Study the association between indothelin-1 with age and body mass index in women with poly cystic ovarian syndrome

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Abstract
Polycystic ovarian syndrome (PCOS) is a common inflammatory endocrine disorder that affects women of reproductive age and considered risk factor for infertility. Endothelin-1 (ET-1) is an indicator of endothelial injury dysfunction and is elevated in women with androgen excess polycystic ovary syndrome (PCOS). Objective: this study aims to detect relation between endothelin-1 with age and bmi in pcos compare to control group. Subject and method: Case control study enrolled 120 women classified according to BMI (20-35) kg/m² and Age (20-40) years.60women diagnosed with PCOS by dr..Anfal AL.kaabi is agynecologist and by sonar and60 apparently healthy . PCOS cases were also classify to three subgroups (A, B, C) accroding to infertility (normal which no infertility, primary infertility was 40, secondary infertility was 20). The samples were collected in the morning fasting state from November 2020 to May 2021. Biochemical variables BMI calculated weight divided by square height in meter(km/m²). measure serum ET-1 (ng/ml) were assessed by ELISA. measure hormons (LH,FSH,F.testo) in cycle day two(CD2) by minividas .measure lipid profile (total cholestrol TC, high density lipo protrin HDL, low density lipoprotein LDL, very low density lipoprotein VLDL and triglyceride TG) by colorimetric assay using spectrophotometer. Measure insulin by ELISA assay. and fasting blood sugar by maglumi device. Results: elevated ET-1 obesity and increasing with age. The mean levels of biomarkers among infertility groups in women with PCOS shown that all parameters were significantly differed among the groups (p values <0.05) . level of LH; Testosterone; TG; Insulin; W\H and LH/FSH ratio were shown a positively significant increasing with secondary infertility cases. While FSH; HDL and fasting blood sugar were decreased significantly in the same group Conclusion: Endothelin-1 may be pre indicator diagnostic markers for PCOs -Endothelin was increasing with age; negative relation between ET-1 and HDL which is may be indicator for heart disease.
Keyword: polycystic ovarian syndrome, endothelin 1

1. Introduction

Polycystic ovary syndrome (PCOS) is the most common gynecological disease in women at reproductive age, which is characterized by metabolic and endocrine abnormalities, as well as chronic inflammation (Lambertini et al,2017; Seyyed Abootorabi et al, 2018).
The prevalence is generally considered to be between 6-20%, depending onthe definition and the population studied (Azziz et al, 2016). PCOS is due to acombination of genetic, environmental, and endocrine factors, which are the main causes of female ovulatory infertility (Szczuko et al, 2017).
The most common risk factors for the progress of PCOS include family history of PCOS,fast food diet habits, lack of physical exercise, body mass index and waist circumference (Begum et al, 2017).
The clinical manifestations of PCOS are acne, amenorrhea or oligomenorrhea hirsutism, hyperinsulinemia, infertility, and mood disorders (Yvette C et al,2015).
Women with PCOS are more likely to develop many metabolic and reproductive health complications that include dyslipidaemia, abnormal glucose level, insulin resistance, miscarriage, gestational diabetes, mood disorders,hypertensive disorders, preeclampsia, prediabetes, type 2 diabetes, obesity,obstructive sleep apnea, cardiovascular disease, stroke, chronic kidney disease,renal failure, breast cancer, endometrium cancer and others (Escobar-Morreale,2018).
The diagnosis of PCOS is dependent on two of the following three findings: intermittent or absent menstrual cycles, high circulating levels of testosterone (T) or hirsutism (Gorsic et al, 2019) and ultrasonographic findingsof the ovary (12 or more small follicles that are between two and nine mm indiameter in both ovaries) (Kadri etal, 2021).
Other conditions that produce similar symptoms include Thyroid disease, hyperprolactinemia and hyperlipidemia (Trent et al, 2020).
History of the disease: The earliest description of the polycystic ovary dates back to the 17th century (Azziz et al, 2016). Stein-Leventhal first described Polycystic ovaries syndrome in 1935, as infertility, menorrhagia, hirsutism, enlarged ovaries (El Hayek et al,2016; Mohammad et al, 2017), amenorrhoea, chronic novulation andobesity(Escobar- Morreale, 2018).

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The history of diagnostic the PCOS begins as the following: In 1990, the National Institutes of Health suggested Hyperandrogenism and Oligo-anovulation as the two criteria and the two are needed to diagnose PCOS(Fox, C.W, et al, 2019), which is the first formal attempt to classify PCOS.

