Study of Some Biochemical Parameters and Fatty Acids Composition in Blood Serum of Women with Polycystic Ovary Syndrome

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الخلاصة

صممت هذه الدراسة لمقارنة مستويات بعض المتغيرات الكيموحياتية و المكونات الدهنية وتركيب أحماضها الدهنية في مصل الدم النساء المصابات بتكيس المبايض المتعدد . تضمنت الدراسة (25) عينة من النساء اللاتي تم تشخيص الحالة ل ديهن باستخدام جهاز الامواج فوق الصوتية وتم جمع العينات من مستشفى البتول التعليمي في مدينة الموصل، تتراوح أعمار المرضى مابين (٢٥-٤٠) سنة، ومقارنتها مع (٢٥) عينة سيطرة. وتم قياس عدد من المتغيرات الكيموحياتية في مصل الدم ، إضافة الى تحليل الأحماض الدهنية وتقدي ر نسبتها المئوية في المكونات الدهنية لمصل الدم (استر الكوليستيرول، الدهون الفوسفاتية والكليسيرايد الثلاثي) وذلك باستخدام تقنية كروماتوكرافيا الطبقة الرقيقة بعد ذلك تم إعادة أسترة الأحماض الدهنية وقياس النسبة المئوية لها باستخدام جهاز كروماتوكرافيا الغاز الشعري. أشارت النتائج إلى وجود اختلاف كبير في المكونات الدهنية وتركيب أحماضها الدهنية في مصل الدم المرضى مقارنة مع مجموعة السيطرة.

ABSTRACT

This study was designed to compare the level of some biochemical parameters and lipid fractions and percentage of fatty acids in serum of women with Polycystic ovary syndrome (PCOS), the study include (25)

patients (females) who were diagnosed by ultrasonography, the sample collection is from Al-Bitol teaching hospital in Mosul city, the age is between (25-40) year and compared with (25) normal woman with same age were collected as control and measurement of a number of biochemical parameters in serum,as well as analysis and measurement of percentage of fatty acids in the fatty component of serum (cholesterol ester, phospholipids and triglyceride) by applying thin layer chromatography (TLC) and then re-esterfication fatty acids and measurement percentage of fatty acids applying capillary gas chromatography (CGC). the result of this study show that there is a significant differences in the level of studied biochemical parameter and fatty acids percentage in patients compared with the control group.

INTRODUCTION

The polycystic ovary syndrome (PCOS) is considered as a common disease as it affect is about 30% of female all over the world (1), in which there is irregularity in menstrual cycle and it is lead to delay in pregnancy but it not cause infertility (2). The polycystic ovary syndrome is characterized by presence of small size follicles inside the ovary and specially under the external wall of ovary (3). the polycystic ovary syndrome is due to hormonal disturbance such as (LH, Estrogen and Dopamine) hormones (4) which lead to menstruation disturb and ovulation disturbance is usually accompany by hypertension, weight gain and hirsute in some areas of body specially the chin and chest (5). The polycystic ovary syndrome is probably a mixed group of related conditions, in it is full from there is hirsutism, amenorrhoea, infertility and ovarian abnormalities in the from of follicular cysts and a thickened capsule preventing ovulation(6), the condition may be discovered during investigations for infertility, breast development is usually normal but endometrial proliferation varies from the unstimulated state to hyperplasia(7).

Materials and Methods

1. Samples collection:-

In this study the blood samples were collected from patients after fasting period for (10-12) hours and (5)ml of blood from each subject was collected and serum was separated from it and then divided in to two parts: 1st part measurement of the following parameters glucose, total cholesterol(TC), high density lipoprotein cholesterol(HDL-C), triglyceride(TG), low density lipoprotein cholesterol(LDL-C) by enzymatic methods using kites(8,9), very low lipoprotein cholesterol(VLDL-C) was measurement theoretical(10), and phospholipids(PL) by colorimetric method (11). the 2nd part was stored at (-18)°c until measurement of fatty acids.

