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Correlation between serum carnitine level and Soluble Receptors for Advance Glycation End Products(sRAGE) in Clomiphene Citrate Resistant- PCOS Women

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Abstract

The most frequently diagnosed condition in women at the age of reproduction is the polycystic ovarian syndrome (PCOS).It could be related to a complex endocrine condition, due to its heterogeneity and uncertainty about its etiology, as the clinical highlights of PCOS incorporate those related to reproductive signs such as decreased frequency of ovulation, irregular menstrual cycles, decreased fertility. Carnitine plays a substantial role in weight loss, glucose tolerance, insulin function and fatty acid metabolism. Thus, carnitine plays a crucial role in controlling obesity, insulin resistance, oxidative stress that are associated with PCOS. While, AGEs are a diverse group of reactive molecules that are formed endogenously by non-enzymatic reactions of carbonyl group of carbohydrates with free amino groups of proteins, nucleic acids or lipids. The soluble form of receptors of AGE (sRAGE) could play an important role in management obesity, insulin resistance, hyperandrogenism, oxidative stress which could be related to PCOS. This study aimed to investigate serum levels of carnitine & soluble receptors for advanced glycation end products (sRAGE) in clomiphene resistant PCOS. Besides assessing the correlation between serum levels of carnitine, as well as, soluble receptors of AGE with hormonal (LH, FSH& Testosterone) and metabolic (serum glucose , serum insulin & HOMA-IR) markers in these patients **.**

The study included thirty women with clomiphene resistant PCOS and thirty apparently healthy women, as a control. In order to measure serum total carnitine and serum soluble receptor of advance glycation end product (sRAGE in PCOS and control groups.

The results of our study have shown a decreased serum levels of total carnitine in PCOS group in comparison with control group (48.05 and 59.73 nmol/ml, respectively), but there was no significant elevation in serum levels of sRAGE in patients group as compared with control group. In addition to a significant correlation between serum total carnitine and serum sRAGE levels **(r=0.45, P-value=0.03).**

In conclusion, serum total carnitine level was low in Clomiphene resistant-PCOS patients in comparison with control group. Although, sRAGE levels in clomiphene resistant- PCOS patients were not significantly different from the age and BMI-matching controls, but a significant correlation between serum total carnitine and sRAGE was detected.

Keywords: Poly cystic ovarian syndrome, soluble receptor of advance glycation end products (sRAGE) , SerumTotal Carnitine .

العلاقة بين مستوى الكارنتين والمستقبلات الذائبة للمنتجات النهائية السكرية المتقدمة في مصل المريضات بمتلازمة المبيض المتعدد الاكياس من النوع المقاوم للعلاج بالكلومفين

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الخلاصة

ان الحالة الأكثر شيوعًا عند النساء في سن الإنجاب هي متلازمة المبيض المتعدد الاكياس. يمكن أن يكون ذلك مرتبطًا بحالة الغدد الصماء المعقدة او بسبب عدم التجانس وعدم اليقين بشأن مسبباتها. تتضمن المظاهر السريرية لمتلازمة تكيس المبايض وخاصة تلك المتعلقة بالعلامات الإنجابية مثل انخفاض تكرار الإباضة ، وعدم انتظام الدورة الشهرية ، وانخفاض الخصوبة ، والمبيض متعدد الاكياس بالموجات فوق الصوتية ، وتراكيز مرتفعة للهرمونات الذكرية مثل التستوستيرون ، والتي يمكن أن تساهم في نمو شعر الجسم والوجه المفرط وحب الشباب. وبالتالي، فإن متلازمة المبيض المتعدد الاكياس لها آثار سريرية متنوعة ومختلفة تشمل الخصوبه (فرط الأندروجينية، عقم ,فرط نمو الشعر)، التمثيل الغذائي (ضعف تحمل الكلوكوز، مقاومة الأنسولين، النوع الثاني من داء السكري، زياده خطرالاصابة بامراض القلب) والميزات العقلية (الاكتئاب الموسع، القلق) التي قد تؤدي الى تراجع جودة الحياة.