In 2003, the Rotterdam criteria by the Rotterdam ESHRE/ASRM sponsored PCOS Consensus Workshop Group were puplished, which suggest two of the three following criteria (HA, ovulation dysfunction, and PCOM) (Anagnostis etal, 2018), which is the second formal attempt to classify PCOS.

In 2006, the Androgen Excess and PCOS Society (AE-PCOS) concluded that PCOS should be based only on two criteria, that is clinical and/or biochemical hyperandrogenism with either polycystic morphology or clinical anovulation (Anagnostis et al, 2018), which is the third formal attempt to classify PCOS.

In 2012, the National Institutes of Health (NIH) Evidence-based Methodology Workshop publishe the last report for PCOS diagnosis which suggested two of three following criteria (HA, ovulation dysfunction, and PCOM) (Jungari et al,2020).

Endothelin 1 (ET-1): is a potent vasoconstrictor peptide that is also expressed in neurons. The peptide comprises 21 amino-acids with two intrachain disulfide linkages and was first isolated from the culture supernatant of porcine aorta endothelium cells in 1988 (M. Yanagisawa, 1988).Endothelin derives from "big endothelin" a prepropeptide that is cleaved by endothelin-converting enzymes to produce mature endothelin (R. M. Kedzierski and M. Yanagisawa, 2001). endothelin has attracted intensive interdisciplinary interest because of its unique profile as an endothelium-derived vasoactive factor with a powerful and characteristically long-lasting vasopressor activity. Thus, whereas cellular mechanism of endothelin action appears to be similar to classic vasoconstrictor substances such as angiotensin II and norepinephrine, the slow time course of the regulatory mechanisms of its biosynthesis and secretion resembles more that of inflammatory cytokines. These characteristics make this family of small peptides unique within the realm of intercellular mediators with cardiovascular relevance (M. Yanagisawa, 1994).Endothelin is expressed in three isoforms called ET-1, ET-2, and ET-3, with slightly different amino-acid sequences and different distribution in various tissues. Accordingly, three different genes encoding the endothelins have been identified in the human, rat, and pig genome (Inoue A, 1989).Furthermore, three ET receptor subtypes called ETA, ETB and ETC have been described. ET-1 is present in the aqueous humour at concentrations several times higher than in plasma, presumably because it is secreted by the ciliary epithelium and not derived from plasma (Lepple-Wienhues, 1992).The vascular endothelium modulates local vascular tone by releasing relaxing factors such as nitric oxide, prostacyclin, and endothelium-derived hyperpolarizing factors as well as the potent vasoconstrictor peptide endothelin-1 (R. F. Furchgott and J. V. Zawadzki, 1980). Endothelin-1 is one of the several circulating molecules of endothelial injury products. ET-1-mediated vascular tone and metabolic function are abnormal in obesity and diabetes (Traupe et al. 2002; Cardillo et al. 2004; Lteif et al. 2007; Mundy et al. 2007). ET-1 also plays a role in adipogenesis and lipolysis (Bhattacharya and Ullrich 2006; van Harmelen et al. 2008), thus endothelin blockade may be particularly feasible for preventing cardiovascular and renal disease in patients and may also be suitable for treating obesity and its associated complications, such as insulin resistance (Berthiaume et al. 2005). Moreover, endothelin contributes to glycemic control and glucose uptake (Rachdaoui and Nagy 2003; Said et al. 2005) and development of type 1 diabetes (Ortmann et al. 2005) play a role in the early events of endothelial dysfunction, and has been used as a marker of ab- normal vascular reactivity (Yanagisawa M, 1989). Elevated endothelin-1 (ET-1) levels have been reported in some insulin-resistant states such as obesity (Ferri C, 1997), diabetes mellitus (DM) (Hopfner RL, 1999), and hypertension.Studies estimating ET-1 levels in women with PCOS. preliminary results by Paradisi et al. (Paradisi G) was shown abnormal endothelium vascular response in obese PCOS (PCOS-OB) women. On the cardiac vascular risk side, there are many biochemical markers (hyperandrogenism, estrogen/progesterone imbalance, IR, compensatory hyperinsulinemia, and low SHBG) which tell us that PCOS should be regarded as a high-risk state. The non-correspondence of alterations of biomediators of CV risk, early atherosclerotic processes, and CV events in PCOS later in life could be a product of lacking clinical data (prospective longitudinal studies) with adequate cohorts that are followed long enough, the extreme heterogenicity of the cohorts of PCOS included in the studies, or of their inadequate phenotypization in terms of biomediators of CV risk, in part due to the inadequacy of the methods used to measure it. In the meantime, the understanding of the underlying biochemical processes of CV risk in PCOS shifts attention to specific subgroups of the PCOS population which potentially carry most of the risk for CV events. (Srdan Pandurevic, 2021).Since Abdominal adiposity and obesity are often present in PCOS. Mounting evidence indicates that adipose tissue is involved in innate and adaptive immune responses. Continuous release of inflammatory mediators such as cytokines, acute phase proteins, and adipokines perpetuates the inflammatory condition associated with obesity in women with PCOS, possibly contributing to insulin resistance and other long-term cardiometabolic risk factors. (Ojeda-Ojeda, et al., 2013).On the other hand, PCOS may confer an adverse cardiovascular risk profile involving increased circulating AGEs and increased

