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Effect of BMI on chemerin in serum on femals undergoing ICSI

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***Abstract*---**Back ground: Serum chemerin levels are associated with both body fat percentage and BMI, and elevated chemerin levels are associated with metabolic problems and inflammatory shifts. Aim: The aim of this study was to evaluate the effect of BMI in serum and follicular fluid chemerin and Leptin level on patients undergoing ICSI. Patients and methods: This study was performed by participating 44 women undergo ICSI procedure devided in to two groups based on BMI in to non-obese group, theirBMI<30 and obese group there BMI>30 . Both groups are infertile women who attend high institute of infertility diagnosis and assisted reproductive technologies at Al-NAHRAIN university In AL-KADHIMIA city in BAGHDAD. From NOVEMBER 2021 to the end of MAY 2022. Blood sample was drown from each patient at day of oocyte pick up for serum chemerin measurements Also follicular fluid is collected for chemerin and Leptin level. Results: The result in non obese group showed a significant positive correlation was found between Serum chmrin levels, indicating a strong effect size (p< .001). This suggests that as Serum chemerin levels rise, so will FF chemerin. A significant positive correlation of r =0.92 was found between No of oocytes retrieved and Number of metaphase II, indicating a strong effect size (p<.001, This shows that as the number of metaphase II oocytes increases as the retrieved oocytes number increase. A significant positive correlation of 0.66 was found between Number of embryo and G1 embryo, indicating a large effect size. This implies that as Number of embryos increases, so does G1 embryo. A significant positive correlation of 0.65 was found between Number of embryos and Grade 2 embryo, indicating a strong effect size. This implies that as Number of embryo increases, so does Grade 2 embryos. within obese group; S chemerin, FF chemerin. Number of oocytes retrieved, germinal vesical, Number of metaphase I, Number of metaphase II, Number of embryo, G1

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embryo, G2 embryo, Maternal age, AMH ng ml, FSH , LH, There were no significant correlations between any pairs of variables. Conclusion: Serum chemerin had a significant correlation with oocyte maturity and embryo grading and there was no significant difference between obese and non obese patients regarding serum chemerin level.

***Keywords*---**BMI, ICSI, chemerin.

Introduction

Failure to become pregnant after at least 12 months of regular, unprotected sexual contact is a sign of infertility for both sexes. It is estimated that infertility affects more than a billion people of reproductive age around the world. Infertility affects 48 million couples and 186 million individuals worldwide, according to current estimate.(WHO)(Zivari-Ghader et al, 2018). Infertility in women can be caused by a variety of conditions, including problems with the ovaries, uterus, fallopian tubes, or endocrine system. There are two types of infertility: primary and secondary. In the case of primary infertility, a woman has never been able to conceive; in the case of secondary infertility, she has had at least one previous pregnancy. (Ghaffari and Arabipoo, 2018). Preventing, diagnosing, and treating infertility all fall under the umbrella of fertility care. Most countries, particularly those with low and middle incomes, still struggle to provide equal and equitable access to reproductive health care. National universal health insurance benefit packages rarely give high priority to reproductive health care. Chemerin is a tiny chemotactic protein that is involved in the early stages of the immune response. Chemerin is an adipokine that has a role in energy homeostasis and reproductive function regulation. When chemerin was first identified in 1997, it was thought to be merely a protein linked to healthy skin function that stood in stark contrast to psoriasis. A global regulator of chemotactic, adipokine, autocrine/paracrine, adipogenic, angiogenic, and reproductive functions, chemerin has recently come into the spotlight due to the discovery of multiple receptors for the chemerin protein and a large number of associations with various pathologies. .(Fischer and Beck-Sickinger, 2022). Chemerin levels in the body and in the blood are linked to BMI and obesity-related indicators . Chemerin has been linked to adipogenesis, glucose homeostasis, food intake, and body weight in previous research including gain or loss of function. (Marti, et al., 2021), Female reproductive functions in mammals are influenced by their nutritional status and body composition. The aim of the study was to evaluate weather there is any relation of BMI on chemerin level in serum and follicular fluid with oocyte and Embryo quality in women with infertility candidate for fresh intracytoplasmic sperm injection

