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Study of socio-clinical risk factors and the pathophysiological variation of biological and oxidative stress markers of polycystic ovary syndrome in women

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الإهداء

إذا كان الإهداء جزء من الوفاء أهدي هذه المذكرة الى :

الينبوع الذي لا يمل العطاء, الى من كان دعائها سر نجاحي و بوجودها عرفت معنى الحياة إلى من حاكت سعادتي بخيوط منسوجة من قلبها الى رمز الحب والحنان أمي الغالية أدام الله عليها صحتها ، إلى من مهد لي طريق العلم واعطى فاكثر العطاء. إلى من بذل جهد السنين من أجل أن أعتلي سلالم النجاح، إلى من علمني الصبر الى من احمل اسمه بكل فخر ابي العزيز اطال الله في عمره .

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الإهداء

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Abstract

Polycystic ovary syndrome is one of the most common hormonal disorders and represents one of the causes of women's inability to have children. Therefore, the aim of our work is an evaluation of some risk factors and some biological and oxidative stress markers among polycystic ovary syndrome (PCOS) Women in El-Oued region. Our risk factor study was conducted on a group of 76 women who were diagnosed by specialized doctors (38 healthy and 38 polycystic ovaries), with 11 women volunteering to provide blood, saliva and urine samples to study the biological markers (hematological, biochemistry, and oxidative stress markers) for the examination and follow-up of polycystic ovaries. About risk factors study, our results showed a strong correlation between polycystic ovaries and social, economic behaviors as well as diet, and on the other hand, genetic factors. But the high levels of Nervous, menstrual period and year of taking pills are the most dangerous factors (OR=3.11; OR=9.205; OR=5.940) respectively, Conversely playing sport and movement in daily life are considered the important protective factors against this disease. Our result demonstrated that a significant increase ($P > 0.05$) in blood glucose, total cholesterol and Triglycerides levels and a significant decrease ($P > 0.01$) in HDL level in women with PCOS patients as compared to controls. The results of the hematological analysis show a significant decrease in WBC ($P < 0.000$), MCV ($P < 0.05$) HGB, RBC, CMH but a significant increase ($P < 0.05$) in sedimentation rate in the PCOS patients group as compared to the control group. Our result showed also that a significant increase ($P < 0.05$) of MDA level and SOD activity ($P < 0.05$) in serum and saliva of patient group compared to control group. In conclusion, several social and nutritional factors, as well as disorders of the reproductive organs, contributed to the spread and development of polycystic ovaries in El-Oued region. In addition to a change in blood components, biochemical parameters and their relationship to oxidative stress, this contributes to disease development and the occurrence of complications.

Key Words: PCOS , Risk factors , Oxidative stress ,Women, El-Oued region .

الملخص

تعد متلازمة تكيس المبايض من أكثر الاضطرابات الهرمونية شيوعًا وتمثل أحد أسباب عدم قدرة المرأة على الإنجاب. لذلك ، فإن الهدف من عملنا هو تقييم بعض عوامل الخطر وبعض المعايير البيولوجية بما فيها الإجهاد التأكسدي لدى النساء المصابات بمتلازمة تكيس المبايض في منطقة الوادي. أجريت دراسة تحديد عوامل الخطر على مجموعة من 76 امرأة تم تشخيصهن من قبل أطباء متخصصين (38 سليمَة و 38 مريضة بتكيس مبايض) ، مع 11 امرأة متطوعة لتقديم عينات الدم واللعاب والبول وهذا لدراسة المعايير البيولوجية (مكونات الدم ، والبيو كيميائية، و معايير الإجهاد التأكسدي) لفحص ومتابعة تكيس المبايض. حول دراسة عوامل الخطر ، أظهرت نتائجنا وجود علاقة قوية بين الاصابة بتكيس المبايض وبعض السلوكيات الاجتماعية بما فيها النظام الغذائي وايضا بروز دور العامل الوراثي في ذلك. لكن تبقى العصبية والتوتر و تناول حبوب منع الحمل هي أخطر العوامل (OR = 3.11 ؛ OR = 9.205 =؛ OR = 5.940) على التوالي ، على العكس من ذلك فان ممارسة الرياضة والحركة في الحياة اليومية تعتبر عوامل حماية للمرأة من الاصابة بهذا المرض. بالنسبة للدراسة البيولوجية، أظهرت نتائجنا زيادة معنوية (P> 0.05) في نسبة الجلوكوز في الدم ومستويات الكوليسترول الكلي والدهون الثلاثية وانخفاض كبير (P> 0.01) في مستوى HDL لدى النساء المصابات بمتلازمة تكيس المبايض مقارنةً بالشاهدات. كما اظهرت نتائج تحليل الدم انخفاضًا معنويا (P<0.05)في كريات الدم البيضاء ،كريات الدم الحمراء، حجم الكريات الحمراء, الهيموغلوبين ، CMH ولكن ايضا تبين ان هناك زيادة معنوية (P <0.05) في سرعة ترسب الدم عند مجموعة مرضى متلازمة تكيس المبايض مقارنة بالمجموعة الشاهدة. من جهة اخرى النتائج المتحصل عليها أظهرت زيادة معنوية (P <0.05) في مستويات بيروكسيد الدهون ونشاط انزيم SOD في مصل ولعاب مجموعة المرضى مقارنة بالمجموعة الشاهدة. في الختام ، هناك العديد من السلوكيات الاجتماعية والغذائية الخاطئة، وكذلك اضطرابات في وضائف بعض الأعضاء التناسلية ، التي ساهمت في انتشار وتطور متلازمة تكيس المبايض عند النساء في منطقة الوادي. بالإضافة إلى تغير في مكونات الدم ، وبعض المعايير البيو كيميائية وعلاقتها بالإجهاد التأكسدي ، مما يساهم في تطور المرض وحدوث المضاعفات وتعقيدات تصل الى حرمان المرأة من الانجاب.

كلمات مفتاحية : تكيس مبايض , عوامل خطر , اجهاد تأكسدي، نساء، منطقة الوادي.

Abbreviation list

PCOS : Polycystic ovary/ovarian.

CAT: catalase .

RNAs : Ribonucleic Acid Synthesis.

RNS: Reactive Nitrogen Specie.

ROS :Reactive Oxygen Specie.

CKD : chronic kidney disease

CVDs : cardiovascular diseases

Cu SO 4 : Copper sulfate.

DNA: deoxyribonucleic acid.

DTNB :5,5′-Dithiobis(2-nitrobenzoic acid.(

EDTA :Ethylene di-amine tetra-acetic acid .

FAA: free fatty acid.

FNS : Hematological analysis.

GPX :glutathione peroxidase.

GSH: Reduced Glutathion.

HCl : Hydrochloric acid.

HDL: High density lipoprotein

HGB: Hemoglobin.

HgbA1c: Hemoglobin A1c.

Abbreviation list

H2O2:Hydrogen Peroxyde.

LDL : Low density lipoprotein.

LYM: Lymphocytes.

MDA: Malondialdehyde.

Met : Methionine.

NaCl :Sodium Chloride.

NADPH : Nicotinamide adenine dinucleotide.

NBT :Nitroblue tetrazolium.

NEUT: Neutrophil.

OR :Odds Ratio .

ORAC: Oxygen Radical Absorbance Capacity.

OS :Oxidative stress.

P : P values.

PLT: Platets.

SOD: Superoxide Dismutase.

TBA: Thiobarbituric acid.

Tc: Total cholesterol.

TCA :Trichloroacetic acid .

TG: Triglyceride.

TSH: Thyroid-stimulating hormone.

US :United States.

Abbreviation list

Utox: Urine toxicology.

Vit c : Vitamin c.

WBC: White Blood Cell.

ROS: reactive oxygen species.

