Relationship of fatty acid composition and some biochemical parameters in women with ovarian cancer

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Abstract

The study was designed to estimate the effect of ovarian cancer disease on the serum level of fatty acids (with its percentage) and some biochemical parameters in the serum of females with ovarian cancer.

The present study dealt with the determination of the fatty acid composition of serum cholesteryl ester CE and phospholipids PL and measured some of biochemical parameters included (high density lipoprotein cholesterol HDL-c, total cholesterol TC, glutathione GSH, malondialdehyde MDA and uric acid U.A. in the serum of healthy females and females with ovarian cancer.

The study includes 30 women with ovarian cancer from hospital of oncology and nuclear medicine in Mosul also 50 apparently healthy females included in this study, the measurement of percentage of fatty acids, in lipids extraction from serum cholesterly ester and phospholipids by thin layer chromatography TLC, and measurement of percentage of fatty acid by capillary gas chromatograph CGC has been done.

The results showed a significant increase p≤0.001 in n6-polyunsaturated fatty acids, they also exhibited as significant decrease in n3 polyunsaturated fatty acid and level of total cholesterol, HDL-c, GSH, on the other side the result showed a significant increased in MDA and uric acid level in p≤0.001.

It was concluded that the levels and the type of the polyunsaturated fatty acid might have a correlation with ovarian cancer on the biochemical parameters comparison with control group.

INTRODUCTION

Ovarian cancer is an important cause of morbidity and mortality among women. Over the past 40 years, the rate of ovarian cancer mortality has increased among women 65 years of age and older. The incidence of ovarian cancer increases with age, from 1.5% annually among women 20-30 years of age to 49% in women 60-69 years of age (1). The etiology of ovarian cancer is not very clear, some hypothesis about the etiology of...
epithelial ovarian tumors have been advanced (2). The incessant ovulation hypothesis suggests that proliferation and repair of the surface epithelial cells following ovulation may increase the chance of an abnormal repair process, leading to malignant cell and subsequent development of ovarian cancer. Under this hypothesis, any factor that inhibits or prevents ovulation would reduce the risk of ovarian cancer (3).

Risk factors tent to be related to those affecting hormonal and reproductive events. Putative mechanisms for ovarian cancer related to hormonal factors are excessive levels of circulating gonadotropins associated with lower parity and decreased number of ovulatory cycles from higher parity resulting in reduced mitotic events in the ovary (4).

Risk factors include personal or family history of (breast cancer or ovarian), cigarette smoking, obesity, increasing age, use of high dose estrogens for long periods (2) and potential dietary. Some studies have suggested that saturated fat consumption may result in an increased risk of epithelial ovarian cancer (5). The unsaturated fatty acid were inversely associated with the risk of ovarian cancer risk (6).

Fats are broadly divided into saturated and unsaturated fatty acids with polyunsaturated, category, there are two families of essential fatty acids for health (n3,n6) the body needs them but can not manufacture them. In addition to their contribution to meeting energy needs, intake of dietary fat must be sufficient to meet requirements for EFAs (7). Excessive dietary fat intake has been linked to an increased risk of obesity, coronary heart disease and cancer (8,9). The role of causing cancer preventing and treatment has been the subject of extensive research. The n6 fatty acids apparently signal asset of genes to spur the tumors growth (9). Dietary n3 polyunsaturated fatty acids have been found to reduce pathologic cell growth and some researches show the n6 fatty acids have effects on lowering lipid levels in serum and has shown that, in cooperation with n3 fatty acids, they directly or in directly modulate various functions at the cellular level, such as gene expression (10). Lipid peroxidation is biological pathway concerning peroxiation of cell membrane phospholipids and polyunsaturated fatty acids (PUFA) by reactive free...
oxygen radicals. Aldehydeic products such as malondialdehyde (MDA) and hydroxyl nonenal are produced consequently (11). Free radicals (FR) cause oxidative damage to nuclear DNA and consequently somatic mutations such as base changes, deletion and chromosomal strand breaks are developed (12,13).