2. Extraction and Separation of lipids from serum:

Serum samples were treated with methanol and chloroform to extract lipids(10), lipids extract was separated into three parts cholesterol

ester (CE), triglyceride(TG), phospholipids(PL) using thin layer chromatography (TLC).(11)

3. Transmethylation of fatty acids:

In this study analysis and esterfication of fatty acids by using tri-floro boron (BF_3) in Methanol(16%)(12).

4. Measurmeant of percentage of fatty acids:

Measurement of fatty acids in the three lipid fractions was performed by Capillary Gas Chromatography (CGC) Shimadzo 2010, column type TR-WAX, and length 30m, in industry center (Syria).

5. Statistical analysis:

Results were analyzed statistically for biochemical parameters and the percentage of fatty acids using ***t***-test, $P<0.05$ was considered statistically significant (13).

Results and Dissociation

1- Serum Glucose:

The results showed that a significant increase in serum glucose in woman with Polycystic Ovary Syndrome(PCOS) compared with that control group as indicated in table (1) this increase may be due to insulin resistance which leads to increase serum glucose (14) or due to insulin metabolism defect (15),This result is agreement with other studies(16,17).

2- Lipid fractions:

The results showed that a significant increase in total cholesterol (TC) in woman with (PCOS) compared with that of control group as indicated in table (1) this increase may be due to increase in (TC) synthesis as a result of insulin resistance in woman with (PCOS) (18).and the results showed that a significant decrease in (HDL-C) in patients comparison with control group as show in table (1) the cause of that may be due to close relationship to the elevated activity of plasma CETP (cholesterol ester transfer protein) which promotes the lipoprotein cholesterol of HDL to be transfered to other lipoprotein in patients (19). on the other hand, the results showed that there is significant increase in (LDL-C) may be due to defect in hepatic receptor (Apo B100) which plays an important role in increasing (LDL-C) through decreasing transport of (LDL-C) to hepatic tissue(20). But the results of (TG) and (VLDL-C) in patients showed unsignificant results compared with control group as show in table (1), the same results were obtained in other new studies(21,22,23). whereas the results showed that a significant increase in (PL) this increases may be due to action of hepatic lipases which lead to abnormality metabolism of lipids specially phospholipids (24) and may cause of smoking which is play import role of increase (PL) in serum(25).

Table(1): Serum Biochemical Parameters from PCOS and control group

Biochemical Parameters mmol/l	PCOS 25	Control 25	P value
Glucose	6.86±1.65	4.35±0.23	<0.001
TC	6.83±1.30	4.67± 0.83	<0.001
HDL-C	0.87±0.20	1.41±0.21	<0.001
LDL-C	6.12 ±0.51	4.76±0.11	<0.001
TG	2.32±0.47	1.92±0.86	0.1
VLDL-C	1.04 ±0.08	0.86±0.03	0.12
PL	179± 11.54	165±9.87	<0.05

Values:Mean ± SD

3- Percentage of fatty acids:

The percentage of fatty acids was measured by using (CGC) through comparison of results with standard sample composed of (12) fatty acids, as indicated in fig(1): from the result analysis of standard sample of fatty acids and table (2)demonstrated a retention time (Rt) of these standard fatty acids.