ET-1 levels. These two proatherogenic molecules are highly likely to interact with each other, since their circulating levels are positively correlated in women with PCOS, independently of obesity, insulin resistance and hyperandrogenemia.

Subject and material: This study was carried out by chemical and Biochemistry department, College of Medicine, University of Karbala and the cases individuals were obtained from Gynecological and Obstetric Hospital / Babil teaching from the period November 2021 to May 2022 study included 60 women of PCOS (patients) and another 60 apparently healthy women (controls) with age range (20 - 40) years and bmi(20-35) kg/m². some women were excluded they suffer from hyper-tension, pregnancy, diabetes mellitus, thyroid disease, women taking oral contraceptives , biochemical parameter and clinical signs of hyperandrogenism. Blood samples are collected in the morning at fasting state from vein puncture during the follicular phase of the menstrual cycle . The samples put in to gel tubes and left for 15 minutes at room temp-erature to clotting then separated by centrifuge at 3000 rpm. for 10 min, and then measurement of serum endothelin -1 by eliza, hormones Testosterone, Follicle Stimulating Hormone(FSH), Luteinizing hormone (LH)were measured by VIDAS,fasting Insulin was measured byELIZA,and parameter of fasting blood glucose was measurment by maglumi device,lipid profile measure by spectrophotometer

2. Results

Table 1: Mean levels of biomarkers in women with PCOS compared to control group			
Biomarkers Mean ± 2SD	Study groups		P value
	Control (N= 60)	Patient (N= 60)	
LH (mIU/ml)	4.87±1.88	9.03±7.25	**<0.001[S]**
FSH (mIU/ml)	6.71±2.06	5.42±2.96	**0.002[S]**
F. TESTO (pg/ml)	0.96±0.35	1.81±0.78	**<0.001[S]**
TC. (mg/dl)	148.58±37.46	173.01±53.07	**0.017[S]**
TG (mg/dl)	108.87±22.58	145.47±66.24	**<0.001[S]**
HDL (mg/dl)	44.17±7.78	37.22±9.40	**<0.001[S]**
VLDL (mg/dl)	21.75±4.47	29.19±13.51	**0.002[S]**
LDL (mg/dl)	82.17±41.89	106.53±47.43	**0.009[S]**
FBS (mg/dl)	95.69±8.28	91.77±26.60	**0.004[S]**
ET1 (ng/ml)	54.37±6.31	92.31±62.49	**<0.001[S]**
VISFATIN (ng/ml)	10.48±3.15	25.11±43.33	**<0.001[S]**
IR	3.46±1.54	3.35±3.79	**0.006[S]**
W\H	0.86±0.09	0.92±0.19	**0.011[S]**
LH/FSH	0.74±0.22	1.73±0.87	**<0.001[S]**
p<0.05 considered significantly different, [S]= Significant, [NS]= Nonsignificant			

Results of Table (1) were indicated a significant increased levels of LH hormone, Testosterone, LH/ FSH ratio, endothelin and visfatin, the mean levels of the parameters in patients' group were 9.03; 1.81;1.73; 92.31 and 25.11 respectively.

Different lipid patterns are present in PCOS, including low levels of high-density lipoprotein cholesterol (HDL-C), high triglyceride (TG), total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) and significantly higher lipoprotein

Table (2): Correlation coefficients between mean levels of Endotheline-1 and biomarkers among women with PCOs		
ET1		
Biomarkers	Correlation coefficient (r)	P Value
LH	0.5	<0.001 [S]
FSH	0.1	0.582[NS]
F. TESTO	0.5	<0.001[S]
T.C	0.1	0.2217[NS]
TG	0.3	0.025[S]
HDL	-0.3	0.006 [S]
VLDL	0.2	0.044[S]
LDL	0.1	0.126[NS]
FBS	0.3	0.001[S]
IR	0.3	0.007[S]
W\H	0.3	0.015[S]
LH/FSH	0.2	0.057[NS]
p<0.05 considered significantly different, [S]= Significant, [NS]= Nonsignificant		

In table 2, correlation between endothelin-1 and biomarker show highly positive correlation with LH, F.testo, FBS, IR positive with TG,VLDL,W/H and negative correlation with HDL.

