مجلة المثنى للعلوم الصرفة
AL-Muthanna Journal of Pure Sciences (MJPS)
VOL.(4), NO.(1), 2017

Assessment of some physiological criteria in obese and lean patients with PCOS in Sammawa City

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Received 26-5-2016, Accepted 29-12-2016, Published 4-1-2017
DOI: 10.18081/2226-3284/017-1/1-23

Abstract

Back ground: The cases of polycystic ovary syndrome have increased among Iraqi women lately. The current study is conducted to measure lymphocyte and granulocyte cells of patients with polycystic ovarian syndrome by using acount 60 apparatus, measuring of CD marker (CD4, CD8, CD56) of patients with PCOS by using ELISA apparatus, measuring of hormonal levels (LH, FSH, Testosterone) of patients with PCOS by using Minividas apparatus.

Results of the current study reveal a significant difference among women without PCOS and PCOS patients in lymphocytes and granulocytes count in the four age groups. These findings reveal clear increase in the CD8, CD4 and CD56 in patients with PCOS group compared to women without PCOS group. The results show there is a clear and significant difference among women without PCOS and PCOS patients in lymphocytes and granulocytes count according to the weight in three weight categories. While in both four and five weight categories do not have significant differences among women without PCOS and PCOS patients. The results show a significant difference among women without PCOS and PCOS patients both of (CD4, CD8) in (51– 65) kg, (66-80) kg and (81–95) kg while CD56 does not have a significant difference among women without PCOS and PCOS patients in three weight categories. While in both (96– 110) kg, (111– 125) kg four and five do not have a significant differences among women without PCOS and PCOS patients.

The findings reveal also a significant difference in lymphocytes and granulocytes count among women without PCOS and PCOS patients in both of (Mediterranean group, Meaterian1 (Red meat) group Vegetarian group, Meaterian2 (white meat) group. The result show has a significant difference among women without PCOS and PCOS patients in both of (CD4, CD8) in the first three food groups, Meaterian 2 (White Meat) group a significant difference among women without PCOS and PCOS patients in CD4 while CD8 does not have a significant difference among women without PCOS and PCOS patient, CD56 result shows no significant difference among women without PCOS and PCOS patients in all food groups studied.

The aim is to compare lymphocytes and granulocytes, CD markers, in lean and obese PCOS patients using two different control groups matched with age and weight.

Key word: PCOS, Lymphocytes cell, granulocytes cell, CD markers, Obesity.

Materials and methods

The blood sample were collected from patients with PCOS during the period (November 2014 to May 2015) in the women clinic of Al-Sammawa city. The study included 80 patient women with PCOS age from (14– 37) years compared to women without PCOS group 30 healthy women age from (14– 37) years. Then divided the patients in to five groups depending on the weight: G1 (51–65) kg, G2 (66-80) kg G3 (81– 95) kg, G4 (96-110) kg, G5 (111-125) kg.

Results

The PCOS patients in (14-37) age group have a significant difference in lymphocytes and granulocytes compared to women without PCOS group. The PCOS patients in (14-19) age group have no significant difference in CD4, CD8, CD56 compared to women without PCOS group. The PCOS patients in (20-25) age group have a significant difference in CD4, CD8. While CD56 have no significant difference in PCOS patients compared to women without PCOS group. The PCOS patients in (26-31), (32– 37) ages group have a significant difference in CD4, CD8, CD56 compared to women without PCOS group.

Conclusion: Increase lymphocyte number in the case of obesity in women with polycystic ovarian syndrome, decrease granulocyte number in patients with PCOS. Clear increase in CD8 in patients with PCOS, increase in CD4 and CD56 in patients with PCOS.

Introduction:

Polycystic ovarian syndrome (PCOS) is one of the most common causes of ovulatory infertility affects 4-12% of women in reproductive age **(**Knochenhauer *et al.*,1998; Sheehan, 2004; Majumdar and Singh,2009). In 1935, Stein and Leventhal first described the association of polycystic ovaries, amenorrhea, hirsutism, and obesity, for this reason PCOS is also known as Stein Leventhal Syndrome (Majumdar and Singh, 2009; Moran and Teede,2009). It is a condition that causes irregular menstrual periods and elevated levels of androgens (male hormones) in women. The elevated androgen levels can sometimes cause excessive facial hair growth, acne, and/or male-pattern hair thinning (Legro *et al.*, 2007). The disease is present at birth but does not cause symptoms until puberty; genetic component, clinical features of this disorder may change throughout the lifespan, starting from adolescence to post menopausal age. No effort has been made to define difference in the phenotype and clinical presentation according to age (Hamzeh and Balen,2006; Renato and Alessandra, 2006; Fernandes, 2005)**.**

The etiology of PCOS is not fully known, but many environmental and genetic factors may cause PCOS, such as diet, pollution, sedentary lifestyle and stress that contributes to its development (Goodarzi,2008). The PCOS is often associated with obesity, insulin resistance and metabolic syndrome (Frayyeh,2014) in recent years ,neutrophil to lymphocyte ratio (NLR) has gained a popularity in the detection of inflammation in different inflammatory diseases such as PCOS, Diabetes mellitus (DM), Ulcerative colitis and Hypertension ,and it has been shown that NLR is correlated with High sensitivity –C– reactive protein (hs. CRP)

levels (Keskin Kurt *et al*.,2014; Celikbilek *et al*.,2013; Imtiaz *et al*.,2011). Similarly, increased levels of granulocytes can also be used as a marker to detect inflammation neutrophil to lymphocyte ratio (NLR) is a newly introduced systemic inflammation marker that has been linked to mortality and morbidity in many diseases (Keskin Kurt *et al*.,2014; Azab *et al*.,2012). The increase in lymphocyte numbers and particularly natural killer cell ($CD56^{+}$) cells was significantly enhanced in overweight PCOS, who also demonstrated significantly more pronounced cardiovascular responses (Benson *et al*., 2007). T lymphocytes potentially play a role in the local pathological mechanisms of PCOS (Turi *et al*., 1988 ; Luchetti *et al*., 2004; Sander *etal*., 2006; Wu *et al*., 2007). It has been described that the CD4+/CD8+ T ratio is altered in both peripheral blood of women with PCOS with diminished expression of the CD8+T subset (Turi*etal*.,1988) and in the infiltrating ovarian T lymphocytes of am urine PCOS-model (Luchetti*etal*., 2004).