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Summary

Introduction

Introduction

Polycystic ovary syndrome (PCOS) is a chronic and heterogeneous disorder of the endocrine system, which manifests itself as menstrual dysfunction, infertility, hirsutism, acne and obesity (Bani M.,2017). Previous research has shown that both genetic and environmental factors are associated with the negative sequelae of PCOS, including obesity and infertility (Franks, Hardy,2006). PCOS is usually only diagnosed when complications develop that significantly reduce the patient's quality of life (e.g., hair loss, acne, alopecia and infertility-related problems) (Azziz, R. et *al*.,2004).

The global prevalence of PCOS is estimated to be 8–21% of women of reproductive age (Bozdag,G et *al*.,2016). This high prevalence, and its association with ovulation and menstrual disorders, infertility, hair loss, and metabolic complications, highlights the large financial burden caused by PCOS (Chen, X et *al*.,2008) .Although PCOS can occur at any age, from menarche onwards, most cases are diagnosed between the ages of 20 and 30 years old (Bremer, A. A.,2010). Globally, 1.55 million women of reproductive age experience PCOS, resulting in 0.43 million disability-adjusted life-years (DALYs). In 2017, the age-standardised incidence rate of PCOS, among women of reproductive age, was 82.44 per 100,000 population, which was 1.45% higher than in 2007 (Ganie, M. A. et *al*.,2019).In 2019, there were 6,647,566 prevalent cases of PCOS in the MENA region, with an age-standardised point prevalence of 2079.7 per 100,000 women, which represents a 37.9% increase since 199010. PCOS accounted for 236,312 incident cases in 2019, with an age-standardised rate of 77.2 per 100,000 women, which has increased 33.7% since 1990 . In 2019, the regional number of YLDs was 59,835, with an age-standardised rate of 18.7 YLDs per 100,000 women, an increase of 36.1% since 1990 .(Miazgowski,T.et *al* ., 2019).

Introduction

Polycystic ovary syndrome (PCOS) is a public health important disease, affecting at reproductive age and associated with reproductive, metabolic, and psychological dysfunction (Murri M et *al* .,2013). Despite a long history of studies on PCOS, its etiology is still unknown about the pathophysiology of PCOS, have explained the roles of inflammatory state, endothelial injury, oxidative stress, and genetic mechanisms. (Moran C et *al* ., 2010).

Oxidative stress is referred as the imbalance between oxidants and antioxidants and the generation of excessive amounts of reactive oxygen species (ROS). A lot of investigations have revealed that oxidative circulating markers are significantly increased in patients with PCOS compared with the normal and are considered as a potential inducement of PCOS pathogenesis.Oxidative stress is now recognized to play a central role in the pathophysiology of many different disorders, including PCOS.(Lennon SV et *al*., 1991).

In light of these data, the aim of our work is based on the realization of two following.

complementary aspects:

- The first part: is an evaluation of some risk factors associated with PCOS disease in women of El-Oued region.
- The second part: is an biological study concerns the determination of the variation and signification of some biochemical, hematological and oxidative stress markers in the prognostic and following up of PCOS in women.

First part

Bibliographic part

I. Generality on polycystic ovary syndrome

I.1. Definition

Polycystic ovary/ovarian syndrome (PCOS) is a set of symptoms related to an imbalance of hormones that can affect women and girls of reproductives age 1-7 is defined (Azziz et *al*., 2004) and diagnosed by a combination of signs and symptomes of androgen excess ovarian dysfunction and polycystic ovarian morphology on ultrasound 2 this infromational booklet provides guidance for those who are concerned that they may have PCOS and those who have already been diagnosed .there is also infromation on current efforts to understand aı treat PCOS (Trikudanathan et *al*., 2015) .

I.2. Types of PCOS

I.2.1. Insulin resistance PCOS

According to the nutritionist, it occurs in 70 per cent of the cases. As cells become numb to the effect of insulin, a condition called insulinoma arises which causes this type of PCOS. Abdominal weight gain, sugar cravings and fatigue are among its symptoms. Regular exercise and movement are helpful to treat this. Avoid high sugar food and opt for a balanced diet. Reduce stress and sleep well to manage insulin levels. Supplements of magnesium, chromium, NAC and inositol can help.

I.2.2. Adrenal PCOS

This occurs during a massive stressful period. Marked indicators are high levels of cortisol and DHEA. Reduce stress levels through yoga, meditation and good sleep. Avoid high-intensity exercise. Magnesium, vitamin B5 and vitamin C can help support the adrenal glands and nervous system.

I.2.3. Inflammatory PCOS

This type of PCOS occurs due to chronic inflammation. Poor diet and unhealthy lifestyle lead to increased testosterone levels, which causes PCOS. High C reactive protein (more than 5), headaches, unexplained fatigue, skin issues like eczema are some of the symptoms. Maintain good gut health by balancing gut bacteria, improving digestive enzymes and repairing leaky gut tissue. Avoid food that triggers inflammation. Help yourself with natural anti-inflammatories such as turmeric, omega 3 fatty acids and antioxidants like NAC.

I.2.4. Post-pill PCOS

This occurs after stopping the intake of oral contraceptive pills. According to Pooja, "The artificial Progesterone causes a party in the ovaries after you stop the pills", and this can cause PCOS. Taking the pills can stop the symptoms temporarily but can worsen the condition once stopped. This type of PCOS is a temporary situation and reversible. Good sleep and low stress can help. Nutrients like magnesium, vitamin E, vitamin B6 and zinc are helpful.

I.3. Symptoms of PCOS

Hormones are the body's chemical messengers In women with PCOS, two hormones – insulin and androgens (male-type hormones) – are produced in higher levels. This imbalance can result in signs and symptoms including (Zawadzki J et *al* 1992):

- Irregular periods (more or less often) or no periods
- Hair growth on face, stomach and back
- Loss or thinning of scalp hair
- Acne (pimples) that can be severe
- Weight gain

- Emotional problems (anxiety, depression poor body image)
- Difficulties getting pregnant
- Increased risk of type 2 diabetes with earlier onset

Symptoms of PCOS can vary from woman to women and also change over time (Azziz R,et *al* .,2009) .

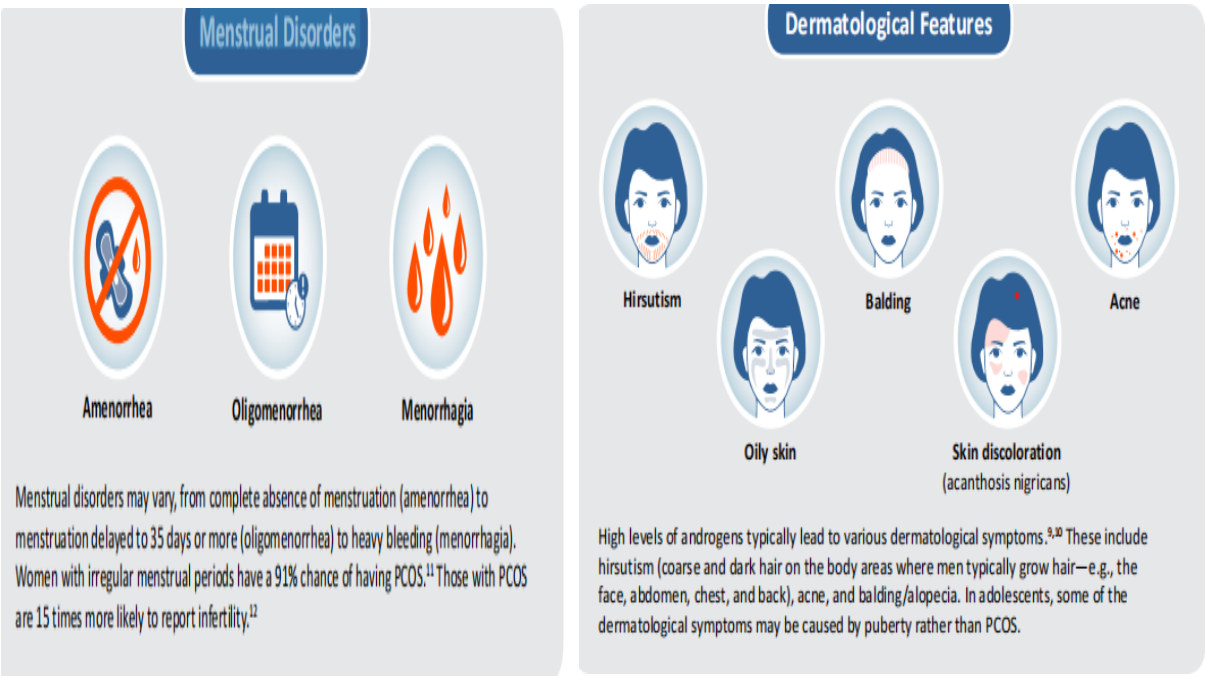

Figure 01: Symptoms of PCOS

I.4. Causes of PCOS

The cause of PCOS is not yet known but it often runs in families. If any of your relatives (mother, aunt's sisters) are affected with PCOS, your risk of developing PCOS may be increased. The symptoms are related to abnormal hormone levels:

* Testosterone is a hormone that is produced in small amounts by the ovaries in all women

- Women with PCOS have slightly higher than normal levels of testosterone and this is associated (Setji TL et *al*., 2008). With many of the symptoms of the condition.

- Insulin is a hormone that controls the level of glucose (a type of sugar) in the blood.

PCOS, the body may not respond to insulin (this is known as insulin resistance), so the level of glucose is higher. To try to prevent the glucose levels becoming higher, your body produces even more insulin high levels of insulin can lead to weight gain more insulin. High levels of insulin can lead to weight gain, irregular periods, fertility problems and higher levels of testosterone. (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group.2013) .

I.5. Treatment

PCOS treatment focuses on managing your individual concerns, such as infertility, hirsutism, acne or obesity. Specific treatment might involve lifestyle changes or medicatio(Legro RS et *al*., 2013).

a. Lifestyle changes

Your doctor may recommend weight loss through a low-calorie diet combined with moderate exercise activities. Even a modest reduction in your weight, for example, losing 5 percent of your body weight might improve your condition. Losing weight may also increase the effectiveness of medications your doctor recommends for PCOS, and can help with infertility (Radosh L et *al*., 2009).

b. Medications

Combination birth control pills: Pills that contain estrogen and progestin decrease androgen production and regulate estrogen. Regulating your hormones can lower your risk of endometrial cancer and correct abnormal bleeding, excess hair growth and acne. Instead of pills, you might use a skin patch or vaginal ring that contains a combination of estrogen and progestin (Buzney E et *al*., 2014).

Progestin therapy: Taking progestin for 10 to 14 days every one to two months can regulate your periods and protect against endometrial cancer.

Progestin therapy doesn't improve androgen levels and won't prevent pregnancy. The progestin-only minipill or progestin-containing intrauterine device is a better choice if you also wish to avoid pregnancy (Goodarzi MO et *al*., 2011) .

Clomiphene: This oral anti-estrogen medication is taken during the first part of your menstrual cycle.

Letrozole (Femara): This breast cancer treatment can work to stimulate the ovaries.

Metformin: This oral medication for type 2 diabetes improves insulin resistance and lowers insulin levels. If you don't become pregnant using clomiphene, your doctor might recommend adding metformin. If you have prediabetes, metformin can also slow the progression to type 2 diabetes and help with weight loss (Homburg R et *al*., 2013).

Gonadotropins: These hormone medications are given by injection.

Birth control pills: These pills decrease androgen production that can cause excessive hair growth.

Spironolactone (Aldactone): This medication blocks the effects of androgen on the skin. Spironolactone can cause birth defects, so effective contraception is required while taking this medication (Romualdi D et *al*., 2011).

Eflornithine (Vaniqa): This cream can slow facial hair growth in women. **Olysis:** A tiny needle is inserted into each hair follicle. The needle emits a pulse of electric current to damage and eventually destroy the follicle. multiple treatments.(Tang T et *al*., 2012).

Chapter II

II.1.Definition

Oxidative stress is broadly referred to as an imbalance between the generation of the Free radicals which are inherently unstable molecules because of balance between the production of reactive species (reactive oxygen specie ROS and reactive nitrogen specie RNS) and their clearance by the anti-oxidant defense system in favor of the former .(Stefanovic et *al* ., 2019 ; Betteridge , 2000). Moreover, oxidative stress has been proven to be associated with many diseases, including cancer, diabetes, chronic kidney disease (CKD), cardiovascular diseases (CVDs), neurodegenerative diseases, inflammation ,atherosclerosis and ageing (El Assar et *al* ., 2019 ; Shahidi & Zhong , 2015).

II.2. Free radicals

II. 2.1. Definition

A free radical can be defined as an atom or molecule containing one or more unpaired electrons in valency shell or outer orbit and is capable of independent existence. Both ROS and RNS collectively constitute the free radicals and other non-radical reactive species (Phaniendra et *al*., 2015; Kehrer et *al*., 1993) (table 01 and table 02). These radicals can be produced in cells by losing or accepting a single electron, therefore, behaving as oxidants or reductants (Lobo et al ., 2010). The main process of ROS generation in mitochondria could be schematically presented as $O_2 \rightarrow O_2^{-\bullet} \rightarrow H_2O_2 \rightarrow {}^{\bullet}OH$(Luo et *al* ., 2019).

Table 01 : Reactive oxygen species (ROS)(Rahman et *al* ., 2012)

Radicals	Non-radicals
Superoxide: O_2^-	Hydrogen peroxide: H_2O_2
Hydroxyl: OH^-	Hypochlorus acid: HOCL
Peroxyl: RO_2^-	Hypobrromus acid: HOBr
Alkoxyl: RO^-	Ozone: O_3
Hydroperoxyl: HO_2^-	Singlet oxygen: Δg

Table 02 : Reactive nitrogen species (RNS)(Rahman et *al* ., 2012)

Radicals	Non-radicals
Nitric oxide: NO^-	Nitrogen dioxide: NO_2
Nitrous acid: HNO_2	Nitrosyl cation: NO
	Nitrosyl anion: NO^- NO^-
	Dinitrogen tetroxide: N_2O_4
	Dinitrogen trioxide : N_2O_3
	Peroxynitrite: $ONOO^-$
	Peroxinitrous acid: ONOOH
	Alkylperoxynitrites: ROONO

II. 3.Antioxidants

Antioxidant state necessary for Natural physiological function. An imbalance in the case of oxidation / antioxidants can cause injury to the tissues of the organism (Vidhya et al ., 2018). Antioxidant defense protects biological systems from free radical toxicity and includes both endogenous and exogenous molecules , that function interactively and synergistically to neutralize free radicals, (Krishnamurthy & Wadhwani , 2012 ; Birben et al., 2012). The term

'antioxidant' refers to any molecule stable enough to donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity to damage a target molecule . (Li et al ., 2016) . The antioxidants are present in significant amounts in commonly consumed fruits, vegetables, beverages (juices, tea, coffee), nuts and cereal products. (Mirończuk-Chodakowska et *al*., 2018). In a biological system, antioxidants can be categorized as enzymatic or non-enzymatic (Zhao et *al* .,2016).

II.3.1. Endogenous antioxidants

- **Enzymatic antioxidant**

Enzymatic Activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) constitute a first line antioxidant defense system which plays a key and fundamental role in the total defense mechanisms and strategies in biological systems (ghodaro & Akinloye , 2017). SODs are metal-containing proteins that catalyze the removal of superoxide, O_2 is converted by SOD to H_2 O_2 , which is decomposed to water and oxygen by CAT, preventing hydroxyl radicals production (Krishnamurthy & Wadhwani, 2012 ; Liguori et al ., 2018). However, catalase is absent in the mitochondria, hence the reduction of H_2O_2 to water and lipid peroxides to their corresponding alcohols is carried out by Glutathione Peroxidase (GPx). (ghodaro & Akinloye , 2017). Additionally, GSH-Px converts peroxides and hydroxyl radicals into nontoxic forms by the oxidation of reduced glutathione (GSH) into glutathione disulfide and then reduced to GSH by glutathione reductase (Birben et *al* ., 2012).

