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Research Article

PHYSIOLOGICAL AND GENETIC STUDIES IN UTERINE FIBROMA OF WOMEN

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Abstract- Background: Uterine leiomyoma (UL), also named as uterine fibroid (UF), is the most common benign gynecologic tumors in reproductive aged women arising from the smooth muscle cells of the endometrium. The exact etiology of the disease is not clearly understood, but working hypothesis suggested that genetic predisposition, prenatal hormone exposure and the effect of hormones, growth factor and xenoestrogen cause fibroid growth. A striking feature of uterine fibroids is their dependency on the ovarian steroids estrogen and progesterone. Oxidative stress has been shown to be a major player in common pro fibrotic gynecologic disorders such as fibroids, endometriosis and postoperative adhesions. The X-ray repair cross -complementing group 1 (XRCC1) gene is an important component of DNA repair and encodes a scaffolding protein that participates in the base excision repair (BER) pathway and number of its single nucleotide polymorphisms (SNPs) have been considered as a modifying risk factor for a variety of cancer types.

Aims and objectives: In this study, we aimed to measuring serum concentrations of prolactin and estradiol hormones, evaluating some oxidative stress markers (e.g., malondialdehyde (MDA), Hydrogen peroxide (H2O2) and nitic oxide (NO)), assaying the activity of some antioxidant enzymes (e.g., superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (Gpx)) and evaluating serum concentration of some biochemical markers (e.g., alanine transaminase (ALT), aspartate transaminase (AST), acid phosphatase (ACP), total protein (TP), and albumin and globulin). Our study is also done to investigate whether XRCC1 Arg194Trp and Arg399Gln polymorphisms are related to uterine leiomyoma disease or not.

Methods: Whole blood DNA was extracted from 85 UF patients and 85 healthy volunteers. Tetra-primer amplification refractory mutation system (ARMS) was performed for the detection of XRCC1 Arg399Gln and Arg194Trp polymorphisms.

Results: Our results investigated that serum concentrations of prolactin and estradiol hormones and oxidative stress markers significantly increased in contrast impaired antioxidant status.

Conclusion: Our study indicated that the Arg/Gln and Gln/Gln genotypes are associated with higher risk of uterine leiomyoma than the Arg/Arg genotype.

Keywords- Fibroid disease, Estradiol¸ Prolactin, PCR, XRCC1.

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Introduction

Uterine leiomyomas (UL), also called uterine fibroids (UF) or myomas, are the most common benign gynecological tumors of women in reproductive age [1]. UL are benign tumors arising from the smooth muscle cells of the myometrium [2]. UL are one of the most common benign tumors occurring in 20% to 40% of women in their reproductive years [3].UL are the most common neoplasm of the female genital tract, and despite their high prevalence, little attention has been paid to the cause and pathogenesis of this disease because it seldom undergoes malignant transformation [4]. This disease disrupts the functions of the uterus, where it has many complications, including (but not limited to) excessive uterine bleeding, anemia, and loss of the fetus. Moreover, it causes preterm labor, obstruction of labor, pelvic discomfort, and urinary incontinence and may mask malignant tumors. By the time they reach 50 years of age, nearly 70% to 80% of women will have had at least one fibroid; severe symptoms develop in 15% to 30% of these women [1, 5]. UL are monoclonal tumors of the smooth muscle cells of the myometrium and consist of large amounts of extracellular matrix (ECM) that contains collagen, fibronectin and proteoglycan [6-7]. Similar to most benign tumors, uncontrolled cell proliferation increases the size and the growth of UL. As it is induced from the meaning of the word “fibroid”, uterine fibroids have the characteristic features of fibrosis. A hallmark of uterine leiomyoma is the excessive deposition of ECM [8].