Aim of the study:

Measurement of lymphocytes and granulocytes in patients with polycystic ovarian syndrome.

Measurement of CD markers (CD4,CD8,CD56) in patients with PCOS.

Materials and Methods:

The blood sample were collected from patients with PCOS during the period (November 2014 to May 2015) in the women clinic of Al-Sammawa city. The study included 80 patient women with PCOS age from (14– 37) years compared to the women without PCOS group 30 healthy women age from (14–37) years. Then divided the patients in to five groups depending on the weight: G1 (51– 65) kg, G2 (66– 80) kg, G3 (81– 95) kg, G4 (96-110) kg, G5 (111-125) kg.

Immunological tests

Human CD4 (Cluster of Differentiation4) ELISA Kit

Principle of the test

This ELISA kit uses sandwich–ELISA as the method. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human CD4ELISA kit. Standards or samples are added to the appropriate micro ELISA plate wells and combined with the specific antibody. Then abiotinylateddetection antibody specific for Human CD4 ELISA kit and Avidin-Horseradish peroxidase (HRP) conjugate is added to each micro plate well successively and incubated free components are washed away. The substrate solution is added to each well only those wells that contain Human CD4ELISA kit ,biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color The enzyme–substrate reaction is terminated by the addition of asulphuric acid solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450nm± 2nm. The OD value is proportional to the concentration of Human CD4 ELISA kit.

Procedure:

1-Add100ML standard or sample to each well. Incubate 90 minutes at 37c°.
2-Remove the liquid. Add 100 MLBiotinylated Detection Ab.Incubate 1 hour at 37c°.
3-Aspirateand wash3times.
4-Add100ML HRP conjugate. Incubate 30 minutes at 37c°.
5-Aspirate andwash5times.
6-Add90 ML substrate Reagent. Incubate 15 minutes at 37c°.
7-Add50 ML stop solution. Read at 450nm immediately.
8-calculation of results.

Calculation of results

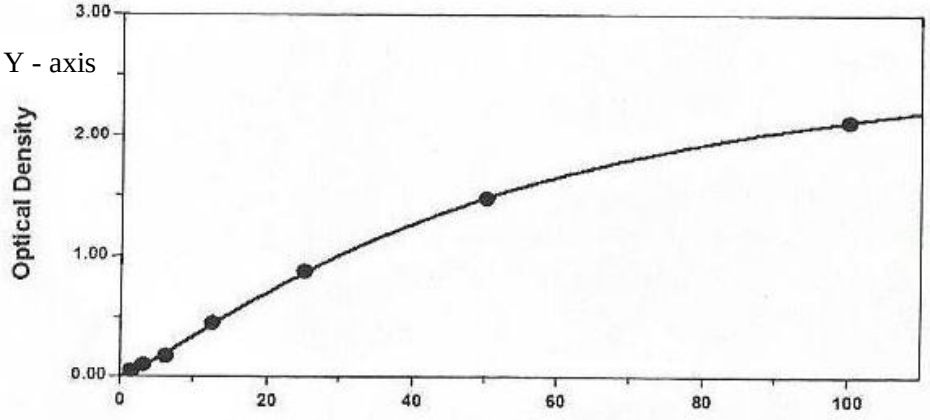
Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Createa standard curve by plotting the mean OD value for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. It is recommended to use some professional software to do this calculation, such as curve expert 1.3 or 1.4.In the software interface, abest fitting equation of standard curve will be calculated using OD values and concentrations of standard sample. The software will calculated the concentration of samples after entering the OD value of samples. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the concentration of the sample surpasses the upper limit of the standard curve, you should re-test it after appropriate dilution. The actual concentration is the calculated concentration multiplied dilution factor.

$$Concentration\ sample = \frac{O.D\ \ sample}{O.D\ stander} \times consentration\ stander$$

Typical data

As the OD values of the standard curve may vary according to the conditions of actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects). the operator should establish standard curve for each test. Typical standard curve and data below is provided for reference only.

Concentration (ng/mL)	100	50	25	12.5	6.25	3.13	1.56	0
OD	2.392	1.585	0.82	0.44	0.25	0.152	0.108	0.06
OD – OD $_{blank}$	2.332	1.525	0.76	0.38	0.19	0.092	0.048	0



X - axis

Human CD8(Cluster of Differentiation8)ELISA kit

Principle of the test

This ELISA kit uses Sandwich-ELISA as the method. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific toCD8. Standards or samples are added to the appropriate micro ELISA plate wells and combined with the specific antibody. Then abiotinylated detection antibody specific for CD8and Avidin-Horseradish peroxidase (HRP) conjugate is added to each micro plate well successively and incubated. Free components are washed away the substrate solution is added to each well only those wells that contain CD8, biotinylated detection antibody and Avidin– HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of asulphuric acid solution and the color turns yellow. The optical density (OD) ismeasured spectrophotometrically at a wavelength of 450nm± 2nm.The OD value is proportional to the concentration of CD8.

Procedure

1-Add 100ML standard or sample to each well.Incubate90mintues at 37c°.
2-Aspirate and add 100 MLBiotinylated Detection Ab. Incubate 1hourat37c°.
3-Aspirate and wash 3times.
4-Add 100ML HRP conjugate. Incubate 30 minutes at 37c°.
5-Aspirate and wash 5times.
6-Add 90 ML substrate Reagent. Incubate15 minutesat37c°.
7-Add 50MLstopsolution. Readat 450 nm immediately.
8-calculation of results.