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يعدالكارنتين اليساري وهو مشتق من احد الأحماض الأمينية، لايسين. حيث وجد ان الكارنيتين اليساري- أيزومر نشط حيويًا. ويتم إنتاجه بشكل رئيسي في الكبد ويعاد امتصاصه في الكلى، ثم ينتقل إلى الأنسجة الأخرى. يتركز أكثر في الأنسجة التي تستخدم الأحماض الدهنية كطاقة أساسية لها، مثل الهيكل العظمي والعضلة القلبية. في هذا الصدد، يلعب ل- كارنيتين دورًا حيويًا في توليد الطاقة عن طريق نقل الأحماض الدهنية من العصارة الخلوية إلى الميتوكوندريا. يلعب الكارنيتين دورًا مهمًا في فقدان الوزن وتحمل الجلوكوز ووظيفة الأنسولين واستقلاب الأحماض الدهنية. وبالتالي يلعب الكارنيتين دورًا حاسمًا في السيطرة على السمنة ومقاومة الأنسولين والإجهاد التأكسدي المرتبط بـ متلازمة المبيض المتعدد الاكياس.ان منتجات السكريه النهائيه المتقدمه هي مجموعة متنوعة من الجزيئات النشطة التي تنتج داخليًا بواسطة التفاعلات غير الإنزيمية لمجموعة الكاربونيل من الكربوهيدرات مع مجموعات أمينية من الدهون والبروتينات والأحماض النووية. وقد لوحظت مستويات مرتفعة من منتجات السكريه النهائيه المتقدمه المصلية في المرضى الذين يعانون من السكري، والشيخوخة و مقاومة الأنسولين ومؤخرًا متلازمة المبيض المتعدد الاكياس.

هدفت الدراسة الحالية إلى البحث في:تقييم مستويات الكارنتين في المصل و الشكل القابل للذوبان من مستقبلات منتجات السكريه النهائيه المتقدمه في مصل الدم في مرضى متلازمة تكيس المبايض المقاومة للكلوميفين، مقارنة بالنساء الصحيحات.اضافة الى دراسه العلاقه بين التواجد المحتمل للارتباطات المعنوية احصائيا بين مستويات المصل من الكارنيتين، والمستقبلات القابلة للذوبان لمنتجات السكريه النهائيه المتقدمه وكذلك مع الهرمونات(هرمون الملوتن، هرمون التحوصل، البرولاكتين والتستوستيرون) والتمثيل الغذائي (الجلوكوز في المصل، الأنسولين في المصل) في هؤلاء المرضى.

اشتملت الدراسة على ثلاثين امرأة مصابة بمتلازمة تكيّس المبايض المقاومة لـ الكلوميفين وثلاثين امرأة ذات صحه جيده على ما يبدو، كمجموعة مقارنة. من أجل قياس مستوى الكارنتين الكلي ومستوى المستقبلات القابلة للذوبان لمنتجات السكريه النهائيه المتقدمه في مصل الدم، إلى جانب العلامات الهرمونية والكيميائية الحيوية في مجموعه متلازمة تكيس المبايض و مجموعه السيطره.

أظهرت نتائج الدراسة الحالية انخفاض مستوى الكارنيتين الكلي في مصل الدم لمجموعة متلازمة تكيس المبايض بالمقارنة مع المجموعة الضابطة (٤٨,٠٥، ٥٩,٧٣ نانومول/ مل، على التوالي) وأظهرت أن مستوى المستقبلات القابلة للذوبان لمنتجات السكريه النهائيه المتقدمه في مصل الدم قد زاد بشكل طفيف في مجموعة المرضى مقارنة بالمجموعة الضابطة. بالإضافة إلى الارتباط الهام بين مستوى الكارنتين ومستوى المستقبلات القابلة للذوبان لمنتجات السكريه النهائيه المتقدمه في مصل الدم (م = ٠,٤٥ ، قيمة ف = ٠,٠٣) في مجموعة المرضى.

يستنتج مما ورد في النتائج: انخفاض مستوى الكارنيتين في مصل الدم لدى مرضى متلازمة تكيس المبايض مقارنة بالمجموعة الضابطة. ومع ذلك، زيادة غير كبيرة في مستويات المستقبلات القابلة للذوبان لمنتجات السكريه النهائيه المتقدمه في مصل الدم في مرضى متلازمة تكيس المبايض مقارنة مع المجموعه الضابطه. بالإضافة إلى ذلك، تم الكشف عن وجود ارتباط معنوي سلبي كبيرفي مصل الدم بين مستوى الكارنيتين ومستوى المستقبلات القابلة للذوبان لمنتجات السكريه النهائيه المتقدمة لدى مرضى متلازمة تكيس المبايض المقاومة للكلوميفين.