The exact etiology of the disease is not clearly understood. However, research studies show that genetic predisposition, prenatal hormone exposure and the effect of hormones, growth factor and xenoestrogen contribute to fibroid growth [9]. There are also predisposing factors of this tumor that have been identified, including age (late reproductive years), African-American ethnicity, null parity and obesity [10]. However, the development of fibroids is attributed to genetic, hormonal, and growth factors, especially transforming growth factor beta (TGFb)-related cellular changes [6, 11].A striking feature of UF is their dependency on the ovarian steroids estrogen and progesterone [12]. Several growth factors are elevated in UL and may be the effectors of estrogen and progesterone promotion [10]. Leiomyoma are estrogen-dependent neoplasms [13]. Substantial evidence

indicates an increase in estrogen-binding sites in leiomyoma, and this increase may account for their enhanced sensitivity to estrogen [14-15].UL patients have high level of serum prolactin, with a significant positive correlation with the leiomyoma size and its prolactin production. As the big forms of PRL have a decreased bioactivity, macroprolactin in UL patients may cause hyperprolactinemia. They do not cause clinical symptoms of hyperprolactinemia [16]. Tumor markers are produced in small concentration by normal cells but increase in concentration when produced by tumor cells [17]. Enzymes such as alkaline Phosphatase (ALP), acid Phosphatase (ACP) and Aspartate transaminase (AST) can be used as tumor markers [18]. Serum prolactin and serum total protein (TP) can be used as adjuvant biochemical markers to confirm the diagnosis of uterine fibroids [19]. Moreover, it is shown in [19] that total serum protein level is lower in UL patients than in normal healthy women.

Generally speaking, oxidative stress is defined as an imbalance between the level of free radicals and the antioxidant defense system. It, then, causes irreversible cell damage [20-22]. Oxidative stress has been shown to be a major player in common pro fibrotic gynecologic disorders such as fibroids, endometriosis and postoperative adhesions [23-26]. Evidences support the role of oxidative stress in the development of UL [27]. The X-ray repair cross -complementing group 1 (XRCC1) gene is an important component of DNA repair. It encodes a scaffolding protein, which contributes to the base excision repair (BER) pathway [28, 29]. XRCC1 has been shown to physically interact with DNA polymerase b, poly adenosine diphosphate-ribose polymerases 1 and 2, APE1/APEX1, OGG1, and proliferating cell nuclear antigen. The absence of XRCC1 causes a strong reduction in the levels of its partner ligase III [30-31]. Amongst the studied single nucleotide polymorphisms, the genes Arg194Trp on exon 6 (dbSNP no. rs1799782), Arg280His on exon 9 (dbSNP no. rs25489), and Arg399Gln on exon 10 (dbSNP no. rs25487) are the most interesting studied genes [32]. The literature shows that the DNA repair gene XRCC1 Arg399Gln polymorphism is associated with the risk of many human tumors [33-34].

Materials and Methods

Patients and control

85 women with uterine fibroid (without any other medical illnesses) and 85 healthy participants (women) were included in this study. All UF cases were recruited from the department of obstetrics and gynecology department at the University hospital of Mansoura. Subjects having virus C or B were excluded from the study. Women in the group of control subjects were randomly selected from subjects who were referred to internal medicine clinic for checkup examinations. None of the control subjects had any signs or symptoms suggesting UF. Based on medical history and gynecology questionnaire, all control subjects were free from any other diseases

Sampling

Venous blood samples (5ml) were drawn from each of the patients and healthy women. From these 5ml, (3ml) were transferred immediately to a clean dry plain tube. After removing the needle, the blood was allowed to clot for (10-15) min, at room temperature (RT). Then, the blood has been centrifuged for (10) min, at (3500) rpm to obtain serum for measuring each of E2, PRL concentration, measuring MDA, H2O2 and NO levels, assaying the activity of SOD, CAT, GR and Gpx enzymes, and evaluating some biochemical markers (ALT, AST, ACP, TP, albumin and globulin) concentrations. Another sample of 5ml venous blood from healthy and patient women were collected in EDTA tube for the analysis of XRCC1 Arg194Trp and Arg399Gln polymorphisms.

Hormones assay

Prolactin level was measured by using prolactin ELISA kit according to the method of Uotila *et al*. [35]. However, estradiol level was measured by using enzyme immunoassay (EIA) test kit according to Ratcliffe *et al.* [36].