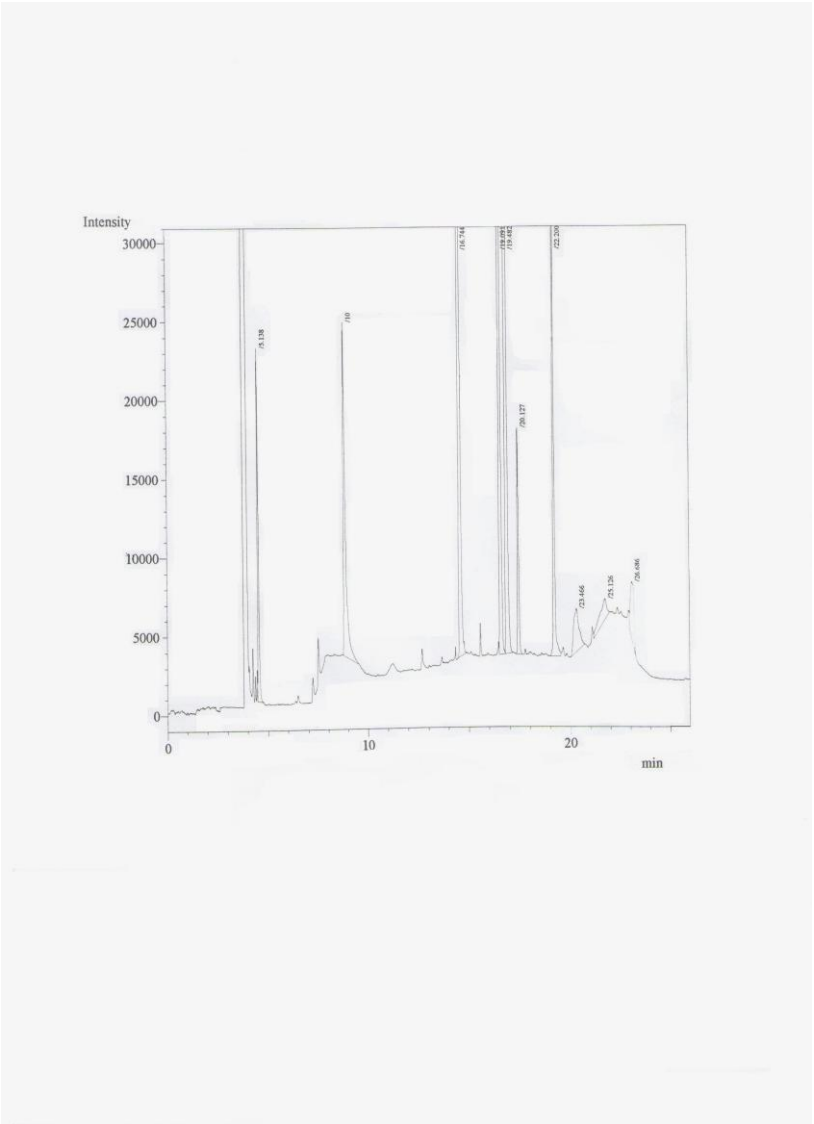

Figure (1):The CGC chart of (12) standard fatty acids

Table (2): standard fatty acids

Standard fatty acids	Symbol	Retention time(min)
Capric acid	C10:0	4.900
Lauric acid	C12:0	5.138
Myristic acid	C14:0	8.500
Palmitic acid	C16:0	10.08
Palmitoleic acid	C16:1	16.74
Stearic acid	C18:0	19.09
Oleic acid	C18:1	19.48
Linoleic acid	C18:2	20.12
Linolenic acid	C18:3	22.20
Arachidonic acid	C20:4	23.46
Eicosapentaenoic acid	C20:5	25.12
Docosahexaenoic acid	C22:6	26.68

3.1- Percentage of fatty acids in (CE) part: Fig (2)

The results showed that a significant increases in percentage of total saturated fatty acids (SFA) in woman with (PCOS) in comparison with control group, as show in table(3), this increasing may be due to abnormality in metabolism of fatty acids in patients(26). also a significant decrease in percentage of total monounsaturated fatty acids (MUFA) and a significant increase in percentage of total polyunsaturated fatty acids (PUFA) in this parts, this increasing may be due to insulin resistance in (PCOS) patients which leads to a big defect in enzymes action which leading to defect in percentage of unsaturated fatty acids (27).

3.2- Percentage of fatty acids in (TG) part: Fig (3)

The results showed that a significant increase in percentage of total (SFA) and total (MUFA), on the other hand a significant decrease in percentage of total (PUFA) in PCOS patients in compared with that of control group, as shown in table (3), this may be due to some type of food(butter fat and hydrogenate vegetable oils) which leads to increase the risk factor of PCOS disease such as trans-fatty acid (28), or may be due to transport (Acetyl-CoA) from different metabolism pathway to pathway causes anabolism of fatty acids (29).