**Aim of study:**

The study was designed to estimate the effect of ovarian cancer disease on the percentage and level of fatty acids and level of some biochemical parameters in serum blood for females with ovarian cancer.

**MATERIAL AND METHODS**

**Subjects and blood sampling**

The study was conducted on free living subjects and was not strictly controlled for nutrient and energy intake. The subjects consisted of 80 females aged 40-55 years, 50 healthy (reference) and 30 with ovarian cancer, (IRRESPECTIVE OF ITS STAGE), they were visitors to oncology and nuclear medicine hospital in Mosul in period from 2007 through October 2009 province to undergo chemotherapy, blood samples were drawn at the approval of hospital management, from all females (who showed no objection) in to plastic tubes and the serum was separated 2h after venipuncture by centrifugation.

Blood serum samples from fasting patients divided in to two parts for lipid analysis and measured biochemical parameters included (High density lipoproteins (HDL), Total cholesterol (TC), Uric acid (U.A), Glutathione (GSH), Malondialdehyde (MDA).

**LIPID ANALYSIS**

Two of the main lipid cheese in this study CE and PL were separated by thin Layer chromatography (Merck silica gel G25nm) with hexane : diethyl ether : formic acid (80:20:2 by volume) After drying, the plates were sprayed with 1% alcoholic solution of 2,7-dichorofluoresine, a nondestructive reagent (7), to visualize each band under UV-light. The PL, CE spots were scraped in to separate vials and their fatty acid were converted to fatty acid-methyl esters (FA-Mes).

Via reaction with 14% (by weight) BF3/methanol at 100° c for 1 hours (17) The proportional composition % of FA-Mes was determined by high resolution capillary gas chromatography (SHIMADZU CORPORATION 2010, Japan) with 30
meter we fused silica column (TR-WAX) in Syria.

The initial temperature was 178°C and the retention time is 30 min and N2 is a carrier gas. A suitable design for this purpose was made as show in fig (1).

The apparatus capillary gas chromatograph shimadzu 2010 Which is using capillary column (TR-Wax) the identify of in dividable fatty acids peaks was ascertained by comparing (18).

A-DETERMINATION THE LEVEL OF BIOCHMICAL PARAMETERS

The sand part of serum can be measurement (total cholesterol, uric acid, high density lipoprotein cholesterol were determined by enzymatic method (19), using kits manufactured by syrbio (Syria) for uric acid, and bicon (Germany) for total cholesterol and HDL-c.

LIPID PEROXIDES

Lipid peroxides was estimated by thiobarbituric acid (T.B.A) reactivity MDA reacts with T.B.A to colored complex that has maximum absorbance at 532 nm using yagi method (20). the reduced glutathione level was determined using the method of beutler and Kelly this method is passed on the development of a relatively stable yellow colour when 5,5 dithio bis-2, nitro benzoic acid (DTNB) is added to sulphhydr compounds the yellow colour developed was measured at 412 nm (21).

STATISTICAL ANALYSIS

for comparison of means between two groups (fatty acids composition of serum CE and PL and biochemical parameters (TC, U.A, HDL-c, GSH, MDA) of healthy females and females with ovarian cancer), using spss soft ware version 10.0 All the data were expressed as mean±standard deviation using T-Test. A value of p≤0.05 indicates statistical significance (22).

RESULTS

The PL and CE fatty acids composition of reference females of females with ovarian cancer show in tables (1, 2). saturated fatty acids exhibited no significal difference between the two groups for PL but the exhibited a significant increase in health group for CE. In general seems the results of saturated fatty acids higher in fameless with ovarian cancer for reference females.
B – The second part of serum for measured biochemical parameters are (HDL-c total cholesterol, MDA, GSH, uric acid).