الكلمات المفتاحية: متلازمة المبيض المتعدد الاكياس، والمستقبلات الذائبة للمنتجات النهائية السكرية المتقدمة، الكارنتين الكلي في مصل الدم.

Introduction

The most frequently diagnosed condition in women at the age of reproduction is the polycystic ovarian syndrome (PCOS) [(1)] . It could be related to a complex endocrine condition, due to its heterogeneity and uncertainty about its etiology. Clinical highlights of PCOS incorporate those related to reproductive signs like decreased ovulation frequency, menstrual irregularities, decreased fertility, ultrasound polycystic ovaries, and elevated male hormone concentrations such as testosterone, which can contribute to excessive body and facial hair development and acne. Hence, Poly cystic ovarian syndrome has important and assorted clinical implications involving reproductive(hyperandrogenicity,hirsutism,infertility), metabolic(compromised glucose tolerance , insulin resistance, type two DM, unfavorable cardiovascular hazard profiles) and mental highlights (expanded depression, anxiety) that may decline life quality [(2)] .

In most cases, ovulation can be induced with Clomiphene citrate (CC) is a selective modulator of oestrogen receptors (SERM), and has been the first-line therapy of patients with anovulation or oligomenorrhea for over 40 years. Clomiphene citrate competes with endogenous estrogen at hypothalamus and pituitary gland receptors, interfering with natural estrogen's negative feedback signaling. Compared to natural estrogen, CC binds in the hypothalamus for weeks rather than days, effectively blocking the replenishment of estrogen receptors. Uninhibited release of GnRH and FSH is due to this hypoestrogenic state. The elevated levels of FSH induce ovarian hyperstimulation and the potential to grow multiple follicles; however, 15-20% of the patients fail to ovulate (CC resistance) and from 60-70% of the patients fail to conceive (CC failure) after 6 cycles of treatment with CC and require alternative treatments. other treatment modalities for Clomiphene resistant PCOS Clomiphene citrate + metformin, gonadotropin and laparoscopic ovarian drilling [(3)] .Currently, insulin resistance and the compensatory hyperinsulinemia affects some 65–70% of women with PCOS. Part of the insulin resistance appears to be independent of obesity and related specifically to PCOS, with abnormalities of cellular mechanisms of insulin action and insulin receptor function having been documented [(4)] .

Approximately 50 percent of PCOS patients suffer from weight gain which can worsen disease symptoms [(5)]. For a few trials, serum carnitine levels for PCOS patients and the impact of carnitine supplements is tested on their weight reduction. In an intersectional analysis [(6)], there was a negative and significant association between plasma level of L-carnitine and body mass index(BMI) in women with PCOS.A study by Ismail et al. among clomiphene resistant PCOS women, getting clomiphene citrate combined with 3000 mg per day of L-carnitine for 12 weeks, resulted in a significant decrease of BMI in the L-carnitine treated group [(7)] . In addition to a cross-sectional study, which concluded that plasma concentrations of L-carnitine had a negative and significant

correlation with HOMA-IR-index in PCOS patients [6].

Hyperinsulinemia and insulin resistance in patients with PCOS are caused by elevated androgen levels [8]. Insulin resistance and hyperinsulinemia can also increase the proportion of LH / FSH and the androgen production [9]. The impact of carnitine on ovarian hormones has been appeared within the past studies [10,11]. In like manner, it shows up that carnitine improves insulin sensitivity which in turn influences androgens and ovarian hormones levels [12]. Correlation between hormonal status such as estrogen, testosterone and serum levels of carnitine and has been investigated in a few researches. In one research, in obese women with PCOS there was an inverted correlation between SHBG and serum carnitine; but there was no correlation between carnitine levels and androgens [13].

On the other side, the advanced glycation end products AGEs are a diverse group of reactive molecules, that are formed endogenously by non-enzymatic reactions of carbonyl group of carbohydrates with free amino groups of proteins, nucleic acids or lipids. Elevated serum AGEs levels have been observed in patients with hyperglycemia, insulin resistance, diabetes, aging, and lately PCOS. High circulating levels of AGEs can cause cellular damage after deposition in different tissues. Late data have shown that AGEs' circulating levels and expression of their pro-inflammatory receptors in the ovarian tissue called as receptor for advanced glycation end products (RAGE) are elevated in women with PCOS [14] .

