (Clomid)® on albino mice offspring's hormones level. Treating mothers with CC doses 0.2 and 0.3 mg/day caused high increasing and variation in Insulin, Insulin-like Growth factor I (IGF-1) and Growth Hormone (GH) levels comparing to control and between male & female, as was clearly noticed on the new offspring of the treated mothers with CC.

Keywords: Offspring; Insulin; IGF-1; GH; Clomiphene citrate

Introduction

There are many ovulation inducing drugs is prescribed nowadays, Clomiphene citrate (CC) is the first choice for women treatment with ovulatory disorder or those with Polycystic Ovaries (PCOS) and have been widely used since 1962 until today. Clomiphene citrate or Clomid® tablets are orally administered, nonsteroidal land usually given on the third or the fifth day of cycle after spontaneous or progesterone induced withdrawal bleeding with 50 mg for five days. The effective dose of CC ranges from 50, 100, 150 mg/day, doses excess 250 g/day is not approved by the FDA [1-3]. During CC treatment which binds to oestrogen receptors(ER) in reproductive system the levels of both (FSH & LH) is indicated to rise and effect ovarian stimulation to produce one or more dominant follicles emerges and matures [2]. Recently some studies suggests that is GH with IGF-1 have an important role in human follicular regulation development and steroidogenesis, any abnormal defect in IGF-1 can leads to folliculogenesis disturbance in PCOS [4,5]. Oral administration of CC does cause a decreasing of IGF-1, GH and Insulin levels or not change it in PCOS [6,7]. Evaluation of GH and IGF-1 can be measured by serum concentration level which is not differ between males and females in healthy adults, whereas GH higher in females [8,9]. The aim of the present study is to evaluate the effect of CC on Insulin, IGF-1 and GH levels in offspring of treated mothers.

Materials and Methods

Animals

All experimental procedures with mice were approved by the ethical guidelines of the animal care and use committee of King Abdulaziz University. Twenty five virgin albino mice of SWR strain female, at age (8 week old) and weighing (23-25 g) were used in the present study. Mice were obtained from animal house unit of king Fahad Medical Research Centre, King Abdulaziz University, Jeddah, Saudi Arabia. Animals were acclimatized to laboratory conditions for one week before to the initiation of experimental treatments and were housed in standard plastic cages and maintained in controlled laboratory conditions room, temperature (20 ± 1°C), and light: Dark cycle (12:12 h) and humidity (65%). Mice were feed ad libitum with standard diet and had free access to tap water.

Experiment design

Animals were divided in to two groups: 1-Control group (five females). 2-Clomiphene citrate treated group (20 female). Mice were oral injected with 0.2 and 0.3 mg/day of CC daily for 2 week, after 2 week every female were housed with a male for mating after female get pregnant males were taken out. On the day 26th after weaning and 8 week blood samples were taken from orbital sinus of mice offspring (22 pre-pubertal, 22 post-pubertal male and 23 pre-pubertal, 23 post-pubertal female) for Insulin, IGF-1 and GH levels were determined by using (Elba Science) ELIZA kits, according to the manufacturer's instructions.

Statistical analysis

Statistical Package for Social Sciences (SPSS version 20) was used. Data were presented as mean (standard deviation). The continuous variables between more than 2 groups as comparison between control, male and female were compared using Onaway ANOVA (LSD) test, and between 2 groups as pre-pubertal and post-pubertal were made using unpaired student "t" test. A probability (P) <0.05 was considered significant. Graphs were made using Prism software for statistics version 5.

Results

Males group

Table 1 and Figure 1 as well for the analysed data showed that Pre-males vs. Pre-control significant difference increase in IGF-1(6.75 ± 3.01 vs. 2.90 ± 0.01, [1]P=0.021), Insulin (14.92 ± 5.59 vs. 5.51 ± 0.01, [1]P=0.0002) and GH (17.42 ± 5.99 vs. 8.31 ± 0.01, [1]P=0.001), but lower significant increase in IGF-1(6.03 ± 2.44 vs. 2.4 ± 0.01, [1]P=0.028) and Insulin (16.75 ± 3.88 vs. 8.89 ± 0.01, [1]P=0.007) in Post- males vs. Post-control group. While the Pre-males vs. Post- males showed high significant increase in GH only (17.42 ± 5.94 vs. 6.04 ± 2.21, [2]P=0.0001).