3.3- Percentage of fatty acids in (PL) part: Fig (4)

The results showed that a significant decrease in percentage of total (SFA),on the other hand a significant increase in percentage of total (MUFA) and (PUFA) in PCOS patients in comparison with control group,as show in table (3), this decreasing or increasing may be due to defect in action of desaturation enzyme (Δ9) desaturase and elongation enzymes (Δ6), (Δ5) in PCOS patients (30).

Table(3): Percentage of fatty acids composition of CE,PL,TG in serum PCOS woman and control group

Fatty acid	CE		PL		TG	
	control	PCOS	control	PCOS	control	PCOS
n	10	5	10	5	10	5
SFA						
10:0	1.0±0.13	0.70±0.05	0.09±0.01	0.69±0.08	0.10±0.04	0.3±0.10
12:0	1.3±0.23	1.62±0.22	1.5±0.31	1.75±0.09	2.00±0.30	6.0±0.15*
14:0	0.56±0.10	1.36±0.30	0.38±0.1	2.95±0.18*	4.0±0.65	5.24±0.38
16:0	6.00±1.52	3.80±1.21*	10.25±2.8	2.43±0.89*	25.0±2.60	24.0±3.34
18:0	3.00±0.47	10.52±2.21*	10.01±1.3	11.75±1.87	5.25±1.24	7.25±0.9*
Total	11.86±2.45	18.00±3.99*	22.23±4.5	19.57±3.11*	36.35±4.83	42.8±4.9*
MUFA						
16:1	1.70±1.20	2.23±0.87	2.30±0.7	5.6±0.87*	3.50±0.35	7.28±1.2*
18:1	18.0±2.54	15.0±2.65*	8.30±1.44	9.26±2.11	20.24±1.24	18.9±1.8*
Total	19.70±3.74	17.23±3.52*	10.6±2.14	14.86±2.98*	23.74±1.59	26.18±3.*
PUFA						
18:2 n-6	20.0±2.43	22.0±3.32*	18.78±3.2	17.65±1.65	18.26±2.77	17.12±1.9
18:3 n-3	2.30±0.44	4.50±0.76*	2.10±0.98	5.74±2.43*	2.85±0.67	1.80±.55
20:4 n-6	6.80±1.77	10.23±1.83*	10.24±3.2	14.28±2.29*	4.85±0.55	3.24±.76
20:5 n-3	1.56±0.5	4.00±0.88*	2.85±0.34	5.56±1.20*	2.90±0.54	2.68±.21
22:6 n-3	2.38±0.91	5.00±0.67*	2.3±0.50	4.5±1.54*	6.65±0.88	2.56±1.3*
Total	33.04±6.0	45.73±7.46*	36.27±8.2	47.73±9.11*	35.51±5.41	27.4±4.7*
n-3	6.24±1.85	13.50±2.31*	7.25±1.82	15.8±5.17*	10.40±2.09	10.04±2.1
n-6	26.8±4.20	32.23±5.15*	29.0±6.40	31.93±3.94*	20.11±3.32	25.36±2.*

Values: Mean ± SD ***: P value < 0.05**

C:\GCsolution\Data\Project1\faty1.gcd

CE

Sample Information

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Sample Name : oil
Sample ID : fat_106
Sample Type : Unknown
Data Name : C:\GCsolution\Data\Project1\faty1.gcd
Method Name : C:\GCsolution\Data\Project1\OIl.gcm