PCOS women have raised serum advance glycation end products (AGEs) and raised expression of the membranous inflammatory receptor (RAGE) seen in their ovaries [15]. The linking of AGEs to it is receptor (RAGE) results in cellular events leading to the production of reactive oxygen species mainly through the activation of (NADPH) oxidase and proinflammatory transcription factor (NF- κB). Subsequently, the development of proinflammatory cytokines (such as IL-1,6,8), regulators of apoptosis like Fas ,bcl-2, adhesion molecules (like ICAM-1 and VCAM-1), and activation of platelets and macrophages [16,17]. Interests, ROS production triggered by receptor of advance glycation end products (RAGE)activation therefore induces a positive up regulating receptor of advance glycation end products (RAGE) expression [18]), thereby pushing the inflammatory processes to be amplified. Induction of RAGE has been recorded in atherosclerosis, inflammatory processes and recently PCOS [16,19].

Serum levels of advance glycation end products (AGEs) contribute to the hormonal alteration found in women with PCOS [20]; for occurrence, it appeared that there is a relationship between serum AGEs and serum testosterone (20 In addition, it has been studied that alters in nutritional AGEs that may improve changes in hormonal status, oxidative stress ,insulin sensitivity in PCOS women [21].As documented that approximately two thirds of women with PCOS are inclined to develop insulin resistance (IR) [8] and eventually diabetes mellitus(DM)[22,23], the DM and IR are frequently worsened by obesity (24)(35). AGEs contributed to the development of insulin resistance (IR) in PCOS [26].

The present study was aimed to investigate serum levels of carnitine & soluble receptors for advanced glycation end products (sRAGE) in clomiphene resistant- PCOS women in comparison with age and BMI-matching non-PCOS women. Besides assessing the correlation between serum levels of carnitine,as well as, soluble receptors of AGE with hormonal (LH,FSH&Testosterone) and metabolic (serum glucose , serum insulin and HOMA-IR) markers in these patients **.**

Subjects and Methods

The present study was a case control study included patients whom diagnosed to have PCOS from those attending Al-Yarmook Teaching hospital / Baghdad, for the period from (October/2019 to April /2020). This study was approved by the Ethics Committee of the College of Pharmacy/University of Baghdad. All participants were informed about the aim and the proposed benefits of the study before they obtained their consent.

The study included thirty women with clomiphene resistant PCOS and thirty apparently healthy women, as a control. The chosen PCOS patients were under supervision , based on the changed Rotterdam criteria, which require, two of the following three appearances: (1) oligo-and/or anovulation (cycle length >35 days), (2) clinical and/or biochemical hyperandrogenism (clinically by evaluation of the hair development based on the altered Ferriman-Gallwey score and/or biochemically based on raised add up to testosterone, raised serum dehydroepiandrosterone sulfate (DHEAS), and androstenedione) and (3) polycystic ovaries on ultrasound (PCO was characterized as the presence of 12 or more follicles in each ovary, each measuring 2-9 mm in diameter, and/or expanded ovarian volume > 10 ml) (27). Table-1 summarizes the subject's characteristics.

Blood specimen was obtained from each participant for assessing hormone levels in serum: LH, FSH, Testosterone and insulin. In addition to some biomarkers: Fasting serum glucose, Homeostatic model assessment for insulin resistance (HOMA-IR),as well as, ELISA measurement of serum soluble receptor of advanced glycation end products AGE(sRAGE) and total Carnitine. Blood was collected at 9:00 am after an overnight fasting between the 2nd and 4th days of a spontaneous bleeding episode of the PCOS group and of a menstrual cycle of the controls. Venous blood specimens (10 ml) were exchanged into gel tubes

permitted to clot and after total clotting; serum is isolated by centrifugation 10 minutes at 3500 to 4000 rpm to get serum. The serum to was isolated into eppendrof's tubes (kept frozen at -20˚C) until their measure , unless something else they analyzed quickly like(FSG,FSH,LH,Prolactin&Testosteron) that were analyzed immediately.