Calculation of results

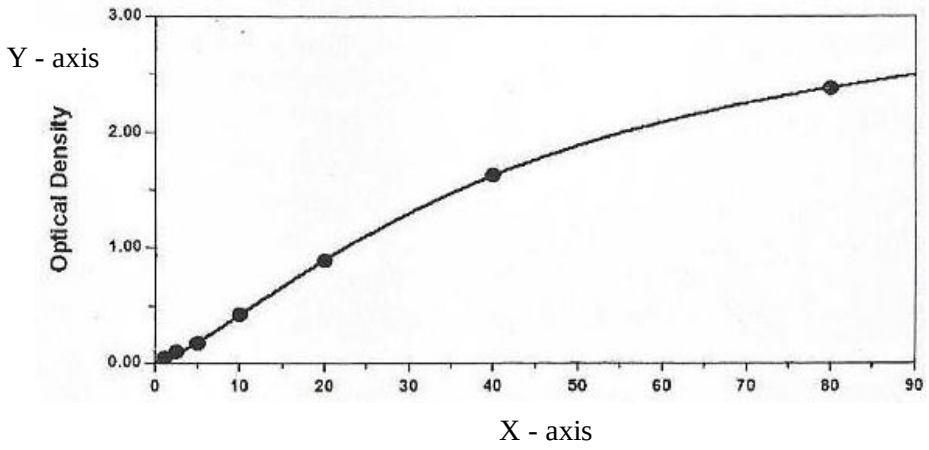
Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Create a standard curve by plotting the mean OD value for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. It is recommended to use some professional software to do this calculation, such as curve expert 1.3 or 1.4. In the software interface, abest fitting equation of standard curve will be calculated using OD values and concentrations of standard sample. The software will calculated the concentration of samples after entering the OD value of samples. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the concentration of the sample surpasses the upper limit of the standard curve, you should re-test it after appropriate dilution. The actual concentration is the calculated concentration multiplied dilution factor.

$$Concentration\ sample = \frac{O.D\ \ sample}{O.D\ stander} \times consentration\ stander$$

Typical data

As the OD values of the standard curve may vary according to the conditions of actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects). the operator should establish standard curve for each test. Typical standard curve and data below is provided for reference only.

Concentration (ng/mL)	80	40	20	10	5	2.5	1.25	0
OD	2.461	1.682	0.936	0.474	0.225	0.165	0.117	0.066
OD – OD_{blank}	2.395	1.616	0.87	0.408	0.159	0.099	0.051	0



Human NCAM/CD56(Neural cell Adhesion Molecule)ELISA kit

Principle of the test

This ELISA kit uses sandwich-ELISA as the method. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to NCAM/ CD56. Standards or samples are added to the appropriate micro ELISA plate wells and combined with the specific antibody. Then abiotinylated detection antibody specific for NCAM/ CD56 and Avidin- Horseradish peroxidase (HRP) conjugate is added to each micro plate well successively and incubated. Free components are washed away the substrate solution is added to each well only those wells that contain NCAM/ CD56, biotinylated detection antibody and Avidin- HRP conjugate will appear blue in color. The enzyme–substrate reaction is terminated by the addition of asulphuric acid solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at awavelength of 450nm± 2nm.The OD value is proportional to the concentration of NCAM/ CD56.

Procedure

1-Add 100ML standard or sample to each well. Incubate 90mintuesat 37c°.

2-Remove the liquid. Add 100 MLBiotinylated detection Ab. Incubated 1hour at37c°.

3-Aspirate and wash 3times.

4-Add 100ML HRP Conjugate .Incubate 30minutes at 37c°.

5-Aspirate and wash 5 times.

6-Add 90ML substrate Reagent. Incubate 15minutes at 37c°.

7-Add 50ML stop solution. Read at 450nm immediately.

8-calculation of results.

Calculation of results

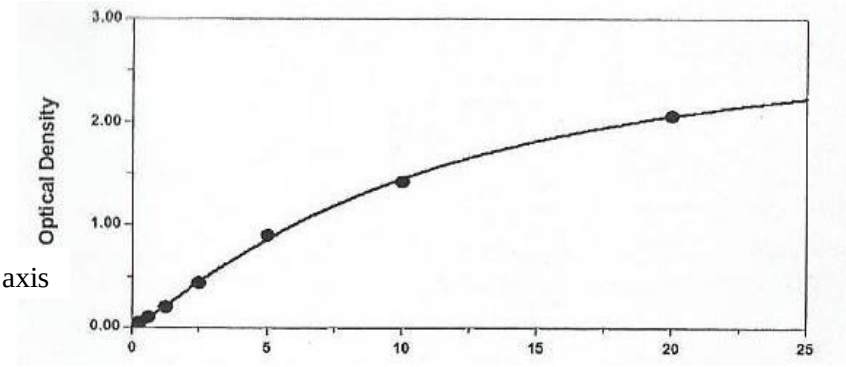
Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Create a standard curve by plotting the mean OD value for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. It is recommended to use some professional software to do this calculation, such as curve expert 1.3 or 1.4.In the software interface, abest fitting equation of standard curve will be calculated using OD values and concentrations of standard sample. The software will calculated the concentration of samples after entering the OD value of samples. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by thedilution factor. If the concentration of the sample surpasses the upper limit of the standard curve, you should re-test it after appropriate dilution. The actual concentration is the calculated concentration multiplied dilution factor.

$$Concentration\ sample = \frac{O.D\ \ sample}{O.D\ stander} \times consentration\ stander$$

Typical data

As the OD values of the standard curve may vary according to the conditions of actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects).the operator should establish standard curve for each test. Typical standard curve and data below is provided for reference only.

Concentration (ng/mL)	20	10	5	2.5	1.25	0.63	0.31	0
OD	2.511	1.647	0.928	0.508	0.26	0.181	0.132	0.082
OD – OD $_{blank}$	2.429	1.565	0.842	0.426	0.178	0.099	0.05	0



Y - axis

X - axis

lymphocytes count counting process

For the usual testing blood samples, all the cells are interlapped to

each other, so the analyzer cannot precisely count the blood cells or calculate their volume distribution. for this reason, samples should be diluted before the counting or calculating the volume distribution. This analyzer has the second dilution function, under the whole blood sample and prediluted blood sample modes, the analyzers second dilution ratio are different.