- **Non enzymatic antioxidant**

Non-enzymatic antioxidants include endogenously produced GSH or dietary compounds (Zhao et *al* ., 2016) . The endogenous non-enzymatic antioxidants are molecules that inter act with RONS and terminate the free radical chain reactions: bilirubin, α-tocopherol (vitamin E), and β-carotene are present in

blood while albumin and uric acid account for 85% of antioxidant capacity in plasma (Wu JQ, Kosten TR & Zhang XY ., 2013).

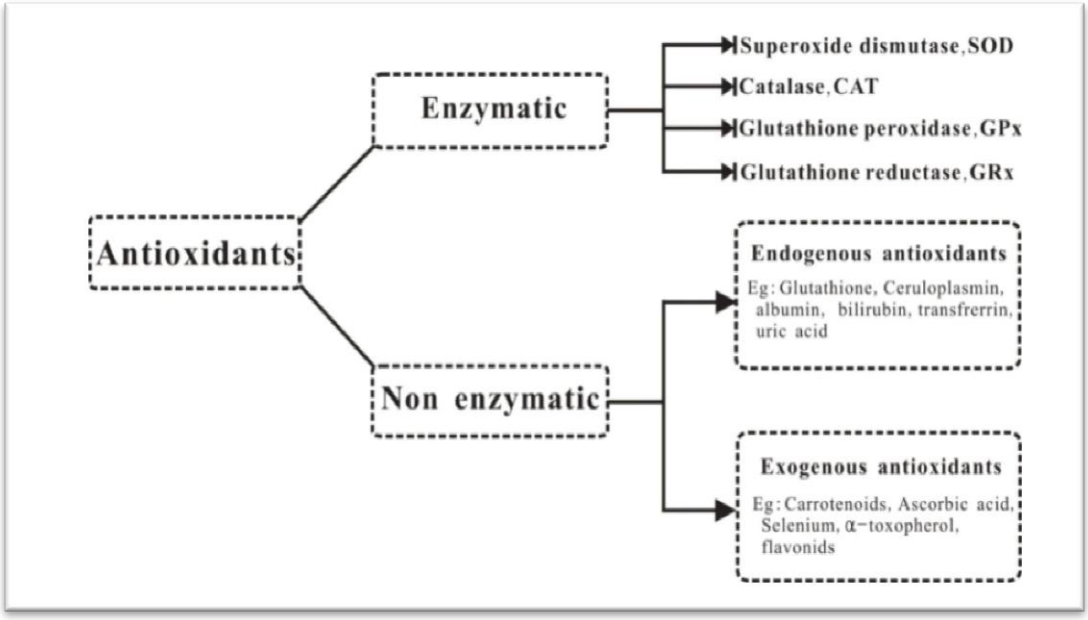

Figure 02 : Enzymatic and non-enzymatic classification of antioxidants (Li et *al* ., 2016)

II.3.2 Exogenous antioxidants

Dietary antioxidants such as vitamin E, vitamin C, carotenoids, some minerals (e.g. ZnMn, Cu, Se) and polyphenols (flavonoids, phenolic acids, stilbenes, lignans) can affect the activity of endogenous antioxidants. Endo- and exogenous antioxidants may act synergistically to maintain or reestablish redox homeostasis (Mirończuk-Chodakowska et *al* ., 2018).

II.4.Oxidative stress and PCOS

Polycystic ovary syndrome (PCOS) is a public health important disease, affecting at reproductive age and associated with reproductive, metabolic, and psychological dysfunction (Zhang J, Fan P, Liu H, Bai H 2012). Despite a long history of studies on PCOS, its etiology is still unknown about the pathophysiology of PCOS, have explained the roles of inflammatory state,

endothelial injury, oxidative stress, and genetic mechanisms (Moran C, Tena G et al 2010).

Oxidative stress is referred as the imbalance between oxidants and antioxidants and the generation of excessive amounts of reactive oxygen species (ROS). A lot of investigations have revealed that oxidative circulating markers are significantly increased in patients with PCOS compared with the normal and are considered as a potential inducement of PCOS pathogenesis. (Murri M, Luque-Ramírez et *al* .,2013)Oxidative stress biomarkers in PCOS.

For investigation of the oxidative stress role in the pathogenesis of diseases, mainly, we have examined oxidative stress biomarkers including MDA and SOD. The evaluation of oxidative stress and antioxidant biomarkers have been suggested as useful tools in estimating the risk of oxidative damage and associated diseases(Dib M, Garrel C et *al* .,2002) and help in the prevention and management of oxidative diseases.

A. Malondialdehyde

MDA results from lipid peroxidation of polyunsaturated fatty acids (Davey MW et *al*., 2005) is stable and can serve as a good biomarker. MDA level in PCOS reported in several studies. One meta-analysis showed that circulating mean MDA concentrations according to the age and BMI were increased 47% in women with PCOS compared with controls .(Northrop-Clewes CA2007). Compared blood MDA level in PCOS patients with healthy controls. They showed the MDA level was significantly higher in the PCOS group but was independent of obesity.(Kuscu et *al* 2007) In another study, (Zhang et al2006). demonstrated that serum MDA levels in PCOS patients were significantly higher than the control group, but BMI and age were not recorded.(Dursun P 2006) In addition,(Dursun et *al* .,2001*)*. studied PCOS patients and found serum MDA levels in PCOS patients were similar to those of BMI and smoking status matched controls (Palacio Jet *al*., 2006).

Compared PCOS patients with BMI and age matched controls. They demonstrated that higher levels of erythrocyte MDA were seen in PCOS patients compared with controls. These results also were found by (Sabuncu et *al* ., 2001).

B. Superoxide dismutase

SOD is an enzyme and an important antioxidant defense that eliminates superoxide anions (O2-), as a major oxygen radical, by catalyzes of them to H_2O_2 and final by GPx converted to water. Several common forms of SOD exist depending on the metal cofactor and the protein fold such as the Cu/Zn type, Fe and Mn types, and the Ni type (Barondeau DP et *al* .,2004).

SOD activity in PCOS reported in several studies. (Sabuncu et *al* .,2004) determined antioxidant status in women with PCOS evaluated blood SOD level in PCOS patients compared with healthy controls. They showed that women with PCOS had higher SOD levels than normal subjects. (Moreover, Zhang et al., 2001) showed that the serum SOD level in PCOS patients was significantly lower than the control group.(Davey MWet *al*., 2005).

Determined the oxidative stress role in endothelial dysfunction in a young, non-obese group of PCOS patients, measured blood SOD level. They demonstrated that SOD levels were significantly higher in a PCOS group than in control group. (Northrop-Clewes CA et *al* .,2007). In 2013, the result of one meta-analysis also showed that the mean SOD activity was 34% higher in PCOS patients than in controls (Murri M et *al*., 2013) .

In another study, (Seleem et *al*., 2014). examined the activity of SOD both in the serum and follicular fluid (FF) from women with PCOS undergoing intra cytoplasmic sperm injection. They showed SOD activity highly significant

decrease both in the mean serum and FF, in PCOS than the control group, and suggested serum SOD activity could be a clinical parameter for determining systemic oxidative stress in PCOS.(El Refaeey AA., 2014) Further studies are needed to examine the mechanism of SOD as the antioxidant defense in PCOS.