Patients Material and Methods

This study was done on 44 infertile females who were undergoing intracytoplasmic sperm injection (ICSI) in the infertility center for High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/ Al- Nahrain University/ Baghdad/ Iraq, during the period from November 2021 to the end of May2022 regardless to the presence or absence of previous trials. The morphological assessment of the oocytes aspirated from infertile females and their resulting embryos was done in the ICSI laboratory of the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies,while the measurement of serum and follicular fluid chemerin was done in a private laboratory

Patients

The present study was done on fourty four infertile females who were subjected to an intracytoplasmic sperm injection (ICSI) cycles. Their ages ranged from 20 to 40 years old. The infertility duration range 1-20 years with both primary and secondary type of infertility. Some of these infertile females were having their first IVF/ ICSI attempt , while others had already undergone at least one cycle before.

Patients assessment

The infertile females who were enrolled in this study underwent flexible antagonist ovarian stimulation protocols, The duration of stimulation and gonadotropin dose depended on female's age, markers of ovarian reserve (such as antral follicle count (AFC), Antimullerian hormone (AMH) levels, and/ or early Follicle stimulating-hormone (FSH), Luteinizing hormone (LH) and Estradiol levels) in addition to type of protocol used in previous IVF trials if present.

All the fourty four infertile females had a normal ovarian reserve, based on their serum AMH level and antral follicle count (AFC), evaluated at day 1 or 2 of the menstrual cycle (follicular phase) which are done routinely on infertility clinic. Some with history of ovulation disorders like polycystic ovarian syndrome (PCOS), and this was decided according to the Rotterdam criteria (oligo-and/or anovulation, hyperandrogenism signs and polycystic ovary morphology on ultrasound examination) . Others were having history of tubal blockage, male factor or unexplained infertility. Basal serum levels of Follicle-stimulating hormone (FSH), Luteinizing hormone (LH) and .The number of dominant follicles for each patient was documented by ultrasound assessment on day of hCG triggering and total number of retrieved oocytes was recorded during the day of ova pick up (OPU).At the same time the follicular fluid was collected and stored in deep freezer for later assessment of serum and follicular fluid chemerin and Leptin levelAfter denudation, the nuclear maturity of the retrieved oocytes was evaluated and these oocytes were classified according to their nuclear maturity (depending on presence or absence of 1st polar body) into germinal vesicle (GV) , metaphase I (MI)),and metaphase II (MII) . Only normal mature oocytes were injected with morphologically normal sperm (ICSI). On day three following sperm injection (ICSI) embryo quality was assessed depending on morphological criteria(Ozturk, 2020).

Inclusion and exclusion criteria

The patients who were included in the present study were selected according to following criteria

Inclusion criteria

- Their age ranged from 20-40 years old.
- These patients were having normal ovarian reserve documented by normal anti Mullarian hormone(AMH),Fsh,Afc
- that should be at least within the satisfactory or normal ovarian reserve .
- They have normal thyroid function test.
- Patients with patent or non-patent tubes (except those with endometriosis).
- Couples with unexplained infertility
- Male factor infertility except those with non obstructive azoospermia.

Exclusion criteria

- Age less than 20 and more than 40
- Patients with poor ovarian reserve diagnosed by high FSH >12 IU/ml and low antral follicle count).and low AMH
- Heavy smokers
- Uncontrolled endocrinological disorders as thyroid dysfunction.
- Male partner with non obstructive azoospermia.
- intrauterine pathology.