Principle of the test

Single channel fluidic systems counting module structure as figure (3.2), consists of counting chamber, valve5, valve7, measuring cup, volume measuring module, negative pressure pump and relevant support system.

The counting module completes the most important function of the analyzer-counting. The electrodes installed on the counting chamber detect the cell pulses when they pass the aperture by coulter principle. The detected pulses are then transmitted to the analog circuit to be amplified, rectified, recognized, threshold adjusted and finally count the cells. when the sample is mixed well in the counting chamber, the machine establishes the vacuum and open valve 9.sample(blood cells) in the chamber is lead to cross the aperture under the negative pressure to generate pulses and finish the counting process.

Procedure

1-The sample blood collection should strictly follow the peripheral blood collection standard of National Health Department and this field. Note at the same time: while in collecting the blood, if the blood does not flow well, slight pressure could be forced distally to the would, prohibiting to exert strength around the puncture area, incase of flowing in the tissue fluid and affecting the test results.

2-while in adding the diluent, the sample tube should be inclined under the probe to let the diluent flow down through the tube wall, incase of generating bubbles.

3-If the blood sample has been placed for along time and not blended well, it will lead to measurement error and unprecise test results.

4-The testing sample can only be stored at room temperature and should be tested with in 4 hours .Do not shake the testing samples violently.

Statistical Analysis

The readings obtained from samples of both groups (with/ without PCOS)were tested and means were compared by using one- way ANOVA test, independent t-test and the least significant difference (LSD) at a 5% level of significance, to evaluate the significant differences, Data were processed and analyzed by using statistical program social science (SPSS 20) and the results were expressed as Mean± SD (Al– Rawi, 2000).

Results and Discussion

Lymphocytes and granulocytes in PCOS patients according to the age

The PCOS patients in (14-19) age group have a significant difference in lymphocytes compared to women without PCOS group. The expression pattern of androgen receptors, which have been documented in lymphoid and non lymphoid cells of thymus and bone marrow but not in mature peripheral lymphocytes, suggests that androgen plays an important part in the development and activation of lymphocytes (Olsen and Kovacs, 2001). This result is agree with (Yilmaz *et al*.,2015).

The PCOS patients in (14-37) age group have a significant difference in granulocytes compared to women without PCOS group. This result is new marker can used in diagnostic the PCOS patients.

The PCOS patients in (20-25) age group have a significant difference in lymphocytes compared to women without PCOS group. The increased number of lymphocytes in the present PCOS group can be an initiating factor of chronic inflammation and disturbed hormone spectrum(Lao xiong *et al*., 2011). This result is similar to other results obtained by(Orio *et al*.,2005).

The PCOS patients in (26-31) age group have a significant difference in lymphocytes compared to women without PCOS group. PCOS is a pro inflammatory disorder and several publications on PCOS show increased levels of circulatory inflammatory markers such as lymphocytes. The reason of increased inflammation in PCOS has not been clarified yet, and it remains uncertain whether it is associated with PCOS itself or the accompanying obesity (Zahorska– Markiewicz *et al*.,2000).This result is agree with (Phelan *et al*., 2013).

The PCOS patients in (32-37) age group have a significant difference in lymphocytes compared to women without PCOS group. Indeed, inflammation has been recognized to play a central role in both initiation and progression of the atherosclerotic process (Alexander,1994). This result is similar with result reported by (Lao xiong *et al*.,2011). Moreover, there are no significant differences among all age groups for lymphocytes, granulocytes of PCOS patients $p>0.05$. Table (1).

Table 1: Determination the lymphocytes and granulocytes cells in patients with PCOS compared to women without PCOS group according to the age.

Age (year)	Lymphocytes count (%)		Granulocytes count (%)	
	Women without PCOS	Patient with PCOS	Women without PCOS	Patient with PCOS
(14 -19)	29.9±7.2	*59.1±11.5	56.6±5.5	*22.1±10.9
(20-25)	33.7±3.3	*59.2±13	55.1±4.2	*24±12.2
(26-31)	33.4±5.2	*57.2±10.1	56.5±4.6	*25.4±11
(32-37)	31.3±6.9	*53.2±9.4	57.1±6	*26.8±10.8

***** Represent a significant difference among women without PCOS and patient with PCOS, and the results are shown as a Mean±SD.at $P<0.05$.

2.CD4,CD8,CD56 in PCOS patients according to the age

The PCOS patients in (14-19) age group have no significant difference at $p<0.05$ in CD4 compared to women without PCOS group. Interestingly, T helper cells have been previously described in ovarian follicles of PCOS women (Gallinelli *et al*., 2003), though their function remains unknown. Similarly, peripheral blood $CD4^+$ T cells also increased after DHEA and rogenisation in the model we describe here. This suggests that androgenisation acts through specific receptors on T cells. It has been proposed that $CD4^+$ T cells express a receptor for DHEA (Meikle *et al*.,1992). This current result agreed with (Moro *etal*., 2012).But using another method flow cytometry to estimate CD4.

The PCOS patients in (14-19) age group have no significant difference at $p<0.05$ in CD8 compared to women without PCOS group. In the uterus, decidual $CD8^+$ T cells display a cytolytic activity since they regulate the invasion of extra villous trophoblasts, a crucial process for normal utero placental development (Scaife *et al*.,2006).

The PCOS patients in (14-19) age group have no significant difference at $p<0.05$ in CD56 compared to women without PCOS group. NK cells have been reported to be increased in endometrial of patients, who had recurrent pregnancy loss (Quenby *et al*.,1999;Clifford *et al*.,1999). It is suggested that increased number and activity of NK cells will cause the stimulation of the secretion of inflammatory cytokines in Th1 cells, the production of TNF-α and nitric oxide with decidual macrophage activation via IFN-γ, and therefore a damage to conceptus via apoptosis (Wegmann *et al*.,1993; Wilson *et al*.,1997;Seyhan *et al*.,2011).