Biochemical marker determination

ALT and AST were assayed by kinetic methods according to the International Federation of Clinical Chemistry (IFCC) [37], using ALT kit and AST kit purchased from Egyptian Company for Biotechnology (S.A.E). ACP was measured by the method mentioned by Hillmann [38]. Serum total protein was measured using a quantitative colorimetric determination method shown by Gornal *et al*. [44]. Albumin was measured by using modified bromocresol green colorimetric method according to Doumas *et al*. [45].

Determination of antioxidant and oxidative stress markers

Malondialdehyde was determined using Thiobarbituric acid (TBA) test according to the method mentioned by Draper and Hadley [39]. Based on *Green et al*. [40] method, serum nitrite level was estimated by colorimetric assay. Also, superoxide dismutase activity was measured according to the method of Nishikimi et al. [41] using colorimetric kit method from Bio diagnostic, com, Giza, Egypt. CAT activity was assayed by colorimetric method kit from Biodiagnostic com Cat, (No. CA 2517) Giza, Egypt that mentioned by Aebi [42]. Serum glutathione peroxidase and glutathione reductase were evaluated by the method mentioned by Beutler [43].

XRCC1 genotyping

DNA was isolated from the whole blood according to Bio spin whole blood genomic DNA extraction kit bio flux (Japan). Tetra-primer amplification refractory mutation system (ARMS) was performed for the detection of *XRCC1* Arg399Gln and Arg194Trp polymorphisms, as previously described by Salimi *et al*. [46].

Statistics

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) for windows, version 10.0. The mean, standard error (SE), correlation and one-way ANOVA were used to evaluate the significance (*p*-value) between the studied variables of the control subjects and uterine leiomyoma patients. A *p*-value <0.05 was considered statistically significant. The frequency of genotypes and alleles was compared between UL women and healthy controls using the Chi-square or Fisher's exact tests. Student's *t*-test was used for comparison of quantitative variables. A p-value < 0.05 was considered as statistically significant. The odds ratio (OR) with 95% confidence interval (CI) was calculated to study the association between SNPs and UL disease [47].

Results

[Table-1] shows the concentration of prolactin and estradiol for both UL patients and healthy subjects. The results show that the serum concentration of prolactin is significantly increased (p = 0.0003) in UL patients, with mean ± SE (44.92 ± 5.51), corresponding to its concentration in healthy control, with mean ± SE (10.99 ± 7.57). It shows also that there is a significant increase (p = 0.0009) in the concentration of estradiol in UL patient, with mean ± SE (140.26 ± 22.43), corresponding to its concentration in healthy control, means ± SE (16.2 ± 3.12).

Table-1 *Prolactin (PRL) and estradiol (E2) concentrations in the blood serum of healthy control and uterine leiomyoma (UL) patients*

groups / parameter	Healthy controls n = 50	UL Patients n = 85	p-value
PRL (ng/ml)	10.99 ± 7.57	44.92 ± 5.51	0.0003*
Estradiol(E2)pg/ml	16.2 ± 3.12	140.26 ± 22.43	0.0009*

Values are means± SE of 50 healthy control and 85 of UL patients. * Significant difference in comparison with the healthy control at p≤0.05.

[Table-2] shows the statistical analysis for some oxidative stress markers. The table shows that serum concentration of oxidative stress markers MDA, H2O2 and NO is significantly increased in UL patients, with mean ± SE (308.6 ± 16.9, 7.99 ± 0.434 and 12.54 ± 0.47) respectively, corresponding to their concentration in healthy volunteers, with mean ± SE (172.6 ± 9.7, 2.69 ± 0.224 and 3.97 ± 0.292) respectively, at p< 0.0001 level.

In [Table-3], results obtained from the analyses of some antioxidant serum activities for both UL patients and healthy subjects are presented. The table shows that serum activities of antioxidant enzyme SOD, CAT, GR and Gpx decrease significantly in UL patients, with mean ± SE (7.53 ± 0.51, 3.94 ± 0.28, 4.06 ± 0.35 and 3.94 ± 0.23) respectively, compared to their activities in healthy

volunteers, with mean ± SE (14.08 ± 0.52, 6.94 ± 0.34, 12.51 ± 0.64 and 8.32 ± 0.57) respectively, at p< 0.0001 level.