Biochemical Assay methods

Measurement of serum total carnitine level using specific ELISA kit (6).Serum Soluble Receptor of Advanced Glycation End Products (sRAGE) Level was estimated by (ELISA) (28),both kits were purchased by Shanghai Yehua Biological Technology/China .While hormonal assessment of LH,FSH,Prolactin &Testosterone were measured by VIDAS specific kits purchased by Biomerieux/France(29-31).Fasting serum glucose was measured kinetically at a wavelength of 642 nm and 37 C and is displayed after about 125 seconds in mg/dl or mmol/L Reflotron /Germany (32).Serum insulin level was measured by chemiluminesce using kit provided by Cobas e411 /Germany (33).

Statistical Analysis

The analyses were conducted using the Statistical Package for the Social Science (SPSS, version 22, IBM, New York, USA). Descriptive statistics (means, standard errors of the mean, frequencies and percentages) of the participants (both patient and control group) were calculated. Independent T-test was used to compare parameter means between the two groups and Pearson correlation was used to measure the correlation between two parameters within each group. A p-value of less than 0.05 was statistically significant.

Table 1 . Subjects Characteristics.

Parameter	Groups	Mean	±SEM	(P-value)
Age(yrs)	Control	**27.43**	**1.115**	**0.234**
	Patient	**29.40**	**1.197**	
Weight (Kg)	Control	**77.20**	**2.117**	**0.397**
	Patient	**74.80**	**1.853**	
BMI (kg/m2)	Control	**29.717**	**0.749**	**0.790**
	Patient	**29.443**	**0.697**	
WHR	Control	**0.908**	**0.023**	**0.879**
	Patient	**0.901**	**0.021**	
Height (cm)	Control	**161.16**	**1.195**	**0.238**
	Patient	**159.30**	**1.465**	
Prolactin (ng/ml)	Control	**18.86**	**1.58**	**0.038**
	Patient	**25.57**	**1.87**	
LH (MIu/ml)	Control	**4.30**	**0.38**	**0.701**
	Patient	**4.19**	**0.41**	
FSH (MIu/ml)	Control	**5.85**	**0.48**	**0.135**
	Patient	**5.26**	**0.48**	
Testosterone (ng/ml)	Control	**0.47**	**0.04**	**0.160**
	Patient	**0.43**	**0.05**	
FSG(mg/dl)	Control	**99.91**	**2.63**	**0.506**
	Patient	**102.61**	**2.85**	
Insulin (uU/ml)	Control	**7.06**	0.44	0.188
	Patient	**7.89**	**0.43**	
HOMA-IR	Control	**1.81**	**0.15**	**0.164**
	Patient	2.06	0.16	

BMI=Body mass index, LH=Luteinizing hormone, FSH=Follicle –stimulating hormone, FSG=Fasting serum glucose , HOMA-IR= Homeostatic Model Assessment for Insulin Resistance .

RESULTS

Serum total carnitine levels in PCOS patient and control groups:

As presented in **Figure 1**, serum carntine level was statistically lowered in PCOS group as compared to control group (P-value <0.05)

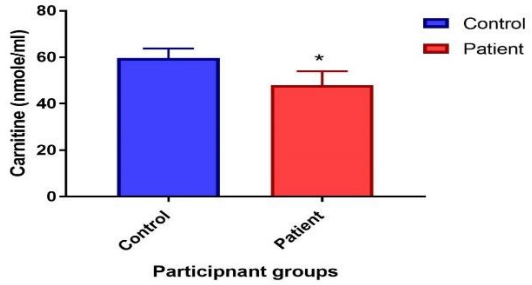

Figure 1. Serum Carnitine Level in Patient and Control Groups

*According to Mann-Whitney test, there is significant P-value < 0.05) difference in Camitine level between the two groups (control group has significantly higher level compared to patient group) Additionally as illustrated in **Table- 2,** there is no significant (P-value >0.05) correlations between glycemic markers (FSG,Insulin,HOMA-IR) with serum total Carnitine level in patient group.

Table 2. Correlations between glycemic markers (FSG, Insulin and HOMA-IR) with serum total carnitine level in patient group

Glycemic marker		Total Carnitine
FBG	**Correlation Coefficient**	**.030**
	Sig. (2-tailed)	**.876**
	N	**29**
Insulin	**Correlation Coefficient**	**.229**
	Sig. (2-tailed)	**.233**
	N	**29**
HOMA-IR	**Correlation Coefficient**	**.174**
	Sig. (2-tailed)	**.367**
	N	**29**

Non-significant (P-value >0.05) correlations according to Spearman correlations.