The PCOS patients in (20-25) age group have a significant difference at $p<0.05$ in CD4 compared to women without PCOS group. Patients with PCOS represent a population with a high risk of developing Insulin resistance (IR), Diabetes mellitus type 2 (DM2), and obesity and with potentially increased risk of cardio vascular disease (CVD). (Niccoli *et al*., 2011; Moran and Teede, 2009). Indeed, it is known that risk factors of cardio vascular disease (CVD) , including dyslipidemia hypertension, oxidative stress, inflammation, and increased frequency of $CD4^+CD28^{null}$ T lymphocytes, are associated with PCOS (Niccoli *et al*., 2011; Martens *et al*.,1997; Giubilato *et al*.,2011). This result was in agreement with (Gallinelli *et al*.,2003). But with using another method flow cytometry to estimate CD4.

The PCOS patients in (20-25) age group have a significant difference at $p<0.05$ in CD8 compared to women without PCOS group. The apparition of cysts increased ovarian T lymphocyte infiltration while diminished the populations of $CD4^+$ and increased the $CD8^+$ T subset. Recently, (Lu *etal*., 2002) found that, after estrogen stimulation, there was a direct relationship between $CD8^+$ enriched T cell population expression and high B cell-produced cytokine levels in rhesus macaque ovaries. In addition ,selective changes in lymphocyte subtype were also reported in premature ovarian failure (Chernyshov *et al*.,2001).This result was in agreement with (Gallinelli *et al*.,2003).

The PCOS patients in (20-25) age group have no significant difference at $p<0.05$ in CD56 compared to women without PCOS group. It is known that NK

activity is very sensitive to neuroendocrine changes (Levy *etal*., 1989) and further studies are needed to verify the hypothesis that such a decrease of NK activity in middle-aged subjects is related to this phenomenon. Recent data suggest that a persistently low NK activity is a predictor of morbidity (Levy *et al*.,1991).

The PCOS patients in (26-31)age group have a significant difference at $p<0.05$ in CD4 compared to women without PCOS group. Higher levels of CD4 in PCOS could be part of the complex pathogenic mechanism of this syndrome .Of course, further studies are needed to better understand the causes and mechanisms underlying the expansion of CD4in PCOS and its possible link with hyperandrogenism. Our study demonstrated the presence of higher levels of CD4 in young women with PCOS. Our finding suggest that both activation of innate immunity as well as dysregulation of adaptive immunity play a path genetic role in this complex syndrome (Niccoli *et al*., 2011). PCOS is a state of inflammatory activation (Moro *et al*.,2012). In addition, this is other result that in agreement with our result ,but with using another method such as (Krishna *et al*.,2015).who used the flow cytometry method to estimate CD4.

The PCOS patients in (26-31) age group have a significant difference at $p<0.05$ in CD8 compared to women without PCOS group. It has also recently become evident that T cells play an important role in generating the inflammatory phenotype in adipose tissue CD4 (+) helper cells are diminished and CD8 (+) effectors T cells are markedly increased in adipose tissue in response to a high-fat diet (Villa and Pratley,2011).

The PCOS patients in (26-31) age group have a significant difference at $p<0.05$ in CD56 compared to women without PCOS group. $CD56^+$ T cells to mucosal immune dysregulation and suggest pathological significance for mechanisms modulating $CD56^+$Tcell frequency. Although perturbations in $CD56^+$ T cell frequency may not be unique to IBD, specific modulation of $CD56^+$T cell trafficking oreffectors function may represent targets foregut-specific therapeutic intervention (Cohavy and Targan, 2007). This result was in agreement with (Matteo *et al*., 2010). But with using another method flow cytometry to estimate CD56.

The PCOS patients in (32-37) age group have a significant difference at $p<0.05$ in CD4 compared to women without PCOS group. In the ovary, $CD4^+$ T helper cells and macrophages, through the secretion of cytokines, metalloproteinases and other inflammatory mediators, orchestrate tissue remodelling and apoptosis, both of which are involved in folliculogenesis, ovulation and CL formation (Wu *et al*., 2004).

The PCOS patients in (32-37)age group have a significant difference at $p<0.05$ in CD8 compared to women without PCOS group. The enriched $CD8^+$ T cell expression could be involved in the high levels of cytokines, such tumor necrosis factor, reported to be increased in the cystic pathology(Araya *et al*.,2002;Korhonen *et al*., 2002; Peral *et al*., 2002; Sayin *et al*., 2003; Deshpande *et al*.,2000;Gallinelli *et al*.,2003).

The PCOS patients in (32-37) age group have a significant difference at $p<0.05$ in CD56 compared to women without PCOS group. Increase of the absolute number of NK cells expressing

the marker CD56 in the peripheral blood of women with PCOS.

There are a significant differences in CD4 and CD8 means among the different age groups for patients with PCOS ($p<0.05$), while CD56 means are not have significant differences among the different age groups ($p>0.05$). Table(2).

Table2: Determination the CD markers in patients with PCOS compared to women without PCOS group according to the age.

Age (year)	CD4 (ng/µL)		CD8 (ng/µL)		CD56 (ng/µL)	
	Women without PCOS	Patient with PCOS	Women without PCOS	Patient with PCOS	Women without PCOS	Patient with PCOS
14-19	3.55±1.9	6.67±2.9	6±5	10±10.9	5±1	4.3±1.8
20– 25	2.8±1.3	*7.6±8	5±3.9	*20.3±23.9	2.4±1	3.1±1.6
26– 31	3.6±2.7	*13.9±13	2.9±1.3	*14.6±13.8	3.6±1.4	*5.4±3.3
32– 37	1.6±0.4	*6.3±2.9	6.4±3.3	*17.8±14.3	2.7±1	*8.7±7.9

* Represent a significant difference among women without PCOS and patient with PCOS, and the results are shown as a Mean± SD. at $P<0.05$.

3.Lymphocytes and granulocytes in PCOS patients according to the weight

The PCOS patients in (51-65) kg have significant difference at $p<0.05$ in lymphocytes compared to women without PCOS group. Previous studies demonstrated that proinflammatory T lymphocytes are also present in visceral adipose tissue and contribute to adipose tissue inflammation and the development of glucose intolerance before the recruitment of macrophages (Wu *et al*., 2007). A recent elegant study by (Nishimura *et al*., 2009). Elucidated the role of T lymphocytes in adipose tissue inflammation in obesity.