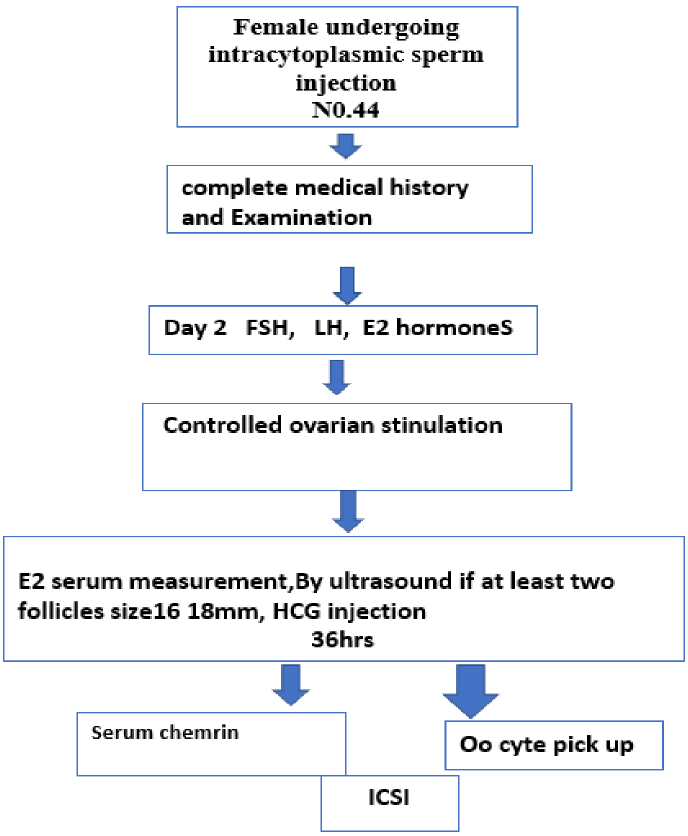

Figure 1: Study protocole

Methods

Intracytoplasmic sperm injection cycle (ICSI)

In all fourty four infertile females, serum FSH, LH and estradiol (E2) were measured on day 1 or 2 of the cycle just before starting ovarian stimulation

Gonadotropin releasing hormone (GnRH) antagonist protocol

The flexible GnRH antagonist protocol was used in this study in which gonadotropins were initiated on the second day of menstrual cycle in the doses of (Gonal- F 150-300IU) and when the leading follicle reached a diameter of 12- 14 mm, GnRH antagonist (Cetrotide) was administered in a dose of 0.25 mg daily until day of HCG injection The ovarian response to stimulation was monitored by measuring the serum E2 concentration and by ultrasound assessment of follicular growth. Ovulation was triggered with an injection of 250 µg human chorionic gonadotropin (hCG) (Ovitrelle) when at least two follicles reached the diameter of 17 mm or more on ultrasound examination. At ovulation triggering day, the hormonal ovarian response was also evaluated by quantifying serum estradiol (E2) level

Oocyte retrieval and follicular fluid collection

Oocytes were retrieved by trans-vaginal ultrasound-guided aspiration 34-36 hours after HCG injection. For each patient, all follicles were aspirated without flushing; cumulus-oocyte complexes were isolated. Oocyte retrieval was done under general or sometimes spinal anaesthesia according to the patient condition. Normal saline solution was used to clean vagina thoroughly. Aspiration was achieved by single lumen needle using aspiration media as a flushing medium. Aspiration pump pressure was set around 120 mm Hg