	Pre-pubertal		Post-pubertal	
Parameters	**Control**	**Treated**	**Control**	**Treated**
Insulin growth factor- 1 (pg/ml)	2.90 ± 0.01	6.75 ± 3.01	2.41 ± 0.01	6.03 ± 2.44
Significance	0	[1]P=0.021	0	[1]P=0.028; [2]P=0.353
Insulin (ng/ml)	5.51 ± 0.01	14.92 ± 5.59	8.89 ± 0.01	16.75 ± 3.88
Significance	0	[1]P=0.002	0	[1]P=0.007; [2]P=0.181
Growth hormone (ng/ml)	8.31 ± 0.01	17.42 ± 5.94	5.04 ± 0.06	6.04 ± 2.21
Significance	0	[1]P=0.001	0	[1]P=0.699; [2]P=0.0001
Data are expressed as mean +/- standard deviation. [1]P: Significance versus control; [2]P: Significance pre-pubertal versus post-pubertal. Comparison between groups was made using OneWay ANOVA test.				

Table 1: Comparison of the measured hormones in different studied groups of male mice.

Females group

In Table 2 and Figure 1 analysed data shown low significant increase in Insulin and GH only in Pre-females vs. Pre-control Insulin (10.39 ± 4.70 vs. 4.20 ± 0.01, [1]P=0.030), GH (13.47 ± 5.75 vs. 6.80 ± 0.01, [1]P=0.016). But there were no statistically significant difference in Post-females vs. Post-control group. While in Pre-females vs. Post-females the significant decrease was found in GH only in this group GH (13.47 ± 5.75 vs. 10.01 ± 2.91, [2]P=0.010).

	Pre-pubertal		Post-pubertal	
Parameters	**Control**	**Treated**	**Control**	**Treated**
Insulin growth factor 1 (pg/ml)	2.70 ± 0.01	5.67 ± 2.55	3.57 ± 2.37	4.87 ± 2.47
Significance	0	[1]P=0.054	0	[1]P=0.389; [2]P=0.276
Insulin (ng/ml)	4.20 ± 0.01	10.39 ± 4.70	10.40 ± 1.73	11.43 ± 4.69
Significance	0	[1]P=0.030	0	[1]P=0.713; [2]P=0.440
Growth hormone (ng/ml)	6.80 ± 0.01	13.47 ± 5.75	10.00 ± 0.00	10.01 ± 2.91
Significance	0	[1]P=0.016	0	[1]P=0.997; [2]P=0.010
Data are expressed as mean +/- standard deviation. [1]P: Significance versus control; [2]P: Significance pre-pubertal versus post-pubertal. Comparison between groups was made using One-way ANOVA test.				

Table 2: Comparison of the measured hormones in different studied groups of female mice.

Male and Female Groups

All results in Table 3 and Figure 1 as well are shown high significant difference in IGF-1, Insulin and GH levels in all males and female groups in both stages per-pubertal & post-pubertal as the following:

Pre-males vs. Pre-females Insulin (14.92 ± 5.59 vs. 10.39 ± 4.70, ^{1}P=0.005), GH (17.42 ± 5.94 vs. 13.47 ± 5.75, P^{1}=0.029). Post-males vs. Post-females Insulin (16.75 ± 3.88 vs. 11.43 ± 4.69, ^{2}P=0.0001), GH (6.04 ± 2.21 vs. 10.01 ± 2.91, ^{2}P=0.0001. Pre-males vs. Post-females Insulin (14.92 ± 5.59 vs. 11.43 ± 4.69, P^{3}=0.029), IGF-1 (6.75 ± 3.01 vs. 4.87 ± 2.47, P^{3}=0.028), and GH (17.42 ± 5.94 vs. 10.01 ± 2.91, P^{3}=0.0001). Pre-females vs. Post-males Insulin (10.39 ± 4.70 vs. 16.75 ± 3.88, P4=0.0001), GH (13.47 ± 5.75 vs. 6.04 ± 2.21, P4=0.0001).

Parameters	Pre-pubertal		Post-pubertal	
	Control	Treated	Control	Treated
Insulin growth factor 1 (pg/ml)	6.75 ± 3.01	17.42 ± 5.94	6.03 ± 2.44	4.87 ± 2.47
Significance	0	^{1}P=0.203; ^{4}P=0.628	0	^{2}P=0.115; ^{3}P=0.028
Insulin (ng/ml)	14.92 ± 5.59	10.39 ± 4.70	16.75 ± 3.88	11.43 ± 4.69
Significance	0	^{1}P=0.005; ^{4}P=0.0001	0	^{2}P=0.0001; ^{3}P=0.029
Growth hormone (ng/ml)	17.42 ± 5.94	13.47 ± 5.75	6.04 ± 2.21	10.01 ± 2.91
Significance	0	^{1}P=0.029; ^{4}P=0.0001	0	^{2}P=0.0001; ^{3}P=0.0001

Data are expressed as mean +/- standard deviation. ^{1}P: Significance pre-pubertal male versus pre-pubertal female; ^{2}P: Significance post-pubertal male versus post-pubertal female; ^{3}P: Significance pre-pubertal male versus post-pubertal female; ^{4}P: Significance post-pubertal male versus pre-pubertal female. Comparison between two different groups was made using independent sample "t" test.