The PCOS patients in (51-95) kg have significant difference at $p<0.05$ in granulocytes compared to women without PCOS group. This result it's the new marker can used in diagnostic the PCOS patients.

The PCOS patients in (66-80) kg have significant difference at $p<0.05$ in lymphocytes compared to women without PCOS group. Obesity is associated with a low level of chronic inflammation, which may arise in part from immune cell infiltration into adipose tissue (Hotamisligil, 2006). Multiple subsets of immune cells are found in the adipose tissue of obese animals and humans, including macrophages, T and B lymphocytes, and NK cells (Behan *et al*., 2013). T or B lymphocytes may contribute to obesity-induced adipose tissue inflammation and glucose intolerance (Behan *et al*.,2013).

The PCOS patients in (81-95) kg have significant difference at $p<0.05$ in lymphocytes compared to women without PCOS group. Although a clear role of adipose tissue in immune surveillance has not been identified, expanded adipose tissue in obesity harbors several activated immune cell subsets, including T cellsand macrophages. It is established that obesity- associated chronic inflammation causes insulin resistance. However, the provenance of adipose tissue– derived

inflammation and biological relevance of ARTs in path physiology of obesity is not fully understood (Yang *et al*.,2010).

There are no significant differences between PCOS patients and women without PCOS in lymphocytes and granulocytes means in the two weight groups (96-110 and 111-125) kg, because of there are not women without PCOS for those weight groups in this study.

Moreover, there are no significant differences among all weight groups for lymphocytes, granulocytes of PCOS patients at p>0.05. Table(3).

Table3: Determination the lymphocytes and granulocytes in patients with PCOS compared to women without PCOS group according to the weight.

Weights	Lymphocytes count (%)		Granulocytes count (%)	
	Women without PCOS	Patient with PCOS	Women without PCOS	Patient with PCOS
(51-65)kg	32.3±4.7	*62.3±16.9	56.4±4.6	*20.9±3.9
(66-80)kg	32.8±5.9	*55.9±10.5	55.7±4.9	*26±11
(81-95)kg	34.2±5.5	*59.4±12.8	58.2±7.3	*23.4±12.4
(96-110)kg	-	57.5±7.9	-	25.5±8.6
(111-125) kg	-	54.5±9.6	-	25.4±12.1

* Represent a significant difference among women without PCOS and patient with PCOS, and the results are shown as a Mean ± SD. at P<0.05. (-)There are not women without PCOS for those weight groups in this study.

4.CD4, CD8, CD56 in PCOS patients according to the weight

The PCOS patients in (51-65) kg have significant difference at p<0.05 in CD4 compared to women without PCOS group. Adaptive immune cells, including CD4 and CD8 T lymphocytes, also contribute in development and maintenance of inflamed adipose tissue in obesity. (Suganami and Ogawa, 2010).$CD4^+$ T cells and T regulatory cells are reduced in murine obesity and similar data have also been found in humans (Nishimura *et al*., 2009; Feuerer *et al*., 2009).

The PCOS patients in (51-65) kg have significant difference at p<0.05 in CD8 compared to women without PCOS group. The increased numbers of local effector and memory $CD8^+$ T cells described in adipose tissue of obese subjects (Duffaut *et al*., 2009; Nishimura *et al*., 2009). Interestingly, obesity as a multifactorial disease is associated with low grade systemic inflammation that can potentially influence naïve to memory CD8 T cell differentiation (Khan *et al*., 2014).That $CD8^+$ T cell infiltration precedes accumulation of macrophages in adipose tissue obesity, $CD8^+$ T cells are required for adipose tissue inflammation and $CD8^+$ T cells have major roles in macrophage differentiation, activation and migration thus, $CD8^+$ T cells are crucially involved in initiating inflammatory cascades in obese adipose tissue. That $CD8^+$ T cells are essential for both the initiation and maintenance of adipose inflammation, strongly suggest that there is a relay involving both $CD8^+$ T cells and macrophages in obeseadipose tissue that propagates local adipose inflammation (Nishimura *et al*.,2009).

The PCOS patients in (51- 95) kg have no significant difference at p<0.05

in CD56 compared to women without PCOS group.

The PCOS patients in (66-80) kg have significant difference at $p<0.05$ in CD4 compared to women without PCOS group. The contribution of CD4 lymphocytes to inflammation in obesity is not limited to their increased numbers. They also directly contribute to the dysregulation of other cells in the inflamed adipose tissue (Sullivan, 2012).

The PCOS patients in (66-80) kg have significant difference at $p<0.05$ in CD8 compared to women without PCOS group. Obesity is associated with increased$CD8^+$ T cells in adipose tissue (Rausch *et al*.,2008). Moreover, depletion of $CD8^+$ T cells in diet-induced obesity resulted in decreased accumulation of macrophages in to obese VAT as well as improved insulin sensitivity. Conversely, adoptive transfer of $CD8^+$ T cells in to CD8-deficient mice increased infiltration of macrophages in to VAT as well as expression of the inflammatory cytokines IL-6 and TNF-α, along with development of insulin resistance following high– fat diet (Nishimura *et al*., 2009). These finding suggest a critical role for $CD8^+$Tcells in the development to inflammation and insulin resistance in obesity. More recently, (Jiang *et al*.,2014), confirmed VAT accumulationof $CD8^+$T cells in obesity and examined the mechanism of $CD8^+$ T cell accumulation in adipose tissue.

The PCOS patients in (81-95) kg have significant difference at $p<0.05$ in CD4 compared to women without PCOS group. That the anti-inflammatory $CD4^+$ Th2 cells play a suppressive role in the development of obesity-related inflammation and IR, and the shift in Th1/ Th2 ratio toward the pro inflammatory Th1 phenotype might be responsible for the polarization from M2 to M1 ATMs (Mraz and Haluzik, 2014).