Soluble Receptors of Advanced Glycation End Products (sRAGE)

Although the mean value of the patients group was (2.28) (ng/ml) in terms of sRAGE was double the value of the control group (1.09) (ng/ml), the difference was non-significant probably because of high variation and small sample size (**Figure- 2**).

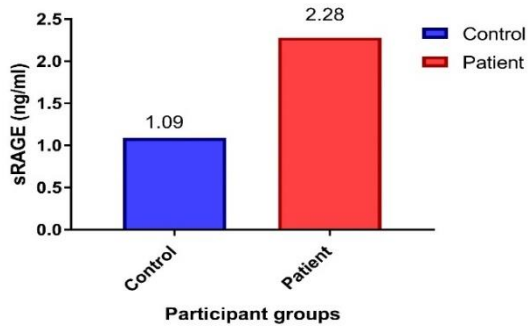

Figure2. Mean Values of sRAGE levels in Patient and Control Groups

Furthermore, there is a significant (P-value <0.05) positive correlation between serum carnitine values and serum sRAGE levels **(r=0.45, P-value=0.03)** in patient group (Figure-**3**).However, no significant correlation was detected for either one with the studied sex hormones (**Table -3**).

Table 3. Pearson's correlations between serum carnitine and s RAGE with sex hormones in patients group

Parameter		Carnitine	sRAGE	Prolactin	LH	FSH	Testosterone
Carnitine	Correlation Coefficient	1.000	.452*	-.259	.084	.026	.340
	Sig. (2-tailed)	.	.030*	.175	.664	.894	.071
	N	29	23	29	29	29	29
sRAGE	Correlation Coefficient	.452*	1.000	-.066	-.179	-.160	.009
	Sig. (2-tailed)	.030*	.	.760	.402	.456	.968
	N	23	24	24	24	24	24
Prolactin	Correlation Coefficient	-.259	-.066	1.000	-.016	.080	-.149
	Sig. (2-tailed)	.175	.760	.	.934	.674	.432
	N	29	24	30	30	30	30
LH	Correlation Coefficient	.084	-.179	-.016	1.000	.324	.244
	Sig. (2-tailed)	.664	.402	.934	.	.081	.193
	N	29	24	30	30	30	30
FSH	Correlation Coefficient	.026	-.160	.080	.324	1.000	-.273
	Sig. (2-tailed)	.894	.456	.674	.081	.	.145
	N	29	24	30	30	30	30
Testosterone	Correlation Coefficient	.340	.009	-.149	.244	-.273	1.000
	Sig. (2-tailed)	.071	.968	.432	.193	.145	.
	N	29	24	30	30	30	30

*** Correlation is significant at the 0.05 level (2-tailed).**

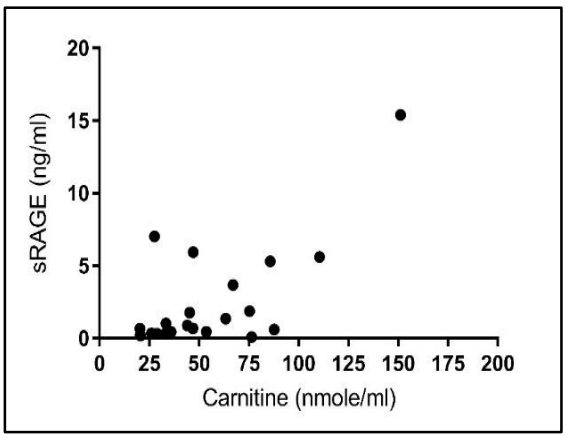

Figure 3. The correlation between patient serum carnitine and sRAGE levels *(r=0.45, P-value=0.03)*

Discussion

According to the results in (**Figure-1**) that shows there is a significant difference in serum total carnitine level between PCOS patients and control group(P=**0.026**) and the average level of serum total carnitine in control group is higher than the average of total carnitine in PCOS group and this is due to insulin resistance and hyperandrogenism which are the foremost Crucial highlights of PCOS, which is related with reduced serum total carnitine levels[6,34]. Additionally the hyperinsulinemia and insulin resistance may be influenced by excess production of androgens, which in turn affects the liver cells leading to reduced SHBG generation and rise of androgens[35,36]. In expansion, both insulin resistance and hyperandrogenism are correlated with dyslipidemia, obesity and consequently risk factors for cardiovascular illnesses [8]. Studies have appeared that serum carnitine levels in obesity and metabolic syndrome diminish following insulin resistance. Moreover, carnitine supplementation leads to decrease in weight, BMI, waist to hip ratio, body fat mass (FM) and increased basal metabolism [37].