In- vitro fertilization/ Intracytoplasmic sperm injection (IVF- ICSI procedure

- After oocyte retrieval, the cumulus was denuded 38 to 42 hours after hCG administration. Stripping was accomplished by placing the cumulus–oocyte complex in 80 IU/mL of hyaluronidase in Ferti Cult flushing media which contains 4g/L human serum albumin (HSA) and HEPES (4-(2-hydroxyethyl)-1- piperazineethanesulfonic acid) for 30 to 60 seconds, rinsing five to six times in ferticult flushing media which consists of HSA, HEPES, phenol red and 10 mg/L. gentamycin, and then mechanically removing the cumulus from the oocyte using a EZ-Grip micropipettor170 and 135 µ
- The sperm was first visualized under 40 magnification and then was immobilized in 10% polyvinylpyrolidone which consists of HSA, HEPES, PVP, MEA≥80%, endotoxin<1IU/ml using the tip of the injection needle. It was then aspirated tail- first into the injection needle.
- The oocyte was grasped with holding pipette at 9 o’clock using gentle suction, and then was rotated such that the first polar body was located at either the 6 o’clock or 12 o’clock position.
- injection pipette was pushed through the zona at the 3 o’clock position and advanced to the outer surface of the oolemma. The oolemma was penetrated

by direct penetration technique. Entry into the oocyte was confirmed by free flow of cytoplasm into the injection pipette.

The cytoplasm was then slowly injected back into the oocyte until the sperm was seen to pass the tip of the injection pipette and slide into the cytoplasm. The procedure was concluded by gently removing the injection pipette.

Results

All women aged below 40 years old. The most frequently observed category of age within the Non-Obese group was (20- 30) years old (n = 17, 53.12%). While the most frequently observed age group within the Obese group was (31- 40) (n = 11, 91.67%). For Non-Obese, the age had an average of 31.34±5.60 (SE_M = 0.99, Median = 30.00). For Obese, age had an average of 34.50 ± 3.83 (SE_M = 1.10, Median = 34.50). The result of t-test comparison was not significant, t (40) = -1.79, p = .080, which suggests the mean of females' age was not significantly different between the Non-obese and Obese groups. The results are presented in Table 4-1. A bar plot of the means is presented in Figure 1.

Table 1: The age wise distribution of the infertile females included in the study

Age in years	Non-Obese N= 32	Obese N=12	Total N=44	P- value
	N (%)	N (%)	N (%)	
(20- 30)	17 (53.125%)	1 (8.3%)	18 (40.91%)	0.0078[a]
(31- 40)	15 (46.87%)	11 (91.66%)	26 (59.1%)	
Total	32 (72.73%)	12 (27.27%)	44 (100%)	
Mean± SD	31.34± 5.60	34.50± 3.83	N =32	0.0800[b]
Median ± SE_M	30.00±0.99	34.50±1.10	N = 12	

[a] chi squared, [b] T test

the absence of PCOS was the most frequently seen Within the non-obese group, (n = 28, 73.7%). As well as within the obese group, (n = 10, 26.3%). The findings of the Fisher exact test were inconclusive, with an OR of 0.93 and a p value of 1.000. This indicates that PCO and obesity might not be related to one another in the study subjects. In another ward, there was not a significant difference between the expected frequencies and the frequencies that were actually observed. The outcomes of the Fisher's exact test are summarized in Table 2

Table 2: percentages of PCOS within obese and non-obese women

	PCOS		
Obesity	No	Yes	total
Non-obese	28 (73.7%)	3 7(5.0%	31 (73.8%)
Obese	10 (26.3%)	1 (25.0%)	11 (26.2%)
	38 (90.5%)	4 (9.5%)	42
Fisher's exact test	P = 1.000		

The most frequently observed Type of infertility within the non-obese group was primary (n = 21, 65.62%). While, the most frequently observed Type of infertility within the Obese patients was secondary infertility (n = 6, 54.55%). The Fisher exact test results were not significant with an OR = 2.24 and p =.295, To put it another way, this means that the observed frequencies did not differ from the expected frequencies. The Frequencies, percentages and Fisher's exact test findings are shown in Table 3 below.

Table 1 frequency table for Association of type of infertility with obesity

	Type of infertility		
Obesity	primary	Secondary	
Non-obese	21 65.6%	11 34.4%	32 (74.4%)
Obese	5 45.5%	6 54.5%	11 (25.6%)
	26 (60.5%)	17 (39.5%)	43
Fisher's exact test P = 0.295450074			

An absence of previous IVF cycle had the frequency of (n = 23, 71.88%) within the non-obese women The same observation was noticed within the Obese group was (n = 6, 50.00%). Frequencies and percentages are presented in Table 4.