Table-2 *Oxidative stress markers (MAD, H2O2 and NO) concentration in serum of healthy subjects and UL patients*

Groups / Parameter	Healthy Control n = 50	UL Patient n = 50	p-value
MDA(nmol/ml)	172.6 ± 9.7	308.6 ± 16.9	< 0.0001*
H2O2(µM)	2.69 ± 0.224	7.99 ± 0.434	< 0.0001*
NO(µmol/L)	3.97 ± 0.292	12.54 ± 0.47	< 0.0001*

Values are means± SE of 50 healthy control and 50 UL patients. *Significant difference in comparison with the healthy control at p≤0.05

Table-3 *Serum activities of antioxidant GR, SOD, CAT and Gpx enzymes in healthy control and uterine leiomyoma (UL) patients*

Groups / Parameter	Healthy Control n = 50	UL Patient n = 50	p-value
SOD(U/ml)	14.08 ± 0.52	7.53 ± 0.51	< 0.0001*
CAT (U/L)	6.94 ± 0.34	3.94 ± 0.28	< 0.0001*
GR(ΔOD/min)	12.51 ± 0.64	4.06 ± 0.35	< 0.0001*
Gpx (ΔOD/min)	8.32 ± 0.57	3.94 ± 0.23	< 0.0001*

Values are expressed as means ± SE of 50 healthy control and 50 uterine leiomyoma (UL) patients. *Significant difference in comparison with the healthy control at p≤0.05.

Further statistical analysis has been done to study the impact of elevation in serum estradiol levels and its relation to oxidative stress markers, antioxidants and other biochemical markers, where the results are shown in [Table-4]. The results show a significant increase in the concentration of MDA in UL patients with elevated serum estradiol (means ± SE: 371.4 ± 29.2) compared with those with normal serum estradiol levels (means ± SE: 281.65 ± 19.1) at p=0.01, whereas H2O2 and NO concentrations showed non-significant differences between normal and elevated serum E2 levels at P>0.05.

The data also revealed non-significant changes in either of SOD, CAT, GR and Gpx enzyme activities as well as ALT, AST and ACP, enzyme activities and TPr, A and G concentrations in normal serum E2 levels compared with their concentration in elevated serum E2 levels for UL patients at P>0.05 level.

Table-4 *Comparison of serum oxidative stress markers (MDA, H2O2 and NO), antioxidants (SOD, CAT, GR and Gpx) and some biochemical markers (ALT, AST, ACP, TPr, A and G) in normal and elevated serum estradiol levels for uterine leiomyoma (UL) patients.*

	Factor	Normal Estradiol (≤ 160 ng/mL) n = 57 UL	Elevated Estradiol (> 160 ng/mL) n = 28 UL	p-value
Oxidative stress	MDA (nmol/ml)	281.65 ± 19.1	371.4 ± 29.2	0.01*
	H_2O_2(µM)	7.6 ± 0.51	8.9 ± 0.78	0.16
	NO (µ mol/ L)	11.99 ± 0.55	13.8 ± 0.84	0.07
Antioxidants	SOD (U/ml)	7.7 ± 0.6	7.1 ± 0.93	0.58
	CAT (U/L)	4.03 ± 0.34	3.7 ± 0.52	0.61
	GR(ΔOD/min)	3.7±0.4	4.86± 0.262	0.13
	G px(ΔOD/min)	3.86 ± 0.28	4.12 ± 0.43	0.61
Biochemical markers	ALT (U/L)	12.65 ± 0.87	10.95 ± 1.5	0.33
	AST (U/L)	16.56 ± 0.79	14.28 ± 1.38	0.16
	ACP (U/L)	2.88 ± 0.43	2.92 ± 0.76	0.95
	TPr (g/dl)	6.56 ± 0.15	6.8 ± 0.27	0.45
	A (g/dl)	3.8 ± 0.07	3.84 ± 0.12	0.71
	G (g/dl)	2.85 ± 0.18	3.07 ± 0.35	0.58

Values are expressed as means ± SE for 57 UL patients with normal serum estradiol (E2) and 28 UL patients with elevated serum estradiol (E2) levels. *Significant difference at p≤0.05 level.