1-HDL-c

Based on the data presented in this study it can be concluded that females with ovarian cancer exhibited a significant decreased in HDL-c (P≤0.001) was (30.140±3.99) mg/dl were compared with healthy females were (39.215±1.9)mg/dl.

2- Total cholesterol

The females with ovarian cancer were a significant decrease in total cholesterol (P≤0.001)were (129.90±8.344)mg/dl were compared with the healthy females were (198.79±4.466)mg/dl.

3-MDA

In this study we shows the level of lipid peroxidation as assessed by MDA level was significantly higher in ovarian cancer females (4.080±0.752)µmol/L as compared with healthy women was (2.231±0.319)µmol/L.

4-GSH

The level of GSH which the non enzymatic antioxidant show significantly lower in ovarian cancer patients (2.682±1.197) µmol/L as compared with normal patient (8.699±0.263) µmol/L.

5-Uric acid

Based on the data in this study showed a significant increased in uric acid level in ovarian cancer females (7.919±1.413)mg/dl are compared with normal females are (5.450±0.568) mg/dl.

Dissection

Diet could contribute to the etiology of ovarian cancer through modulation of the, endogenous hormonal milieu or through antioxidant and anticarcinogenic mechanisms, this results agree with some studies have suggested that high intake fats or a rich food with saturated fatty acids may be increase the risk of ovarian cancer(23,24).

The DNA damage response acts as a mechanism to prevent cancer development. the saturated Fatty acids(SFAS) play a negative role in DNA damage response palmiticacid, as well as stearic acids and nristic acid, compromised the induction of P21 and bax expression in response to double stranded breaks and DNA, While inhibition or knockdown of FASA enhanced these cellular events. SFAS appeared to regulate P21 and bax expression via Atr-P53 dependent
and independent pathways (25). In the other wise monounsaturated fatty acids showed significant increase in females with ovarian cancer for CE and to certain extent for PL, we can see increase oleic acids while the palmitic acid significant decrease in females with ovarian cancer, as regards n6 polyunsaturated fatty acids (PUFAS), the reference female showed significant increase in C18:2 n6 (linoleic acid) and C20:4 n6 (arachidonic acid) for both PL and CE and there was significant difference in the level of C18: n3 linolenic acid and C20:5 n3 in the case of n3 PUFAS, tables 1, 2 show a significant decrease in their in females with ovarian cancer.

One of the principal mechanisms by which MUFA could exert a beneficial effect on longevity membranes to lipid peroxidation a destructive process that also generates many mutagenic, carcinogenic, and DNA-modifying short chain organic compounds. That sensitivity increase exponentially with the number of double bounds per fatty acid molecule MUFA should thus be ideal as means of avoiding the negative effects of both saturated and unsaturated fatty acid PUFA (26), the n3 and n6 families are necessary for proper growth and body function they can not be synthesized by humans and must be obtained from the diet (27) they are several putative mechanisms where by long –chain n3 fatty acids may modulate the carcinogenic process. the mechanisms include 1-suppression of arachidonic acid derived eicosanoid biosynthesis, which results in altered immune response to cancer cells and modulation of inflammation, cell proliferation, apoptosis, metastasis, and angiogenesis 2- influences on transcription factor activity, gene expression, and signal transduction, which leads to changes in metabolism, cell growth, and differentiation 3-alteration of estrogen metabolism, which leads to reduced estrogen stimulated cell growth 4- increased or decreased production of free radicals and reaction oxygen species and 5-mechanisms involving insulin sensitivity and membrane fluidity on basis of these multiple mechanisms n3 PUFA may have an important on carcinogenesis (28, 29).