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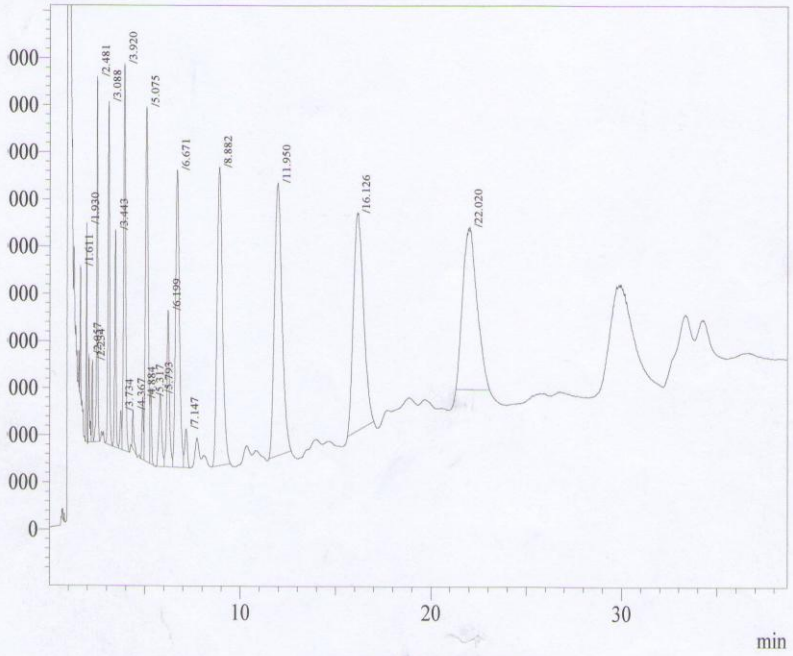

Peak Table - Channel 1

Peak#	Ret.Time	Area	Area%	Height	Mark	Cmpd Name
1	1.611	37207	0.6992	12482		
2	1.930	62052	1.1661	20804		
3	2.057	36797	0.6915	8943		
4	2.234	29610	0.5564	8271	V	
5	2.481	157558	2.9608	36438	V	
6	3.088	202604	3.8073	35616		
7	3.443	114076	2.1437	22738		
8	3.734	23726	0.4459	3968	V	
9	3.920	300295	5.6431	40542	V	
10	4.367	21815	0.4099	3875		
11	4.884	35951	0.6756	6587		
12	5.075	364993	6.8589	37427	V	
13	5.317	44571	0.8376	7685	V	
14	5.793	81605	1.5335	7727		
15	6.199	158346	2.9756	16421	V	
16	6.671	443795	8.3397	31390	V	
17	7.147	39175	0.7362	4085	V	
18	8.882	633036	11.8959	31533		
19	11.950	797605	14.9884	28748		
20	16.126	848759	15.9497	23036		
21	22.020	887892	16.6851	17140		
Total		5321466	100.0000	405455		

Figure (2): The CGC chart of fatty acids in CE part

C:\GCsolution\Data\Project1\faty13.gcd

TG

Sample Information

Analysis Date: 06/05/2009
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Sample ID : fat_106
Sample Type : Unknown
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Method Name : C:\GCsolution\Data\Project1\OIl.gcm

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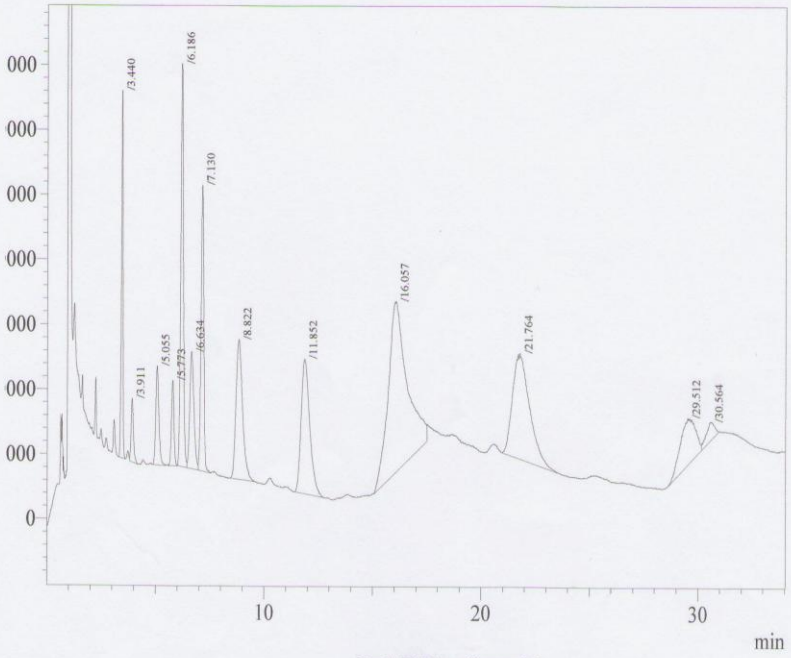