C. Glutathione peroxidase

GPx is an enzyme family that protects the organism from oxidative damage by reducing lipid hydroperoxides to their corresponding alcohols and reduces H_2O_2 to water. The GPx activity evaluation for anti-oxidant defense assessment in PCOS was reported in several studies. (Sabuncu et *al*., 2001). determined oxidant and antioxidant status in women with PCOS. They demonstrated that GPx did not differ between a PCOS group and a healthy control group.(Kassmann CJ et *al* .,2004).

investigated relation between oxidative stress and the antioxidant system in the development of PCOS by measuring GPx activity in PCOS patients and age and sex matched healthy control. Results showed that no significant difference was found between GPx activities in PCOS patients and the control women. (Baskol G et al., 2012) One meta-analysis showed that the mean GPx activity had no statistically significant difference in women with PCOS compared with controls. However, (Savic-Radojevic et al .,2015). showed that GPx activity of PCOS women significantly decreases compared to control (Savic-Radojevic et *al* .,2015).

D. Reduced glutathione

GSH is essential in the regulation of the disulfide bonds of proteins and in the disposal of electrophiles and oxidants. The antioxidant function of GSH is mediated by this redox-active thiol group that becomes oxidized when GSH reduces target molecules (Hammond CL .,2001). GSH evaluation for its

antioxidant effect in PCOS was reported in several studies. One meta-analysis showed that the mean GSH levels were 50% lower in women with PCOS than in controls. (Marí M et *al* .,2009) . demonstrated that GSH was significantly lower in the PCOS patient group than in the control group. In accordance with the findings of Sabuncu et al., Dincer et al. also found GSH levels to be significantly lower in women with PCOS than in the control group. They proposed that GSH depletion might have resulted from increased production of ROS in PCOS patients.(Dinger Y et *al*., 2005).

E. Vitamin C and E

Several vitamins with antioxidant capacities derived from their scavenging of oxidant molecules have been studied in PCOS. Studies on vitamin C show that this vitamin concentrations are lower in PCOS patients than in controls. Kurdoglu et al. reported vitamin C concentrations in serum and Mohan and Vishnu reported in erythrocyte are lower in PCOS patients and the same applies to circulating vitaminE concentrations.(Kurdoglu Z et *al* .,2012),(Mohan S, et *al*., 2009) .

Second part

Experimental part

Materials & Methods

I. Materials and Methods

I.1. Patients and reagents

I.1.1. Study period

The period of our study is 7 Months, (from September 2021 to Mars 2022 ` the medicine service of hospital… (May 8 Hospital and cancer hospital obstetrics Hospital) and medical analysis laboratory of el MEJDE (El Oued) and at the Faculty of Natural Sciences and Life at the University of Echahid Hamma Lakhdar El-Oued.

I.1.2. Epidemiological and Risk factors study

A standard questionnaire was used to obtain the baseline information which Contribute in our study ,with face-to-face interviews by us . This questionnaire asked for 76volunteers divided into 38 healthy group as a control and 38 PCOS patients and used for evaluation of some Risk factors of the disease.

I.1.3. Biological study

For biological study, this study is carried out on 11 volunteers women aged between 18-56years , were divided into two groups ; healthy control women and women with PCOS.

- **Inclusion criteria**

✓ Voluntary live in El-Oued region .

✓ Control group in good health, does not have any pathology.

- **Exclusion criteria**

✓ Persons are suffering from other acute or chronic pathology.

I.1.4 Reagents

Ethylene diamine tetraacetic acid (EDTA), Hydrogen Peroxyde (H2O2) ,Hydrochloricacid (HCl) , Thiobarbituric acid (TBA) , Salicylic acid , Methanol, Tris ,Trichloroacetic acid (TCA) , Copper sulfate (CuSO 4) , Ascorbic acid,

DTNB (5,5′-Dithiobis(2-nitrobenzoic acid)), Sodium chloride (NaCl) , Butylated hydroxytoluene (BHT) ,Phosphate-buffered (KH_2PO 4,K_2HPO 4), folin , NBT , Methionin , riboflavin .

I.2. Methods

I.2.1. Collection of data

We used a questionnaire including social and clinical data for each volunteer, after that collecting the risk and protective factors associated with PCOS.

I.2.1.1. Sample collection

About blood sampling for both groups is done morning fasting . It is performed in the vein of the end of the elbow. Blood samples is collected in two tubes . Dry tubes are centrifuged at 3000 rpm for 10 minutes, then obtained the serum to achieve the dosage of biochemical and oxidative stress markers.

The anticoagulant tube (EDTA) are used to realize the dosage of hematological parameter: WBC, RBC, HB, Lymphocyte,VCM, CMH, TMH, Ht….

I.2.2. Method of Hematological analysis

Hematological analysis (FNS) is performed by the hematology Auto analyzer.

I.2.3. Biochemical parameters assay

Glucose, triglyceride , HDL , LDL , a use commercial kits from Spinreact, (Spainref:glucose-20121, triglyceride-20131, HDL-20113, cholesterol- 20111. and enzyme marker were also measured using commercial kits (Spinreact, ref: alkaline phosphatase-20015).

I.2.4. Method of estimating oxidative stress parameters

I.2.4.1. Malondialdehyde (MDA) assay

MDA was measured according to the method described by (Yagi, 1976). Thiobarbituric acid 0.67% (w/v) was added to aliquots of the sample previously precipitated with 10% trichloroacetic acid (w/v). Then the mixture was centrifuged, and the supernatant was heated (100°C) for 15 min in a boiling water bath. Then cool in a cold water bath for 30 minutes, leaving the tubes open to allow evacuation of the gases formed during the reaction and the absorbance was measured at 532 nm using a spectrophotometer. The concentration of TBARS was determined using the molecular extinction coefficient of MDA (a=1.53 105 M-1.Cm-1).

I.2.4.2. Determination of superoxide dismutase activity assay

The assay method of SOD activity using the NBT by the superoxide anion (O2.), is used as a basis for detecting of presence of SOD by measuring the spectrophotometrically absorbance at 560 nm (Beauchamp and Fridovich,1971).

Operating mode

Collect in tubes	Blank	Sample
EDTA-Met 0.1mM EDTA-13mM Met	1000µL	1000µL
Phosphate buffer (50Mm)	892.2µL	892.2µL
Sample	0	50
Phosphate buffer	1000µL	950µL
NBT (75µM)	85.2µL	85.2µL
Riboflavin (2µM)	22.6µL	22.6µL

Expression of results

$$SOD\ (U/ml)\frac{(DO\ blanc - DO\ sample)}{(DO\ blanc)}$$

Statistical analysis

Statistical analysis is performed by the SPSSV20.0 and Minitab softwares. Results comparisons were carried out by using the Student T test to compare means among the groups, Correlation analysis was carried out using Pearson Correlation test and regression analysis was used for other analysis and statistical data. Differences were considered statically significant at $p < 0.05$.

Results

II. Result

II.2. Risk factors Study of PCOS

II.2.1. Description of study population

Characteristics of the study population are shown in table 03. Women volunteers for this study from El-Oued. Our study found of 76 Participants witch divided to 38 control and 38 PCOS patients. The general data of socioeconomic characteristics of the two groups of subjects include age, number of children, body weight, mass index, job, educational level, blood group , Blood pressure and social case . The results obtained are homogeneous in both control and PCOS patients and do not have any statistically significant differences ($p > 0.05$).