Table 4: frequency table of Association between number of previous IVF cycle with obesity

	Number of previous IVF cycle				
Obesity	No previous IVF	one	two	three	
Non-obese	23	7	2	0	32 (72.7%)
Obese	6	5	0	1	12 (27.3%)
	29(65.9%)	12(27.3%)	2(4.5%)	1(2.3%)	44
Fisher's exact test	P = 0.1509				

The most frequently observed category of Previous IVF reslt within the Non-obese group was failed (n = 8, 88.89%). The most frequently observed category of Previous IVF status within the Obese group was failed (n = 5, 83.33%). Frequencies and percentages are presented in Table 5

Table 5: frequency table of Association between previous IVF status with obesity

	Previous IVF status			
Obesity	Failed	Na	Succeed	
Non-obese	8	23	1	32 (72.7%)
Obese	5	6	1	12 (27.3%)
	13 (29.5%)	29 (65.9%)	2 (4.5%)	44
Chi-squared	1.975			
DF	2			

Significance level	P = 0.3725

FSH levels in non-obese people averaged 5.23 ± 5.01, (Median = 3.00, SEM = 0.91). FSH observations for Obese averaged 3.07 ± 0.58, (Median = 3.32, SEM = 0.22). Statistical analysis found no statistically significant difference between groups, t(42) = 1.75, p =.087. This finding implies that the mean FSH did not differ significantly between the Non-obese and Obese groups. Table 6

Table 6: Hormonal levels in women undergoing ICSI by obesity level

Variable	Non-obese		Obese		
	n	Mean± SD	n	Mean± SD	P [a]
FSH mIU/ml	32	5.17± 4.86	12	2.69± 0.72	0.0874
LH mIU/ml	32	3.83± 2.38	12	3.24± 0.95	0.4143
AMH ng/ml	30	1.99± 0.94	7	2.06± 0.65	0.8512

S. chemerin had an average of 302.40± 199.51 for non-obese (Median =217.64, SEM = 35.27). S. Chemerin had an average of 365.80± 264.48 (Median =253.98, SEM = 79.74) for Obese. The statistical analysis indicated no statistically significant difference between the groups, t(40) = -0.67, p =.509, This finding implies that the mean of S. Chemerin did not differ significantly between the Non-obese and Obese groups. Table 7

Table 7: Mean values comparison of the serum and follicular fluids study parameters by obesity

Variable	Non-obese		Obese		
	n	Mean± SD	n	Mean± SD	P [a]
Serum Chemerin	32	302.40± 199.51	12	351.11± 257.26	0.5092