The results in the present study, agrees with that reported in other studies which have been shown dietary polyunsaturated n3 fatty acids consistently to inhibit the proliferation of breast and prostate and other hormone dependent cancers (endometrium and ovary), for example
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enhanced metabolism of estradiol to inactive catechol estrogen in the case of breast cancer and reduction in circulating testosterone concentration in the case of prostate cancer(30) On the other side show the n6 fatty acids necessary for tumor genesis enhancement, the mechanisms remain unclear, but some studies have shown that n6 PUFAS are required in the case of mammary tumor. Tumor yields increase with the addition of linoleic acid up to a threshold of about 4-5% of total calories(31) Studies animal models show in that a high intake of n6 PUFAS, stimulates several stages in the development of mammary and colon cancer, from an increase in oxidative DNA damage to effects on cell proliferation, free estrogen levels and hormonal catabolism(32). It is cleared that lipid free radicals are one of the main reasons that cause cancer and they are produced from unsaturated fatty acid peroxidation (33). the n6 PUFA are more prone to preoxidation than n3(34), so a high level of n6 PUFA is a negative sign which may cause cancer. The figure 1, 2 show the levels of n3/n6 fatty acids for group of females with ovarian cancer is lower than that for the healthy group, the balance between n3 and n6 fatty acids in the diet is important because of their competitive nature and their essential and different biological roles. In conclusion the level and type of PUFA may have a correlation with cancer. A high level of n6 PUFAs of n3 PUFA may be associated with an increased risk of ovarian cancer.

High density lipoprotein (HDL-c) is one of the major carriers of cholesterol in the blood, it attract particular attention because; in contrast with other lipoproteins, as many physiological functions of HDL influence the cardiovascular system in ways unless HDL is modified pathologically, the functions of HDL that have recently attracted attention include anti-inflammatory and antioxidat activities (35).

The ability of lipoproteins such as HDL to support the proliferation of tumor cells has not yet been investigated, however based on their effect on proliferation of normal diploid cells the suspicion that they play an important role in the proliferation of tumor cells and possibly in the neoplastic process in vivo is reasonable (36), further some studies show the HDL-c could play a role in carcinogenesis through its influence on cell cycle entry, via a mitogen activated protein kinase
dependent pathway or regulation of apoptosis(35).

This study found the relationship of total cholesterol and ovarian cancer, this result may be to the cholesterol is a critical component in cell membranes, and in vitro studies including in ovarian epithelial cells, suggest lipids promote tumor growth. Furthermore cholesterol is known precursor in steroid hormone synthesis, which may underscore the potential mechanism by which lipid lowering medications might impact breast, prostate and ovarian malighancies (37), however, research in to this mechanism has revealed that tumor cells need cholesterol for their growth and proliferation, therefore there is an increased uptake of cholesterol from the blood by tumor cell( 38). Free radicals are capable of altering all major classes of biomolecules such as lipids, nucleic acids and proteins, with changes in their structure and function prime targets of free radicals are the polyunsaturated fatty acids in cell membranes and their interaction results in lipid proxidation( 39). moreover, severe oxidative stress in not only know to cause DNA damage and mutations of tumor suppressor genes which are initial events in carcinogenesis (40) but can also play an important role in the promotion of multi step carcinogenesis( 39). Lipid peroxidation play an important role in the control of cell division (41) the end product of lipid peroxidation, malondialdehyde, due to its high cytotoxicity and inhibitory action on protective enzymes is suggest to act as an tumor promoter and a co – carcinogenic agent( 43). The most important antioxidant compounds found in human physiology (42,43). GSH play a key role in the maintenance of cellular thiolreduxd status, because it is conjugated to many xenobioties and is essential for the optimal functioning of numerous enzymes, it is critical for cellular viability, in general and lymphocyte function in particular (44,45).
The antioxidant defense system is divided into enzymatic and the non-enzymatic system includes compounds synthesized by the human organism such as ceruloplasmine, sexual hormones, Co-enzyme Q and uric acid(16).

Abnormal levels of serum uric acid frequently are found in cancer patients. These alterations generally have been attributed to the malignant process itself. Hyperuricemia in cancer patients (such as those with leukemia, lymphoma, or disseminated cancer) is thought to result from the increased nucleic acid turnover in the rapidly proliferating diseased tissue (46).