In summary, in [Table-5] the results indicated that serum estradiol levels positively correlated (p<0.05) with prolactin, MDA and H2O2 whereas no correlation between serum E2 and NO for UL patients. The results also showed non-significant (p > 0.05) correlation between serum levels of estradiol and (SOD, CAT, GR and Gpx,) antioxidants and biochemical markers (ALT, AST, ACP, TPr, A, and G) for UL patients.

Table-5 *The correlation and the corresponding p-value between estradiol (E2) and prolactin (PRL), oxidative stress markers (MDA, H2O2 and NO), antioxidants (SOD, CAT, GR, and Gpx) and some biochemical markers (ALT, AST, ACP, TPr, A and G) for uterine leiomyoma (UL) patients.*

	Factor	Estradiol	
		r-correlation	p-value
	PRL(ng/ml)	0.43	<0.0001*
Oxidative stress	MDA (nmol/ml)	0.3	0.03*
	H2O2(µM)	0.32	0.02*
	NO (µmol/L)	0.1	0.48
Antioxidants	SOD(U/ml)	0.11	0.47
	CAT(U/L)	-0.07	0.63
	GR(ΔOD/ min)	0.01	0.89
	Gpx(ΔOD/ min)	0.21	0.15
Biochemical markers	ALT(U/L)	-0.04	0.72
	AST(U/L)	-0.47	0.67
	ACP(U/L)	-0.057	0.6
	TP(g/dl)	0.037	0.73
	AL(g/dl)	0.1	0.37
	GL(g/dl)	-0.06	0.64

*Significant correlation with estradiol at p≤0.05.

The results shown in [Table-6] show that Arg/Gln genotype appeared with significant (p = 0.004) high frequency (83.5%) compared with Arg/Arg genotype (10.6%) and Trp/Trp appeared with significant (p = 0.014) low frequency (5.09%) compared with Arg/Arg genotype (10.6%) in UL patients.

Women with the Arg/Gln genotype (OR 3.34; 95% CI 1.45–7.7; P, 0.004) and Gln/Gln (OR 0.07; 95% CI 0.0074–0.703; P, 0.014) were found to have an elevated risk of uterine leiomyoma compared with those with the Arg/Arg genotype.

Table-6 *Genotypes frequency of X-ray cross complementing group 1 (Arg194Trp and Arg399Gln) polymorphisms in healthy control and uterine leiomyoma (UL) patients*

Polymorphism	Controls n= 85	Patients n = 85	p-value	OR (95% CI)
Arg194 Trp				
Genotypes: n(%),				
Arg/Arg	22 (25.9)	15 (17.65)	Ref.	
Arg/Trp	60 (70.6)	68 (80)	0.2 ¶	1.66 (0.79 – 3.49)
Trp/Trp	3 (3.5)	2 (2.35)	0.67 ¶¶	0.59 (0.095-3.6)
Alleles: n (%)				
Arg	104 (61.18)	98 (57.65)	Ref.	
Trp	66 (38.82)	72 (42.35)	0.58	1.16 (0.75 – 1.78)
Arg399 Gln				
Genotypes: n (%)				
Arg/Arg	25 (29.4)	9 (10.6)	Ref	
Arg/Gln	59 (69.4)	71 (83.5)	0.004*†	3.34 (1.45 – 7.7)
Gln/Gln	1 (1.2)	5 (5.9)	0.014*‡	0.07 (0.0074-0.703)
Alleles: n (%), expected				
Arg	109 (64.1)	89 (52.35)	Ref.	
Gln	61 (35.9)	81 (47.65)	0.037*	1.63 (1.05 – 2.51)

The data represent (n) number of appearing genotype. *significant value (p<0.05). Statistics: OR = odds ratio, CI = confidence interval. ¶ Arg/Trp versus Arg/Arg. ¶¶ Trp/Trp versus Arg/Arg. † Arg/Gln versus Arg/Arg. ‡ Gln/Gln versus Arg/Arg.