The PCOS patients in (81- 95) kg have significant difference at $p<0.05$ in CD8 compared to women without PCOS group. Obesity also increases the numbers of $CD8^+$ T lymphocytes (three-to four–times as compared with lean state)along with increased expression of their products, most notably granezyme B and IFNγ. Futhermore, $CD8^+$ T cell infiltration precedes the infiltration of macrophages in to AT, and $CD8^+$Tlymphocytes stimulate M1 macrophage polarization in vitro as well as in vivo (Rausch *et al.*,2008).

CD4, CD8, CD56 of PCOS patients increased in two weight groups (96- 110 and 111- 125) kg, but we are not found healthy women for this weight groups.
Results showed there are no significant difference among all weight groups for CD4, CD8, CD56 of PCOS patients $p>0.05$. except CD56 have significant difference in patients between (51– 65kg and 96-110 kg, 66– 80 kg and 96- 110 kg, 81– 95 kg and 96– 110kg) at $p<0.05$. Table (4).

Table4: Determination the CD markers in Patients with PCOS compared to women without PCOS group according to the weight.

Weights	CD4 (ng\µL)		CD8 (ng\µL)		CD56 (ng\µL)	
	Women without PCOS	Patient with PCOS	Women without PCOS	Patient with PCOS	Women without PCOS	Patient with PCOS
(51 – 65)kg	3.2±1.5	*6.1±2.1	4.5±3.7	*16.5±10	3.4±1.6	3.9±1.9

(66 – 80)kg	**2.8±2.5**	***9.9±10**	**4.6±3.2**	***17.9±23**	**3±1**	**4.3±4.2**
(81 – 95)kg	**2.4±1**	***9.3±10**	**5.2±1.2**	***18.4±18.8**	**3.2±0.8**	**4.6±2.9**
(96-110)kg	**-**	**8.2±10**	**-**	**12.4±8.6**	**-**	**8±6.7**
111-125)kg	**-**	**11.5±7**	**-**	**20±8**	**-**	**8.2±0.4**

* Represent a significant difference among women without PCOS and patient with PCOS, and the results are shown as a Mean ± SD. at P<0.05. (-)There are not women without PCOS for those weight groups in this study.

5. Lymphocytes and granulocytes in PCOS patients according to the diet

The PCOS patients have significant difference at p<0.05 in lymphocytes and granulocytes compared to women without PCOS in the four diet groups (Mediterranean group, Meaterian 1group, vegetarian group,Meaterian2 group)at p<0.05.They were directed to consume unsaturated sources of fat such as nuts, seeds, avocado, and olive oil while limiting overall fat intake. A high intake of vegetables and salads was encouraged, and the intake of added sugars was discouraged. The capacity of dietarycarbohydrates to increase postprandial glycemia may be an important consideration for optimizing metabolic and clinical outcomes in PCOS. Independently of the degree of weight loss, an ad libitum low-GI diet had benefits over and above the benefits of a conventional low-fat diet that was matched closely for macronutrient and fiber content. With only modest weight loss (4–5% of body weight). Diet composition may also influence risk factors of cardiovascular disease, which is a major concern in women with PCOS (Marsh *et al.*,2010).

Moreover, there are no significant differences among all diet groups for lymphocytes, granulocytes of PCOS patients at p>0.05. Table (5).

Table5:Determination the lymphocytes and granulocytes in patients with PCOS compared to women without PCOS group according to the diet.

Type of diet	Lymphocytes count (%)		Granulocytes count (%)	
	Women without PCOS	Patient with PCOS	Women without PCOS	Patient with PCOS
Mediterranean	**32.4±6.2**	**58±11***	**56±5**	***24±11**
Meaterian1	**30.8±6**	***55.6±11.6**	**58.8±4**	***25.7±11.5**
vegetarian	**34.5±3.2**	***45±1**	**55±4**	***36±4.8**
Meaterian2	**33±4.8**	***60±9.9**	**54±4.2**	***23±10**

* Represent a significant difference among women without PCOS and patient with PCOS, and the results are shown as a mean ± SD. at P<0.05.
Meaterian1=Red meat
Meaterian2=White meat

6. CD4,CD8,CD56 in PCOS patients according to the diet

The PCOS patients with Mediterranean diet have significant difference at p<0.05 in CD4, CD8

compared to women without PCOS group. While CD56 have no significant difference between PCOS patients and women without PCOS group. Dietary-induced weight loss may represent an appropriate means of improving hyper androgens and all parameters of the metabolic syndrome in many obese PCOS women (Pasquali *et al*.,1989; Jakubowicz and Nestler,1997). It is generally agreed that energy restriction is required for weight loss. In fact, early improvements in reproductive function, in the absence of apparent weight loss, were probably due to energy restriction per se. However, there is little agreement on what constitutes the optimal diet for women with PCOS (Marsh and Brand-Miller,2005). The resurgence of the ‘‘Atkins diet’’ has generated considerable interestin very low calorie diets in recent years, and these can lead to significantly decreased body weight in PCOS (12% in 24 weeks) and can improve reproductive outcome (Moran *et al*.,2004).

The PCOS patients with Meaterian 1diet have significant difference at $p<0.05$ in CD4,CD8 compared to women without PCOS group. While CD56 have no significant difference between PCOS patients and women without PCOS group. Although low-fat high-carbohydrate diets have been the mainstream approach for weight management, they appear to be no more effective than other dietary patterns that restrict kilojoules (Pirozzo *et al*., 2003).Modifying the type of dietary carbohydrate or glycaemic index (GI) is proposed to both improve the cardiovascular risk profile (Brand *et al*., 1991; Luscombe *et al*., 1999). Increasing the amount of dietary protein at the expense of carbohydrate has been shown to maintain lean body mass in weight loss and reduce abdominal fat in insulin-resistant subjects (Parker *et al*., 2002; Farnsworth *et al*., 2003).

The PCOS patients with vegetarian diet have significant difference at $p<0.05$ in CD4,CD8 compared to women without PCOS group. While CD56 have no significant difference between PCOS patients and women without PCOS group.