Table 3: The mean differences of the ET-1 levels with Age groups in patient						
ET-1	20 - 26 Years		27 - 32 Years		More than 33 Years	
	Patients	Control	Patients	Control	Patients	Control
Mean	69.24	53.29	107.83	53.60	128.72	59.12
Correlation Coefficient r	0.95		0.76		0.81	
P value	<0.001[S]		<0.001[S]		<0.001[S]	

Results were indicated that there was a significant difference in the ET-1 levels in all age groups, p value <0. 001.mean ET-1increase with aging. ET-1 levels were positively and strongly associated with AGEs in both PCOS women and controls

Table 4: The mean differences of the ET-1 levels with BMI groups in patient

ET-1	Normal weight		Overweight		Obese	
	Patients	Control	Patients	Control	Patients	Control
Mean	74.38	54.87	93.57	52.951	95.42	55.24
Correlation Coefficient r	0.86		0.42		0.5	
P value	<0.001[S]		0.06[S]		0.02[S]	

BMI were classified to a sub-group to investigate their effect on the levels of biomarkers, ET-1 levels were demonstrated a significant difference in the normal wight and obese when compared patients to control group, p values were < 0.001, 0.02 respectively.

3. Discussion

The pathophysiology of PCOS is still unresolved. Recent studies suggest that the brain is an important regulator as well as an affected organ in PCOS. The brain containing several receptors (androgen, estrogen and progesterone) and neurons with their neurotransmitters produces an increased pulse frequency of gonadotropin. As a result, an elevated luteinizing hormone (LH) secretion compared to follicle-stimulating hormone (FSH) is observed among patients with PCOS (Coutinho EA, et al, 2019). This altered LH-FSH ratio is responsible for increased ovarian androgen production. On the other hand, hyperandrogenemia reduces the negative feedback of estrogen to hypothalamus leading to increased pulse frequency of LH. This cycle is produced that is responsible for several clinical manifestations of PCOS including hyperandrogenism (Ashraf S, et al, 2019).

*The normal gonadotrophin axis is disturbed in PCOS women; therefore, LH levels increase, and FSH levels decrease, leading to a reversal of LH/FSH ratio (Balen AH, et al,2003).

*Obesity,insulin resistance, and dyslipidemia are PCOS-related morbidities and were found to be correlated with the LH/FSH ratio (Aug, et al, 2020)

*Also, IR plays an important role in the development of PCOS, in which the insulin response increases resulting in hyperinsulinemia as a unique feature in PCOS (L. Mu et al, 2018).

Patients were also shown an increased lipid profile parameters and the waist/ hip ratio, the mean levels of the lipid panel were TC (173.01); TG (145.47); VLDL (29.19); LDL (106.53) and the W/H ratio was (0.9), while in control group: TC (148.58); TG (108.87); VLDL (21.75); LDL (82.17) and the W/H ratio was (0.92).

* Lipid abnormalities are found in women affected by PCOS. A recent study showed that mild hypercholesterolemia is frequently.

Endothelin-1 (ET-1) is an indicator of endothelial injury and dysfunction and is elevated in women with androgen excess polycystic ovary syndrome. Plasma endothelin-1 [ET-1], an important indicator of endothelial injury, is increased in women with PCOS. Secreted through the basolateral compartment of end- othelial cells, ET-1 has vasoconstrictor actions on the vascular smooth muscle in the peripheral circulation via two primary receptor subtypes, ETAR and ETBR. Testosterone is integral to the development of end- othelial dysfunction and hypertension there is a clear androgen-induced EC signalling defect observed, this suggests a global endothelial dysfunction state, which has been suggested for women with PCOS and correlated to the early development of cardiovascular disease. (Usselman, 2019).

ET-1 levels were positively and strongly associated with AGEs in both PCOS women and controls, suggesting that the detrimental effect of AGEs on endothelial cells may involve increased ET-1 production (Christakou Charikleia. et al,.2011). positive correlation between obesity and plasma endothelin-1 (ET-1) levels. (Weil BR,et al ,2011).

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