Discussion

Though their public health impact, little is known about leiomyomas' causes. The most important aspect playing a vital role in fibroids' etiology is unclear. The literature shows the advancement of several theories in this concern. Some of

these theories are based on the increase of some hormone levels (e.g., estrogen and progesterone). It may also be referred to the inherent of genetics. Also, it has been attributed to some growth factors and prolactin levels.

In this study, [Table-1] indicates that serum PRL concentration was significantly higher in UL patients than in healthy volunteers. This finding is in agreement with that shown by Baban [16] and Abdulla and Baban [48] who demonstrated that the PRL levels in UL patients were increased. The results are also in accordance with the data recorded by Baban [19] who showed that serum PRL in patients with UL before surgery is higher than its concentration after surgery, and with the results of Mohammd *et al*. [49] who showed a highly significant increase in PRL serum level in UL females ($p<0.000$) when compared with the healthy females. Such results are also compatible with the data obtained by Levy *et al*. [50] who showed a statistically significant association of fibroids and serum PRL.

Leiomyoma has the ability to synthesize PRL, which increased the evidence that mesenchymal cells origin arising near the paramesonephric ducts are able to express prolactin synthesis genome *in-vivo*. It, thus, induces that this potential genome expression is activated in either of smooth and stromal cells during normal cells transformation into leiomyoma cells [51]. Increase concentration of PRL in this study may be due to local production of PRL, whereas leiomyoma has the ability to synthesize PRL. This also may be due to an increase in the concentration of E2, whereas our result exhibited positive correlation between E2 and PRL in uterine leiomyoma patients.

In the present work, the results also illustrated that the concentration of E2, shown in [Table-1], showed highly significant increase in UL patients when compared with healthy control. Such result is in accordance with the previous study obtained by Dapilah [52] who showed that serum E2 levels in UL patients are higher than those of the healthy controls. Also, Moor *et al*. [53] and Mohammd *et al*. [49] showed that the levels of E2 within UL are higher than in normal myometrium, a result with which ours is in agreement.

UL patients showed a higher proliferative index than normal myometrium through the menstrual cycle [54]. The patients who had higher levels of E2 also had earlier menarche than the controls. Likewise, it is observed that nulliparous women have higher E2 levels than parous women [55]. It is further shown that, during the mid-cycle and luteal menstrual phase, E2 levels increase with age (up to 40 years) among nulliparous women but decline with age among parous women [56].

Our results are consistent with the findings obtained by Bernstin *et al*. [55] and Dorgan *et al*. [56] in that most of our patients in this study were nulliparous. The noted increased risks within women with an early menarche and decreased risks within parous women and women of higher parity are consistent with that study. It supports the hypothesis that the response of myometrial to estrogens may play a key role in these benign neoplasms' etiology [57]. It may open new strategy for novel therapeutic agents.

Oxidative stress has been linked to the pathophysiology of more than 100 human diseases, as well as the aging process [58]. In this study, the results in [Table-2] pointed to elevated levels of lipid peroxidation product MDA as well as H2O2 and NO concentrations. The levels of them were assayed as marker of oxidative stress, where the results showed that all examined samples exhibited decreased mean concentrations of MDA, H2O2 and NO in healthy control in comparison with UL patients. These results indicated to increasing the oxidative stress markers in UL patients with significant differences at $p<0.0001$.

These results were comparable with the previous results obtained by Mohammd et al. [49] who found a highly significant increase in MDA concentration in serum of UL females ($p<0.000$) when compared with the healthy females. Also, Pejic *et al*. [59] showed an increase in the concentration of lipid peroxidation products, as marker of oxidative stress, whereas the level of antioxidants decreased in uterine fibroid patients. Moreover, these findings were in agreement with the findings obtained by Dapilah [52] who found significant differences of serum MDA and ascorbic acid between controls and UL patients. These results, also, are consistent with other results shown by Jyoti *et al*. [60], which revealed increased MDA levels in ovarian, cervical and uterine leiomyoma patients compared with normal control.