Table 3: Comparison of the measured hormones in treated pre-pubertal and post-pubertal male versus female mice.

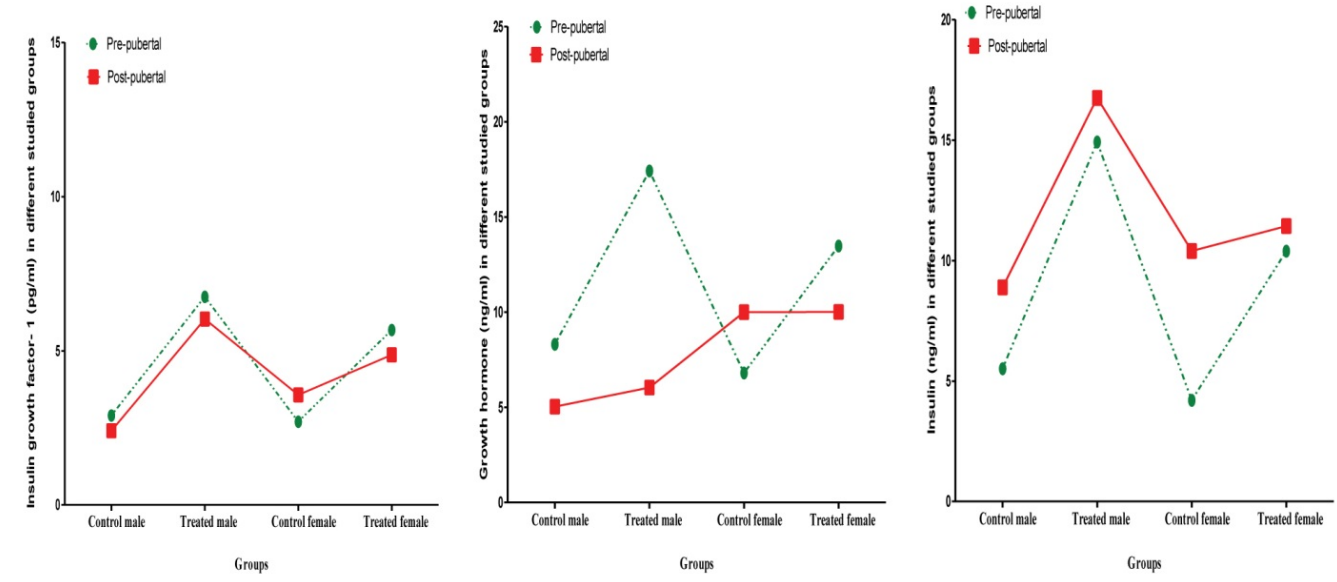

Figure 1: Comparison illustration of measured IGF-1, GH and Insulin levels pre-pubertal and post-pubertal male versus female mice offspring with control.

Discussion

Although, Clomiphene Citrate (CC) considered one of the safest drug given to women with evolutionary disorder since the 60s, rises concern in the medical field on its effects on the newly offspring. Results of this study have shown considerable evidences of disparity in IGF-1, GH and Insulin levels in treated mother's offspring. Where, CC causes significant increasing in GH and insulin levels in males and

females comparing to control and to each other, while the significant increase and differ concentration level for IGF-1 were found in males comparing to control and to females as well, but not in females comparing to control. These findings are consistent with results of previous studies CC treated PCOS patients, were they had higher level of IGF-1 than healthy women [4]. On other hand there were many outcomes of research results showed disagree with present study as what reported in a successful treated cycles by CC in PCOS were resulted significant reduction in IGF-1 level, and no changes in insulin plasma concentrations [10]. Similar findings are also recorded where CC does not affect insulin level in PCOS, but IGF-1 level was decreased [6]. Oral administration of CC lead to 30% decrease in IGF-1 level in PCOS women [7]. Lower level of IGF-1 was found in females comparing to with Growth Hormones Deficiency (GHD) males [11]. In acromegalic patients male the IGF-1 levels was decreased by 40% comparing to normal males level [12]. Blood transmission form the treated mothers to their offspring may cause these highly significant increases and changes in hormones concentrations levels. In conclusion, blood biochemical analysis indicated that CC causes alterations on IGF-1, GH and insulin hormones in treated mother's offspring in albino mice.

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