The beneficial effect of carnitine supplementation on parameters of glucose homeostasis has appeared in past studies [38,39]. Glycemic status disorders are the main common complication in PCOS following insulin resistance. There is a negative correlation in these patients between carnitine

levels and insulin, FBS, HOMAIR. [6,7]. Additionally, carnitine supplementation with every day doses of 250 - 3000 mg appeared to produce significant decrease in glucose, insulin and HOMA-IR values [40]. Carnitine likely improves insulin metabolism variables by directing the activation of gluconeogenic and glycolytic enzymes [41], enhancing glucose oxidation in mitochondria and function as an acetyl-group donor in a high-energy metabolism situation or as a free fatty acid transport molecule that leads to enhance glycemic status and increased insulin sensitivity[42].While the results of present study (**Table-2**) indicated that there is no significant correlation between glycemic markers (FSG,Insulin,HOMA-IR)with serum total carnitine level in patient group.

In patients with PCOS, a frequent low-grade inflammation was observed. In these patients, serum levels of IL-6, TNF-α and CRP increased [43]. The important relationship between oxidative stress circulation and androgen and inflammatory biomarkers levels [44]. These discoveries recommend that hyperandrogenism in PCOS may induce aggravation and upgrade oxidative stress through insulin resistance and hyperglycemia and conversely inflammation induced by hyperglycemia can promote the production of abundance ovarian androgen. In addition, inflammatory markers and oxidizing stress are associated with insulin resistance [45,46].

In this manner the association of oxidative stress and inflammation with hyperglycemia and insulin resistance can lead to hyperandrogenic exacerbations. The antioxidant properties of carnitine are mainly linked to free radical scavenging and the avoidance of free radical formation, preserving the integrity of the electron-transport chain in mitochondria resulting in reduced secretion of ROS holding it under stress conditions, and influencing redox signals by inhibiting nuclear factor-jB resulting in increased production of antioxidant molecules and chemicals [47] .

In spite of the non-significant differences in the mean serum levels of soluble receptor of advance glycation end products(sRAGE) between PCOS patients and control group (P=0.123).However, the mean value of (sRAGE) in patient group (2.28) (ng/ml) was double the value of the control group (1.09) (ng/ml) (Figure-2).This inconsistency , could be related to the size of studied population in this study.That mean the value of sRAGE is much higher in PCOS patients in comparable with non PCOS patients.

Multiple studies give prove for increased oxidative stress in normoglycemic women with PCOS and thus this could account for the elevated levels of serum AGEs in these women [48-50]. On the basis of this prove, the role of AGEs might too be considered to participate directly or indirectly within the pathophysiology of the syndrome, as oxidative stress has been appeared to be involved within the improvement of insulin resistance and hyperandrogenism in PCOS. sRAGE compete with RAGE for ligand binding site (act as a decoy receptor); it may neutralize AGE-RAGE-mediated oxidative damage. Piperi et al. found that the normal glucose level of PCOS women had increased serum levels of sRAGE, which correlated positively with AGE levels.[51]

In the present study, serum sRAGE increased in patients PCOS compared with the control group. However, this increase was not statistically significant and may be explained like other studies have shown that increase AGEs' circulating levels that result from oxidative stress and inflammation accompanied with PCOS that lead to increase expression of their pro-inflammatory receptors in the ovarian tissue called as receptor for advanced glycation end products (RAGE) are elevated in women with PCOS . On the other hand, high levels of the protective anti-inflammatory receptors called soluble receptor for advanced glycation end products (sRAGE) are associated with protection against AGEs [14] .

While other studies have shown an inverse relation between sRAGE and hyperandrogenism and significant effects in PCOS women [52]. In latest years, a few studies, have shown that raised AGEs serum levels in PCOS women. [15].