An increase in serum H2O2 concentration in UL patients is shown in [Table-2] of this study. To the best of our knowledge, this is the first study to measure serum concentration of H2O2 in UL patients. However, the increased concentration of H2O2 in the sera of UL patients in this study may be attributed to the increased rate of oxidative stress as well as the decreased of both CAT and Gpx enzyme activities depending on the fact that CAT and Gpx transformed H2O2 into water and oxygen.

Additionally, our results in [Table-2] show a significant increase in the concentration of serum NO in UL patients. Similarly, Mohammd *et al*. [49] showed a highly significant elevation in peroxynitrite in the serum of UL females compared with healthy ones and suggested that the increase in the levels of the free radicals; NO and super oxide (O2-) producing ONOO- may be occur in UL.

Usually, total nitrates/nitrites levels are used as markers of the activity of nitric oxide synthase and the production of nitric oxide radicals [61]. However, Santulli *et al*. [27] failed to show any difference in serum total nitrates/nitrites in sera of women with and without UL and presented that their results suggested that NO plays a minor role in uterine leiomyoma. Generally, oxidative stress has been shown to be a major player in common profibrotic gynecologic disorders such as fibroids, endometriosis and postoperative adhesions ([23-26]). In agreement with these prior studies, our findings suggested that oxidative stress could play a major role in the pathogenesis of fibroids.

The results of this research in [Table-3] also indicated a decrease in serum of SOD, CAT, Gpx and GR enzyme activities in UL patients compared to their activities in healthy control. Compatible results were also recorded by Chiou and Hu [62] who detected that the activity of SOD in plasma and erythrocytes of patients with cervicitis and UL was lower compared to that of healthy women, and are also in agreement with the data reported by Fletcher *et al*. [63] indicating that fibroid cells have significantly lower antioxidant enzyme activity SOD and CAT mRNA than normal myometrial cells ($p<0.05$). Since uterine fibroids are characterized by an impaired antioxidant cellular enzymatic system, though reduced plasma CAT and SOD expression and activity were expected detected. Accordingly, a similar decrease in plasma SOD activity was also reported by many previous studies in patients with uterine cervical carcinoma [64] and uterine cervicitis and myoma [62]. These results are in agreement with other studies in other associated diseases such as endometriosis, simple hyperplasia [59], ovarian, cervical and uterine cancer [60].

In contrast to the findings of this research, other studies showed that SOD activity in various types of tissues with tumors appeared elevated or unchanged compared to normal tissues ([65-67]). Such contradiction in the results may likely due to the different cell types and the different assays and circumstances in those researches.

Looking for the oxidative chain SOD is considered the first line of defense against oxygen free radicals, catalyzes the dismutation of superoxide anion radical into hydrogen peroxide (H2O2), which can be transformed into water and oxygen by CAT or GPx. Besides hydrogen peroxide, GPx also reduces lipid or non-lipid hydro peroxides while oxidizing glutathione GSH. The oxidized GSH is then reduced by GR [68].

In the present study, the results in [Table-3] illustrated that the activity of both CAT and Gpx in UL patients were decreased comparing with the healthy control. These results are in consistence with the data obtained by Chiou and Hu [62], which showed that patients with cervicitis had increased levels of CAT and GPx activities, while their activities in UL patients were reduced. And with Manoharan *et al.* [69] those authors also found that the activities of these enzymes (SOD, CAT and Gpx) were lower in patients with cervical cancer. Furthermore, a decreased CAT and Gpx activity were also detected by Jyoti *et al.* [60] in ovarian, cervical and uterine cancer compared with control and Mil-kierzenkowska *et al.* [70] who recorded that Gpx activity was statistically significantly higher in control subjects than in cervical cancer patients.