Uric acid is produced as an end product of purine metabolism through hypoxanthine and xanthine and is excreted by the kidneys. Cytolysis of the tumor cells causes a massive release of purines in the circulation that increases the serum level of uric acid(47).

This result is contrary to the proposed antioxidant effect of serum uric acid against cancer and rather indicate high serum uric acid concentration to be independently associated with outcome possibly reflecting more serious prognostic indication, though not necessarily specific to cancer mortality, however, the presence of elevated serum uric acid suggests the clinical importance of monitoring and intervention on this basis(48).

REFERENCES

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علاقة تركيب الاحماس الدهنية وبعض المتغيرات الكيموية في النساء المصابات بسرطان المبايض

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شيماء زهير جلال الدين
جامعة الموصل / كلية التربية / قسم الكيمياء

الخلاصة

صممت هذه الدراسة للتفتيش على تأثير مرض سرطان المبايض على نسبة الاملاح الدهنية ومستوى بعض المتغيرات الكيميائية في مصل النساء المصابات بسرطان المبايض. تضمنت الدراسة دراسة تركيب الاحماس الدهنية للكولستيروفيل المتاسير والدهون الفوسفاتية وقياس بعض المتغيرات الكيميائية المتضمنة ككوليسترول البروتينات، HDL-c، الكولسترول الكلي، الدهون الفوسفاتية، الاملاح، الببتيد GSH، والكولسترول الحادب. في مصل دم النساء المصابات بسرطان المبايض تضمنت الدراسة عينة من المريضات المصابات واللاتي يواجهن مستشفى الأورام والطب النووي التخصصي في الموصل، و50 عينة من النساء الاصحاب حيث تم قياس نسبة الاحماس الدهنية في المكونات الاستر كولستيروفيل والكوليسترول المتاسير والدهون الفوسفاتية بالإضافة إلى دراسة استاتيوناريا تطبيق تقنية كروماتوغرافيا الغازية التي تستخدم درجة الهواء BPL. اتضح من النتائج أن الزيادة في تركيب الاحماس الدهنية كانت مترتبة على زيادة نسبة الدهون المتاسير وانخفاض نسبة الدهون الزيتية. الكولسترول الكلي، الكولسترول المتاسير، الدهون الفوسفاتية، الاملاح، الببتيد GSH، والكولسترول الحادب. أشارت النتائج إلى ارتفاع معدلات الدهون المتاسير والدهون الزيتية وانخفاض نسبة الدهون الزيتية. مستويات HDL-c، الكولسترول الكلي، الاملاح، الببتيد GSH، والكولسترول الحادب. لذلك يمكن الاستنتاج أن مستوى الاحماس الدهنية متعدد الأغراض المزدوجة ونوعها قد يكون له علاقة باحتمالية الإصابة بسرطان المبايض وتأثير سرطان المبايض على مستويات المتغيرات الكيميائية مقارنة بمجموعة السيطرة.

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### Table 1: serum PL fatty acids composition (w %)

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>(Mean ± SE)</th>
<th>Ovarian cancerwomen</th>
<th>p</th>
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<tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:0</td>
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<td>0.603±3.253</td>
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<td>12:0</td>
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<td></td>
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<tr>
<td>16:1</td>
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<tr>
<td>18:1</td>
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<tr>
<td>Polyunsaturated</td>
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### Table 2: serum CE fatty acids composition (w %)

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<td>Total</td>
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<table>
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<th>18:1</th>
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<td>0.396±54.97</td>
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<td>20:5n3</td>
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<tr>
<td>n6</td>
<td>53.00±1.68</td>
<td>0.396±54.97</td>
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**Figure 1.** Separation of major lipid classes by thin-layer chromatography. A suitable solvent system for the bellow would be hexane-diethyl ether-formic acid (80:20:2:v/v/v).(4)