As the AGE-RAGE process is basically linked to hyperandrogenism and insulin resistance, which are major pathophysiological characteristics in patients of PCOS. As well to regular receptors, one type of AGE receptor, missing transmembrane and cytosolic domains, are named soluble receptor of advanced glycation end product (sRAGE). They are discharged out of the cell, and can be recognized in blood circulation [20, 53]. (sRAGE) can link with it is ligands (AGEs) within the blood that lead to prevent the harmful impacts of the (AGE-RAGE) process. sRAGE is at that stage commonly known as good receptor. The values of sRAGE were found to be decreased in obesity and hyperglycemia that have been clarified by the useful function of sRAGE and also its work as decoy to catch the AGEs in blood

circulation avoiding stimulation of RAGE signaling process [54-56]

Obesity is a very frequent highlight of PCOS patients, display in 30 to 75 percent of patients, and act as an aggravating agent within the group of clinical features of the metabolic syndrome [57]. Through past research, had shown that AGEs serum levels are specifically included in adipogenesis as well as generation of inflammatory cells in adipocytes, resulting to abnormalities linked to obesity [58]. These research results suggested a possible part of advance glycation end products in obesity-linked comorbid conditions, as they noticed that AGEs serum levels have been raised, while serum levels of sRAGE have been reduced together with raised BMI [52] .Association testing appeared that, serum sRAGE levels were conversely linked with body mass index, while AGEs had a positive association with body mass index. Specific regression studies showed that BMI had been the major predictor of sRAGE, which assist supported their results [15,20]. As hyperinsulinemia, insulin resistance would be seen as a critical factors and pathophysiological mediators in PCOS [53]. In vitro experiments, it showed that AGEs are associated with insulin resistance pathogenesis. As it had been recorded that serum AGEs to be increased in PCOS independently of the existence of insulin resistance [50] .

According to correlation studies in patient group, as illustrated in (**Table-3**) and **(Figure-3)** there is a significant (P-value <0.05) positive correlation between serum carnitine values and serum sRAGE levels in patient group that mean when the serum carnitine level decrease lead to decrease the serum level of Soluble receptor of advance glycation end products(sRAGE) in PCOS patients.

A soluble form of RAGE, described as soluble C-truncated RAGE (sRAGE) loss both transmembrane and cytosolic spaces of Rage and created after alternative splicing of Rage gene . The sRAGE receptor circulates all through the body and constitute a decoy receptor that binds circulating AGEs, hence avoiding them from association with their pro-inflammatory Rage receptor eventually preventing tissue damage . In spite of the fact that it remains petulant, a decreased sRAGE level shows a increased RAGE Signaling and more pathologies [14].

Carnitine develops maintenance mechanisms for oxidative stress-induced damage to membrane phospholipids, and also keeps up common antioxidant status. It protects cells from reactive oxygen species by acting as a free radical scavenger. Previously, we detailed expanded oxidative stress and diminished antioxidant capacity in patients with PCOS. These perceptions suggest that low levels of antioxidant carnitine may contribute to the hurtful impacts of increased oxidative stress in PCOS patients [34]. According to the results of studies above that revealed the oxidative stress in PCOS patients result from low level of serum total carnitine and (sRAGE) so these results are compatible with findings of present study that show in PCOS patients when the serum total carnitine decrease the sRAGE also decrease.

Because the average of BMI in control group is (29.717) so the control group consider as an overweight group (healthy weight falls between BMI values of 18.5-24.9).In a previous study showed that obesity may be a disorder of energy balance, happening when energy utilization and daily energy intake are not adequate. According to the findings of previous study that confirmed the erum carnitine level decrease when the BMI increase and this compatible with results of our present study.

In another study it appeared that sRAGE is conversely related with BMI, WHR, and fasting glycemia in a non-diabetic population which waist circumference and BMI are independent indicators of sRAGE in a healthy population, and especially in women. This is the primary observation, to the best of our knowledge that describes the relationship of all types of soluble RAGE with cardio metabolic parameters in a healthy population. Therefore, these findings recommend that earlier to any clinical complication, sRAGE plasma levels may reflect a metabolic disturbance status that seems afterward lead to vascular complications and diabetes. This result is supported by the observation that overweight subjects have lower sRAGE levels compared to normal subjects [59].

Conclusions:

1- Carntine might improve weight loss and glycemic status further studies are recommended to prove the exact mechanism of carnitine in patients with PCOS.

2- Soluble receptor of advance glycation end products (sRAGE) increases in PCOS patients in order to reduce the effects of elevated levels of advance glycation end products in PCOS patients since RAGE acts as scavenger receptors.

3- A significant positive correlation between serum total Carnitine and serum soluble receptor of

advance glycation end products in clomiphene resistant PCOS patients had been detected.

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