Previous research has suggested links between dietaryprotein, steroid metabolism and mood (Long *et al*.,2000), and between carbohydrate metabolism, serotonin metabolism, and mood (Latner and Schwartz, 1999).

Markus *et al*., (2000) have suggested that dietary protein enriched in tryptophan may improve coping ability in stress–vulnerable subjects by increasing brain serotonin. However, these studies do not involve dietary manipulations over longer periods, and it is unclear whether the proposed effects would be relevant in this situation (Galletly *et al*.,2007).

The PCOS patients with Meaterian 2 diet have significant difference at $p<0.05$ in CD4 compared to women without PCOS group. While CD8, CD56 have no significant difference between PCOS patients and women without PCOS group. A high intake of saturated fatty acids favors the development of insulin resistance and, conversely, the substitution of saturated fatty acids by monounsaturated fatty acids in the diet improves insulin sensitivity (Vessby *et al*., 2001; Vessby *et al*., 1994). It must be noted that in both the patients with PCOS and the control women the increase in the total fat intake depended mostly on the intake of monounsaturated fatty acids, and the intake of trans fatty acids was even below the current recommendations, possibly reflecting the traditional mediterranean diet of Spaniards. And because saturated and trans fatty acids are

those that favor cardiovascular events and diabetes, the later risk mediated in part by the proinflammatory effects of trans fatty acids (Lopez-Garcia *et al*.,2005). Current lifestyle recommendations for PCOS propose a low fat (~30% of energy, saturated fat ~10% of energy), moderate protein (~15%) and high carbohydrate intake (~55%) and increased consumption of fiber, wholegrain breads, cereals, fruit and vegetables for reduction of associated mortality and morbidities and improvement of insulin sensitivity (Norman *et al*., 2002).

Moreover, there are no significant differences among all diet groups for CD4,CD8,CD56 of PCOS patients at $p>0.05$.Except CD4have significant difference between vegetarian diet and Mediterranean diet, as well as between vegetarian diet and Meaterian 2 diet at $p<0.05$. Table (6).

Table6:Determination the CD markers in patients with PCOS compared to women without PCOS group according to the diet .

Type of diet	CD4 (ng/µL)		CD8 (ng/µL)		CD56 (ng/µL)	
	Women without PCOS	Patient with PCOS	Women without PCOS	Patient with PCOS	Women without PCOS	Patient with PCOS
Mediterranean	2.7±1.4	*8.8±9	4±3	*17.7±15	3.48±1.7	4.2±2.7
Meaterian 1	2.6±1.4	*10.7±12	4.2±2.9	*21±28	2.9±1.4	5.8±6.7
vegetarian	4.2±3.4	*22.2±2	2±0.7	*8.3±2.5	2.8±1.7	4.1±1.1
Meaterian 2	2.6±1.6	*6.9±2.4	7±3	9.4±8	3.6±1.3	4.5±1.9

* Represent a significant difference among women without PCOS and patient with PCOS, and the results are shown as a Mean ± SD. at $P<0.05$.
Meaterian1=Red meat
Meaterian 2=White meat

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الخلاصة:

ازداد شيوع حالات متلازمة تكيس المبيض لدى النساء العراقيات في الآونة الأخيرة، وقد أجريت الدراسة الحالية لقياس الخلايا اللمفاوية والحبيبية في مرضى متلازمة تكيس المبايض باستخدام جهاز العد، قياس CD marker (CD56, CD8, CD4) في مرضى متلازمة تكيس المبايض باستخدام جهاز الاليزا، قياس مستويات الهرمونات (الهرمون اللوتيني، الهرمون المحفز للجريبة، الهرمون الذكري) في مرضى متلازمة تكيس المبايض باستخدام جهاز الـ Minividas.

أظهرت نتائج الدراسة الحالية يوجد فرق معنوي بين مرضى تكيس المبايض والأصحاء في عدد الخلايا اللمفاوية والحبيبية في الفئات العمرية الأربع. أظهرت النتائج يوجد فرق معنوي واضح بين المرضى والأصحاء في عدد الخلايا اللمفاوية والحبيبية وفقاً الى الوزن في ثلاث فئات وزنية (51– 65 كيلوغرام، 66– 80 كيلوغرام و 81– 95 كيلوغرام). بينما في كلا الفئتين الوزنية (96– 110 كيلوغرام، 111– 125 كيلوغرام) لا توجد فروقات معنوية بين المرضى والأصحاء. كشفت النتائج يوجد فرق معنوي بين المرضى والأصحاء في عدد الخلايا اللمفاوية والحبيبية في كل من (مجموعة ذات نظام غذائي مختلط، مجموعة لحوم حمراء، ومجموعة نباتية ومجموعة لحوم بيضاء). وأظهرت النتائج زيادة واضحة في Cluster of differentiation 8، زيادة في Cluster of differentiation 4 و Cluster of differentiation 56 في المرضى الذين يعانون من متلازمة تكيس المبايض مقارنة مع مجموعة السيطرة. اظهرت النتائج يوجد فرق معنوي واضح بين المرضى والاصحاء في كل من (CD4, CD8) بين (51– 65 كيلوغرام، 66– 80 كيلوغرام و81– 95 كيلوغرام) بينما (CD56) لا يملك فرق معنوي بين المرضى والأصحاء في ثلاث فئات وزنية (51– 65 كيلوغرام، 66– 80 كيلوغرام و81– 95 كيلوغرام). بينما في كلا الفئتين الوزنية (96– 110 كيلوغرام، 111– 125 كيلوغرام) لا توجد فروقات معنوية بين المرضى والأصحاء. وأيضا أظهرت النتائج يوجد فرق معنوي بين المرضى والأصحاء في كل من (CD4, CD8) في المجموعات الغذائية الثلاثة الأولى، أما مجموعة لحوم بيضاء يوجد فرق معنوي بين المرضى والأصحاء في (CD4) بينما (CD8) لا يوجد فرق معنوي بين المرضى والأصحاء، بينما (CD56) لم تظهر النتائج أي فروقات معنوية بين المرضى والأصحاء في كل المجموعات الغذائية المدروسة.