Table 03: Description of study population

Parameters		Control	Stroke patients
Age (ys)		34.16±10.10	26.81±5.54
Body Weight (kg)		70.41±13.86	71.60±14.22
Mass index		26.68±4.30	27.42±4.85
Great blood circulation		11.351±1.111	11.081±1.115
small blood circulation		6.378±0.924	6.459±0.153
Job	**Worker (%)**	10.52	11.84
	Retired (%)	39.47	38.15
Educational Level	**Middle school(%)**	5.26	5.26
	High school (%)	11.84	13.15
	University (%)	32.89	31.57
Blood type	**A (%)**	9.2	9.2
	B (%)	5.26	10.52
	O (%)	36.83	32.25
Social case	**Married (%)**	42.10	42.10
	Single (%)	7.89	7.89

II.2.2. Study of socioeconomic and clinic factors

Odds Ratio (OR) values for socioeconomic factors (Table 04) and clinic-pathological factors (Table 05) show that use Spices , genetics Factor and

suffering from obesity are shown To be significant risk factors for polycystic ovary syndrome (OR = 3.478 ; p = 0.019, OR=1.276; p=0.403 and OR = 1.528 ; p = 0.299) respectively , in addition Nervous , year of taking pills , disease recurrence and Mentrual period also shown to be significant risk factors for PCOS in the study population (OR = 3,111; p = 0.394, OR=5,940 ; p=0.001, OR = 2,000; p = 0.000 and OR= 9,205; P= 0,000) respectively . On the contrary ,playing sport and movement in daily life are considered protective factors (OR = 0.869, p = 0.500 ; OR = 0.045, p = 0.000) respectively , Also smoking , fast food , soft drinks , sugars , chemicals , suffering from any other disease , Eat candies , getting into trouble , Use of food additives , eating anti-pain medicines They are not considered as predictors of PCOS in our population since the OR values obtained are not significant.

Table 04: Comparison of socioeconomic factors of PCOS in patients and controls

	Control %	**Patient %**	**OR**	**CI_{95}%**	***P-value***
Smoking			1.375	-3.399 0.556	0.323
Positive	21.05	25			
Negative	28.95	25			
Fast food			1.141	-3.121 0.417	0.500
Positive	13.16	14.47			
Negative	36.84	35.53			
Soft drinks			1.905	-4.763 0.762	0.124
Positive	18.42	26.32			
Negative	31.58	23.68			
Sugars			0.810	-1.993 0.329	0.409
Positive	26.32	23.68			
Negative	23.68	26.32			
Chemicals			1.111	-2.733	0.500

				0.452	
Positive	25	26.32			
Negative	25	23.68			
Playing sports			0.869	-2.475 0.307	0.500
Positive	13.16	11.84			
Negative	36.84	38.16			
Genetics factor			1.276	-3.667 0.484	0.403
Positive	14.47	17.11			
Negative	35.53	32.89			
Suffering from any oth er disease			1.374	-4.170 0.453	0.390
Positive	9.21	11.84			
Negative	40.79	38.16			
Eat candies			1.704	-4.234 0.686	0.179
Positive	19.74	26.32			
Negative	30.26	32.68			
Getting into trouble			0.635	-1.624 0.248	0.238
Positive	34.21	28.95			
Negative	15.79	21.05			
Use of spices			3.478	-10.323 1.172	0.019
Positive	30.26	42.11			
Negative	19.74	7.89			
Use of food additives			1.000	-2.631 0.380	0.597
Positive	15.79	15.79			
Negative	34.21	34.21			
Suffering from obesity			1.528	-4.360 0.535	0.299
Positive	10.53	14.47			
Negative	39.47	35.53			
weight Over			1.115	-2.785 0.446	0.500
Positive	19.74	21.05			
Negative	30.26	28.95			
Movement in daily life			0.045	-0.173 0.11	0.000

Positive	32.89	46.05			
Negative	17.11	3.95			

Table 05: Comparison of the clinic factors of PCOS in patients and control

	Control %	**Patient %**	**OR**	**CI_{95}%**	***P-value***
Pills			0.747	0.258-2.161	0.394
Positive	13.15	10.52			
Negative	36.84	39.47			
Nervous			3.111	1.118-8.147	0.017
Positive	23.68	36.84			
Negative	26.31	13.15			
year of taking pills			5.940	1.908-18.496	0,001
Positive	6.57	23.68			
Negative	43.42	26.31			
childbearing year			0.589	0.238-1.459	0.179
Positive	27.63	21.05			
Negative	22.36	28.97			
cesarean delivery			0.488	0.14761.624	0.188
Positive	11.84	6.57			
Negative	38.15	43.42			
disease recurrence			2.000	1.455-2.749	0.000
Positive	0	25			
Negative	50	25			
Menstrual period			9.205	3.225-26.269	0.000
Positive	14.47	39.47			
Negative	35.52	10.52			
disease detection time			1369.000	82.502-22716.586	0.000
Positive	0	50			
Negative	50	0			
Vitamin D deficiency			1.373	0.557-3.384	0.323
Positive	26.31	22.36			
Negative	23.68	27.63			
take medicine			1.000	0.399-2.509	0.593
Positive	19.73	19.73			
Negative	30.26	30.26			
Eating antibiotics			1.222	0.438-2.872	0.500
Positive	17.10	18.42			

Negative	32.89	31.57			
eating anti-pain medicines			0.899	0.365-2.218	0.500
Positive	18.42	28.94			
Negative	31.57	21.05			

II.2. Study of biological markers and predictive factors

II.2.1.Hematological markers

The results of the hematological analysis for control and PCOS patient are illustrated in table 06. The results of show a significant decrease in WBC ($P < 0.000$), MCV, ($P < 0.05$) HGB, RBC, CMH, CMH but a significant increase ($P < 0.05$) in sedimentation rate in the PCOS patients group as compared to the control group.

Table 06: Haematological parameters levels in control and PCOS patient groups

	Women's groups		
Parameter	**Control**	**PCOS Patient**	***P-value***
Hemoglobin (g/dL)	14.150±0.253	11.167±0.750	**0.011**
Red blood cells (106/ µl)	4.3983±0.0275	4.648±0.0952	**0.028**
Hematocrit	38.160±0.556	36.00±1.43	0.175
MCV (fl)	84.84±4.05	79.23±12.65	0.440
TMH (pg)	31.267±0.488	25±1.33	**0.002**
CMH (pg)	36.600±0.349	32.340±0.474	**0.000**
White blood cells (103/ µl)	9.600±0.524	7.222±0.330	**0.000**
Lymphocytes (%)	2.960±0.107	3.0200±0.0488	0.250
Sedimentation rate 1h (mm)	10.50±1.52	24.75±3.69	**0.006**
Sedimentation rate 2h (mm)	27±4.25	42.75±4.42	**0.009**

II.2.2.Biochemical markers

Concerning biochemical markers table 07, For our result demonstrated that a significant increase in blood glucose (P > 0.05), total cholesterol (P > 0.001) and Triglycerides (P > 0.01) levels and a significant decrease (P > 0.01) in HDL level in women with PCOS as compared to controls . But serum LDL is no significant change (P> 0.05).

Table 07 : Biochemical parameters levels in control and PCOS patient groups

	Women's groups		
Parameter	**Control**	**PCOS Patient**	***P-value***
Blood glucose (g/l)	0.7667±0.0334	1.208±0.219	**0.05**
Triglycerides (g/l)	1.2417±0.0415	11.167±0.750	**0.003**
Total Cholesterol (g/l)	1.848±0.0446	1.5575±0.0337	**0.000**
LDL (g/l)	1.034±0.059	1.026±0.0068	**0.304**
HDL (g/l)	0.4367±0.0114	0.2925±0.0309	**0.002**

II.2.3 biochemical urinary parameters

We note in urine table 08 a significant increase in the value of VO and SG (P>0.500) but there is a slight decrease in the value of URO (P<0.500) and pH (P>0.01) .

Table 08: biochemical urinary parameters levels in control and PCOS patient groups

Parameter	**Control**	**Patient**	***P-value***
Urobilinogen (mg/dl)	2.900±0.224	2.200±1.095	0.226
Specific gravity	1.0296±0.0011	1.0298±0.0008	0.621
pH	6.000±0.000	6.600±0.548	0.070
Volume (ml/day)	3.000±1.000	2.800±0.837	0.621

II.2.4.Oxidative stress markers

The analysis of blood oxidative stress parameters in control and Polycystic ovary patients are shown in table 09 For women our result explained that a significant increase ($P < 0.05$) of MDA level and SOD ($P < 0.001$) , in serum of patient group compared to control group. but in saliva our result explained that a significant increase ($P > 0.500$) of MDA level and SOD activity ($P < 0.001$) , in saliva of patient group compared to control group.