[a] Two-Tailed Independent Samples t-Test

Discussion

Obesity can have a negative impact on fertility. Early onset obesity in women favors the development of oligo anovulation, chronic menstrual abnormalities, and adult-onset infertility. When the body mass index of a woman is greater than 30 kg/m2, obesity can potentially worsen the results of assisted reproductive technologies and pregnancy. Insulin resistance and insulin overproduction may be the key reasons contributing to the connection. Particularly noticeable are these negative effects of obesity in polycystic ovary syndrome.so much more attention should be paid to the impact of obesity on uncertainty in both women and men. (Gambineri, et al., 2019). Age has already been identified as one of the most predictive factors for fertility assessment. The decline in fertility with age is caused by a decrease in the amount and qualy of oocytes,(Jaswa et al., 2021) which also leads to a lower success rate in achievingpregnancy through in vitro fertilization. In the present study, it was found that there was no significant difference in the mean age between the obese and non obese groups ,this finding indicated that both groups had comparable age which is important to eliminate any variable that may affect the results of the study and the effects on IVF results. (ULRICH and MARSH, 2019). In the present study , the results within the non obese group was the absence of PCO have been the most frequently seen, which was the same observation in obese group in another ward, there was a difference between the expected frequencies and the frequencies that were actually observed. And here there is the importance of several methodological issues need to be discussed , the present study has several limitations. For instance, the sample size was quite small. Additionally, the lifestyles (such as their eating habits or how often they exercise), which could have affected their weight regardless of PCOS is not asked. Moreover, some studies have indicated that BMI is not an entirely accurate measure of obesity. (Alfahl et al., 2020) who agree with our results. Also the similarity in the PCO between obese and non obese groups since it has been reported that only one third of the PCOS patients are obese(Toosy, Sodi and Pappachan, 2018;Glueck and Goldenberg, 2019). Basal hormones (FSH, LH and E2) were measured for the females shared in the present study because the determination of 'ovarian reserve' is important before any expensive IVF trial is undertaken. Detection of both low and high responders prior to starting protocol may decrease cycle cancellation rate and side effects, such as ovarian hyperstimulation syndrome (OHSS). One of the most often utilized tests for predicting success in IVF studies is the baseline FSH level. Previous research found that women with an increased cycle basal FSH had a lower ovarian reserve. Several studies since then have found that women with elevate FSH levels, regardless of age, had a poor response to ovarian stimulation protocols, resulting in a reduced pregnancy rate with ART.(Xu et al., 2020). Some studies argued that young age does not protect against the negative effects of decreased ovarian reserve, implying that an elevated basal FSH level is associated with a low response, poor oocyte quality, and a rise in miscarriage rates, leading not only to a decrease in pregnancy rates but also to a rise in miscarriage rates.(Siddiqui, et al, 2019).Other studies, however, have demonstrated that women with elevated baseline FSH levels can still achieve reasonable pregnancy rates with ART , particularly in younger women. (Yang et al., 2022). It was suggested that basal serum LH could predict ovarian response to controlled ovarian hyperstimulation (COH) (Jiao et al., 2021). In this study statistical analysis found no statistically significant difference between groups, This finding implies that the mean AMH ng ml was not statistically different between the non-obese and obese groups and this indicated that the samples which were chosen to compare in the two tested groups were identical in their ovarian reserve. Also in this study a significant positive correlation between AMH and number of retrieved oocyte was observed with a correlation coefficient of =0.44, indicating a moderate effect size p=0.12 and this results were in agreement with (Neha et al ., 2022) who found that AMH level was strongly associated with the number of retrieved oocyte and the availability of embryos suitable for cryopreservation. In this study the statistical analysis indicated no statistically significant difference between the groups, This result reveals that the mean of serum chemerin did not differ significantly between the Obese and Non-obese groups, these results could be due to that Chemerin has been suggested as a potential link between obesity and associated comorbidities in humans Since chemerin was related to parameters of central fat mass and metabolic syndrome these findings suggest an impact of chemerin on factors of the metabolic syndrome in obese persons (Niklowitz et al., 2019). Also the relation of serum chemerin with PCO which is studied by many researchers (Mansoori et al., 2021). Who's found that there is strong correlation between serum chemerin and PCO and in this study there is insignificant correlation between obese and non obese groups regarding PCO that may be leading to insignificant correlation of serum chemerin between obese and non obese . In the current study it doesn't include the relation of this therapy on chemerin levels and on over all metabolic status.There is research found that, Exercise training may decrease serum concentrations of chemerin in individuals with overweight and/or obesity. (Ashtary-Larky et al., 2021)

Conclusion

The present study concluded that

- Evaluation of serum chemerin maybe a promising parameter for the prediction of ICSI cycle success
- Serum chemerin had a significant correlation with oocyte maturity and embryo grading

Recommendation

- Further studies on larger number of patients and compare the level of chemerin in obese and in non obese group in serum could be used as an indicator for oocyte and embryo quality .
- Further studies using different measurement of body weight not only the BMI is needed ,such as fat distribution must included.

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