Peak Table - Channel 1

Peak#	Ret.Time	Area	Area%	Height	Mark	Cmpd Name
1	3.440	138970	4.8680	27900		
2	3.911	35934	1.2587	4861	V	
3	5.055	76019	2.6629	7590		
4	5.773	52546	1.8406	6551		
5	6.186	262948	9.2108	30687		
6	6.634	120378	4.2167	8978	V	
7	7.130	200367	7.0186	21885	V	
8	8.822	226004	7.9167	10768		
9	11.852	286232	10.0263	10349		
10	16.057	794471	27.8293	13129		
11	21.764	447599	15.6789	8102		
12	29.512	175276	6.1397	3599		
13	30.564	38050	1.3328	1412	V	
Total		2854795	100.0000	155813		

Figure (3): The CGC chart of fatty acids in TG part

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Sample Information

Analysis Date: 05/05/2009
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Sample ID : fat_106
Sample Type : Unknown
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Method Name : C:\GCsolution\Data\Project1\OIl.gcm

PL

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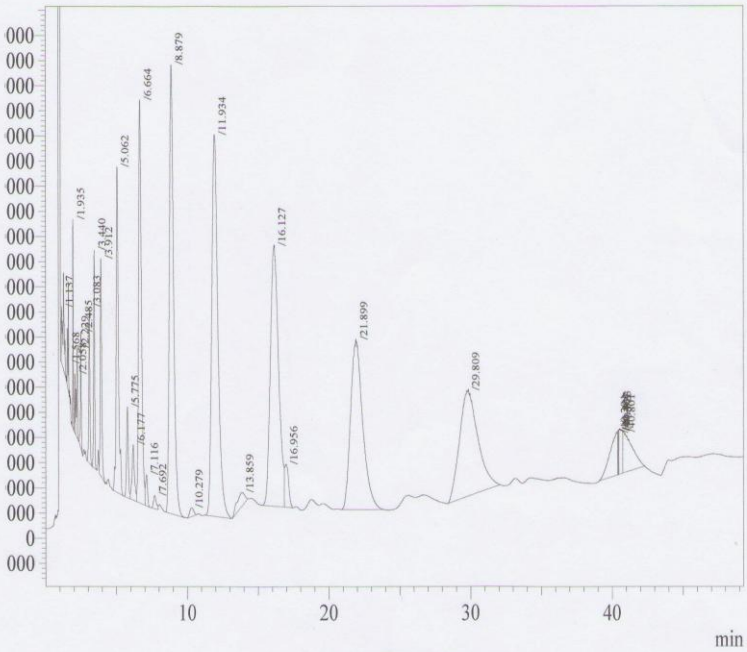

Peak Table - Channel 1

Peak#	Ret.Time	Area	Area%	Height	Mark	Cmpd Name
1	1.137	147495	1.0037	9906		
2	1.568	71674	0.4877	6211		
3	1.935	96998	0.6600	33527		
4	2.058	38943	0.2650	11265		
5	2.229	83581	0.5687	17330	V	
6	2.485	87289	0.5940	21088		
7	3.083	172695	1.1751	29994		
8	3.440	204114	1.3889	42524		
9	3.912	306717	2.0871	42962	V	
10	5.062	704324	4.7927	64534		
11	5.775	153259	1.0429	17957		
12	6.177	130191	0.8859	10967	V	
13	6.664	1124070	7.6490	79782	V	
14	7.116	59063	0.4019	6201	V	
15	7.692	36608	0.2491	2631		
16	8.879	1822237	12.3998	89092		
17	10.279	33471	0.2278	1759		
18	11.934	2275402	15.4834	75789		
19	13.859	108862	0.7408	2719		
20	16.127	2101211	14.2981	51833		
21	16.956	161897	1.1017	8518	V	
22	21.899	2003802	13.6353	33695		
23	29.809	1897273	12.9104	20991		
24	40.301	318468	2.1671	8415		
25	40.401	25768	0.1753	8589	V	

Figure (4): The CGC chart of fatty acids in PL part

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