Table 09: Oxidative stress parameters in the blood of control and Polycystic ovary patient

Parameter		Control	PCOS Patient	*p-value*
MDA (nmol/ml)	saliva	0.838±0.546	0.739±0.603	0.617
	Serum	0.216±0.256	2.084±2.327	0.043
SOD (U/ml)	saliva	1.641±0.317	1.871±0.099	0.001
	Serum	1.374±0.278	1.835±0.034	0.000

Discussion

III. Discussion

Polycystic ovary syndrome (PCOS) is a hormonal disorder common among women of reproductive age. Women with PCOS may have infrequent or prolonged menstrual periods or excess male hormone (androgen) levels. The ovaries may develop numerous small collections of fluid (follicles) and fail to regularly release eggs. Thus, we will discuss the results we obtained from our study.

III.1.Study of risk factors for PCOS

The current study showed that there were statistically significant differences in the different hematological values between women with PCOS and the control group. This finding is consistent with that of (Ucakturk et *al* .,2014) who reported significant differences in hemoglobin, erythrocyte, platelet count, and leukocyte count between women with PCOS and women without PCOS. Similarly, Rashidi et *al*., (2017) reported that there is no significant difference in serum iron levels between cases (PCOS) and controls. However, in study of Han et *al*., (2015), Hormone levels in women with PCOS affect hemoglobin levels. Testosterone is a blood hormone that has a dose-dependent stimulatory effect on erythropoiesis (Berria R et *al*., 2006) and (Coviello AD et *al*., 2007). Moreover, androgen affects bone marrow cells via androgen receptor in the bone marrow. The reduced frequency of menstruation in women with PCOS is thought to cause differences in hemoglobin levels between women without PCOS. Moreover, androgen affects bone marrow cells via androgen receptor in the bone marrow. The reduced frequency of menstruation (Claustres M et *al*., 1988).

In PCOS women there were differences in hemoglobin levels compared to women without PCOS. In the current study, there was a significant change in WBCs between women with PCOS and controls. A previous study reported a positive predictive effect of WBCs and a negative predictive effect of

lymphocytes on insulin resistance in women with PCOS. PCOS has been associated with metabolic disorders, including obesity, (We also noted in our study that the number of obese women is more susceptible to polycystic ovary disease). Obesity has a negative impact on female fertility and is associated with anovulation, miscarriage, and several pregnancy complications (F. Ramezani ,2014).

Abdominal obesity is related to insiulin resistance, chronic anovulation, hyperandrogenism, and inflammation in patients with PCOS (D. R. Meldrum, 2017). Mitochondria may be the main organ that leads to impaired energy metabolism in obese patients with PCOS. Increased oxidative stress can induce obesity by promoting preadipocyte proliferation and adipocyte differentiation by increasing the size of mature adipocytes (S.Furukawa et *al*., 2004).

The results in our study showed that the fluctuation of blood circulation was significantly associated with the risk of developing PCOS. This result is in agreement with study of R. Tsutsumi et al ., (2009). The impaired secretion of pulsatile gonadotropin-releasing hormone (GnRH), a factor responsible for PCOS, originates from the hypothalamus. GnRH causes the pituitary gland to release the follicle-stimulating hormone (FSH) and the luteinizing hormone (LH). these two hormones are needed for the two phases of the menstrual cycle. In PCOS, without these hormones, the egg will not form, and cannot leave the follicle. As a result, the cycle is disrupted, resulting in primary or secondary amenorrhea, which can be of two types. (R. Tsutsumi et *al* ., 2009)

In our study of women with PCOS, we found that in participants experienced a number of distressing symptoms, such as weight gain, facial hair, acne, and fertility problems, which over time led to their diagnosis These results are also similar to a study studied by Asuncion et al . (Asuncion M et *al* .,2000). Consistent with other studies, research participants 9 through 19 reported feeling

low self-esteem and depression, all of which were linked to their medical condition and obesity.

III .2.Biological marker study

We found significant changs in TG, LDL cholesterol, and HDL cholesterol, among patients with PCOS and control subjects. Nevertheless, lower HDL cholesterol were observed in patients with PCOS with higher TG, and cholesterol in PCOS patients compared to controls. Which are in agreement with study of Goldstein et *al*., (2011). In our study low HDL was observed as a dyslipidemia variable in cases. This is similar to findings Goldstein (A, Jutel A et *al* .,2014) where obtained that low HDL has been seen in 93.3 percent of cases with PCOS. The cause of dyslipidemia in PCOS may be hyperinsulinemia and hyperandrogenemia. This allowed adipocytes to undergo increased lipolysis caused by catecholamine and release free fatty acids into the circulation. Increased free liver fatty acids cause VLDL secretion, leading to hypertriglyceridemia. Hypertriglyceridemia leads through the reverse cholesterol transfer pathway to reduced HDL cholesterol and elevated LDL cholesterol levels. The further androgenic priming of adipocytes in early life predisposes PCOS-associated dyslipidemia (Cinar et *al*., 2011). Also our results are in agreement with results of Khomami et *al* (2016) and Jones et *al* (2015) which showed increased cholesterol, triglycerides and decreased HDL in obese PCOS females compared to non-obese and control group. Also, Jayasekara et *al* (2012) showed that in PCOS, there were statistically significant increases in serum cholesterol, LDL and triglycerides relative to the matched control group, while serum HDL was lower in PCOS than control group.

III .3. Syudy of Hematological markers

It has been observed in our study that there is a strong relationship between immunodeficiency and polycystic ovary disease, and this was agreed with Hadigan C et *al*.

Metabolic abnormalities including insulin resistance, dyslipidemia, and increased visceral adiposity are frequently observed in HIV-infected women receiving highly active antiretroviral therapy (Hadigan C et *al* .,1999). In non-HIV-infected women, truncal adiposity and insulin resistance are often associated with features of the polycystic ovary syndrome (PCOS), including amenorrhea, increased androgen levels, reduced SHBG, increased LH to FSH ratio, and multiple small ovarian follicles (Dong KL et *al* ., 1999). Prior studies have demonstrated that the prevalence of insulin resistance and diabetes is more frequent in women with PCOS than an age- and body mass index (BMI)-matched group of women. As many as 35% of women with PCOS have impaired glucose tolerance, whereas 10% are diabetic before the age of 40 yr (Ehrmann DA et *al* ., 1999). Insulin resistance in PCOS is unique in that the ovaries are resistant to insulin-mediated glucose uptake, whereas mitogenic response to insulin is sustained Hyperinsulinemia may contribute to hyperandrogenemia through enhanced LH-stimulated androgen secretion from the ovary (Nestler JE et *al* .,1998) and reduced circulating SHBG.

Among HIV-infected women, little is known regarding the prevalence of PCOS features, e.g. menstrual dysfunction, increased ovarian follicles, and hormonal dysfunction. In a prior study, we demonstrated an increased LH to FSH ratio in a small number of HIV-infected women with lipodystrophy (Hadigan C et *al* .,2000). The purpose of the current study was to characterize ovarian morphology and reproductive indices in a large cohort of HIV-infected women in comparison with healthy age- and BMI-matched control subjects. To our knowledge, prior studies have not assessed PCOS features, including ovarian morphology among HIV-infected women and control subjects similar in age, weight, and race.(Dolan SE et *al* ., 2004).

III .4. Study of oxidative stress markers

One of the basic characteristics of PCOS is increased serum levels of oxidative stress markers (Kucukaydın Z et *al*., 2016). We demonstrated in a previous study that increased oxidative stress in the body could contribute to the pathogenesis of PCOS (Uyanikoglu H et *al*., 2017). Although no consensus has been reached the formation of oxidative stress, one theory is related to the increased number of mononuclear cells that develop secondarily to hyperglycaemia in PCOS patients. Increased amounts of mononuclear cells cause enhanced production of inflammatory cytokines and free oxygen radicals (Isik H et *al*., 2016). Molecular damage due to free oxygen radicals has been suggested to play an important role in the aetiopathogenesis of PCOS (Zuo T et *al*., 2016).

In the case of patients with PCOS, significant values of MDA concentrations were found compared to the PCOS group. This probably suggests a different mechanism of disorders relative to OS in patients with PCOS. Moreover, MDA has been associated with an increase in TChol and especially LDL; therefore, it appears that an increase in LDL are the major cause of MDA (Andrews PJ et *al*., 2011).

Malonyldialdehyde (MDA) is produced enzymatically by the breakdown of unstable hydroperoxides during peroxidation of unsaturated fatty acids (Gürdöl F et *al* .,2008). Measurement of MDA levels in plasma or serum provides a convenient in vivo index of lipid peroxidation and represents a non-invasive biomarker of oxidative stress often clinically employed to investigate radical mediated physiological and pathological conditions (Merendino RA et *al* .,2003). In the present study, MDA was higher in the PCOS group in comparison to the healthy group.

In a study realized by Macut et *al* (2011), oxidative stress factors were measured in 23 women with PCOS and 23 healthy women. In consistent to our

study, MDA was significantly higher in the PCOS group in comparison to the healthy group. In previous study it was observed that, obese women with PCOS were more likely to have oxidative stress .In our study, similar results were also seen (Macut D et *al*., 2011).

Lipid peroxidation is defined as a reaction in which oxidants such as free radicals or unusual species attack lipids with carbon-carbon double bonds, notably polyunsaturated fatty acids (PUFAs), resulting in the extraction of hydrogen from carbon and the introduction of oxygen. as previously described in lipid peroxyl radicals and hydroperoxides (derouiche et *al*., 2020). Glycolipids, phospholipids (PL), and cholesterol (Ch) are all known peroxidation targets. Enzymes including lipooxygenase, cyclooxygenase, and cytochrome P450 can also oxidize lipids (see above, Lipids as Signaling Molecules). Cells can improve cell survival or induce cell death in response to membrane lipid peroxidation, depending on cellular metabolic conditions and repair capacity. Cells induce their own maintenance and survival under physiological or low levels of lipid peroxidation (subtoxic circumstances) by activating constitutive antioxidant defense mechanisms or signaling pathways that regulate antioxidant proteins, which leads to an adaptive stress response. (Jitca et *al*., 2022). In contrast, under medium or high levels of lipid peroxidation (toxic conditions), the extent of oxidative damage outweighs the ability to repair, and cells induce apoptosis or necrotic apoptosis; Both processes ultimately lead to molecular cell damage that can facilitate the development of various pathological conditions and accelerated aging.(Juan et *al*., 2021).

In this study, there was a statistically significant increase in mean serum SOD activity in PCOS compared to the control group. These results were in agreement with (Zhang et *al* .,2008). This systemic elevation in SOD activity due to SOD use may be in response to increased ROS production due to both hyperglycemia and excess free fatty acids. It has been reported that OS in PCOS leads to an inflammatory state that may contribute to co-morbidities such as

abdominal obesity, endothelial dysfunction, dyslipidemia, hyperandrogenism, elevated insulin levels, and insulin resistance (Rajendran S et al 2009; Gonzalez F ,et *al*., 2006). SODs play a critical role in the body's antioxidant defense against oxidative stress. SOD's therapeutic potential and physiological value have been demonstrated in a number of investigations. The enzyme has anti-inflammatory properties as well as the ability to inhibit precancerous cell alterations. Natural SOD levels in the body decline as people age, making them more vulnerable to oxidative stress-related disorders (Robbins D et *al* ., 2014). SOD is used as an anti-aging ingredient and antioxidant in cosmetics and personal care products because of its ability to prevent wrinkles, fine lines, and age spots by reducing free radical damage to the skin. It also aids wound healing, softens scar tissue, protects against UV rays, and reduces other signs of aging. RBC-related illnesses, cystic fibrosis (CF), postcholecystectomy pain syndrome, malignant breast disease, steroid-sensitive nephrotic syndrome, amyotrophic lateral sclerosis, neuronal apoptosis, AIDS, and cancer have all been linked to SOD ((Michalak M et *al* ., 2021). Furthermore, some researches have claimed that SOD activity and Alzheimer's disease have a substantial relationship. Treatment with SOD has also been shown to aid recovery from mustard gas burns. SOD enzymes have been proven to be quite beneficial in numerous animal models of cardiac ischemia-reperfusion injury, inflammation, and cerebral ischemia-reperfusion injury, among others. SOD mimetics (small molecule catalytic antioxidants) have the potential to treat oxidative stress-related illnesses. Synthetic chemicals called SOD mimetics effectively convert O2- into H2O2, which is then transformed into water by catalase (Kocot J et *al*., 2018).

Conclusion & Prospects

Conclusion

Polycystic ovaries are an important cause of infertility in most women in the world. Several international studies have shown the importance of developing diagnostic tools to help control and determine the extent of this syndrome. Several risk factors have been studied to determine which one is actually responsible for the formation of multiple cysts in women's genitals.

Our study results show that genetics Factor and suffering from obestiy are shown to be significant risk factors for polycystic ovary syndrome which indicates the importance of social behavior and diet in causing PCOS. By contrast, the playing sport and movement in daily life are considered protective factors Against the formation of polycystic syndrome in a population El- Oued .

Through the results of this study, we conclude that this disease leads to a significant imbalance in biochemical parameters such as Blood glucose ،Triglycerids, total cholesterols, leukocyte and erythrocyte lineage. This indicates that the body is affected by the metabolic and physiological aspects of this disease

The SOD activity in both salive and Sérum and MDA (Increase), Confirm the presence of oxidative stress associated with PCOS.

In detecting or determining the causes of the disease, what we found through the results of the analysis of MDA and SOD in serum and saliva showed that it contributes to the diagnosis of PCOS diseases. This confirms that oxidative stress is the explanation for the pathophysiological mechanism that causes PCOS through various socioeconomic and clinic risk factors.

Prospects:

Given the importance of these findings, they open up prospects for empirical and other in-depth studies that will allow us to clearly identify the following:

- Determine other factors associated with the risk of developing polycystic ovaries
- A comparative study of risk factors between the El-Oued and other regions.
- Determining the diet and daily life behaviors that reduce the incidence of cystic syndrome.

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Bibliographical references

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Annex

Annex

Annex 01:

Age :…… Marriage age:…...

Weight :…… Pressure gauge :…….

Length :……. Marital Status :……

Education level:……. living location:……..

Job:……. Blood type :……

Clinical factors :

N^0	Questions	Yes	No
01	Are you dynamic in your daily life?		
02	Do you take birth control pills?		
03	Are you nervous in your daily life?		
04	At what age did you start taking birth control pills?		
05	When did you give birth to your first child?		
06	Have you ever had a cesarean delivery?		
07	Does it happen again after every treatment?		
08	Is the menstrual cycle before illness... fluctuating... regular?		
09	When did the disease first appear?		
10	Do you suffer from vitamin D deficiency?		
11	Do you take medication without consulting a doctor?		
12	Do you take antibiotics?		
13	Do you take pain medication?		
14	Did you take paracetamol?		
15	Do you take anti-menstrual drugs?		
16	Do you eat a lot of medicinal herbs?		

Socioeconomic factors:

N^0	Questions	Yes	No
01	Are you addicted to smoke ?		
02	Are you addicted to fast foods ?		
03	Are you addicted to soft drinks ?		
04	Do you eat a lot of sugars ?		
05	Do you practice sports in your daily life?		
06	Has anyone in your family had the same disease as you?		
07	Are you exposed to chemicals in your daily life?		
08	Did you suffer from any other disease before this disease? remember him?		
09	Do you expose to high levels of family stress ?		
10	Do you use spices a lot?		
11	Do you use food additives such as food coloring and flavoring?		
12	Do you suffer or have you been obese?		
13	Are you overweight?		
14	Do you eat a lot of food candies?		
15	Do you expose to work stress ?		