In [Table-3] the results also, revealed a decrease in serum GR activity of UL patients compared with healthy control. The decreased serum GR activity in this study is parallel with the results recorded by Pejic *et* al. [71] who recorded significant reduction of GR activity in all examined groups, namely, polypus endometrii and uterine myoma when compared with healthy control. However, reduced GR activity was observed, similarly, in other investigation that recorded

declines in GR activity among patients with ovarian, cervical and uterine cancer compared with healthy subjects [60]. The decline observed in GR enzyme activity in UL patients, herein, could be reflected in their activity antioxidant status, and consequently could increase the adverse effects resulted from oxidative stress. Recently, perturbations of antioxidant levels and lipid peroxidation, but not oxidative DNA damage as a biomarker of oxidative stress have been reported in uterine myoma patients [72].

In summary, decreased antioxidant enzymes (SOD, CAT, GR and Gpx) activities may be due to increased endogenous production of ROS as evidenced by increased MDA and H2O2 and NO serum concentrations in the present study. Also since the protective antioxidants play a major protective role against oxidative stress, in this study, the decline of serum antioxidant enzyme (SOD, CAT, GR and Gpx) activities in all UL patients corresponding to healthy control may be due to consumption of activated enzymes through prolonged oxidative stress for scavenging ROS and their conversation to hydrogen peroxide by SOD and then H2O2 transformation into water and oxygen by CAT and Gpx, and also by renewal of GSSG to GSH by GR enzyme.

In this research the results in [Table-4] denoted significant increase of serum MDA concentration in UL patients with elevated estradiol levels when compared to its concentration in UL patients with normal estradiol. In contrary no differences show in serum concentrations of both H2O2 and NO of UL patients with elevated serum estradiol levels when compared with their concentrations in UL patients with normal serum estradiol levels. The increased MDA concentration in elevated serum estradiol levels for UL patients may be evidence for a positive correlation between E2 and MDA. This supported the suggestion that the increased oxidative stress in the form of increased MDA concentration may be due to an increase E2 concentration in serum of UL patients.

Also in [Table-4] the results illustrated no significant changes showed in serum antioxidants enzyme (SOD, CAT, GR and Gpx) activities and some biochemical markers (ALT, AST, ACP, TPr, A and G) in both normal serum estradiol UL patients and elevated serum estradiol UL. This is evidence that no correlation present between E2 serums levels and antioxidant enzyme status (SOD, CAT, Gpx and GR) and (ALT, AST, ACP, TPr, A and G) biochemical markers.

The results in [Table-5] suggested that significant positive correlation between serum E2 levels and serum PRL levels($r=0.43$) at $p<0.0001$, MDA ($r=0.3$) significant at $p<0.03$, and H2O2 ($r=0.02$) significant at $p<0.02$ in UL patients. But there is no significant correlation between E2 and other parameters (NO, SOD, CAT, GR, Gpx, ALT, ALT, ACP, TP, AL, and GL). For the best knowledge this is the first research to study the correlation between E2 and PRL, MDA, H2O2, NO, ALT, ALT, ACP, TP, AL, and GL.

The results in [Table-5] show no correlation between E2 and antioxidant enzymes. These results are in agreement with the results obtained by Massafra *et al.* [73] who observed no cycle-phase dependent changes in SOD and CAT activities as well as no correlation between activities of these enzymes and FSH, LH, estradiol or progesterone concentrations. In contrast to these results a recent similar study carried out by Pejic et al. [74] suggested that antioxidant status in UL patients is influenced by the changes in sex hormones estradiol E2 and progesterone during the menstrual cycle and in post menopause. This contradiction may be related to the handling of the specimens, method of determination and /or women species.

The results in [Table-6] suggested no association between genotypes of XRCC1 Arg194Trp polymorphism and the risk of developing UL. In contrast, Arg/Gln and Gln/Gln genotypes of XRCC1 Arg399Gln polymorphism are associated with UL developing. Such results are in agreement with similar results shown in UL women from southeastern Iran obtained by Yaghmaei *et al.* [75] which showed that there was no association between the Arg/Gln genotype of the XRCC1 Arg399Gln polymorphism and UL before adjusting for age, and observed a significant association between this genotype and UL susceptibility after adjusting for age and there was no association between XRCC1 Arg194Trp polymorphism and UL.

Yang *et al.* [76] showed a correlation between Arg280His but not Arg399Gln and Arg194Trp polymorphisms and UL in Chinese population. In another study, Hsieh *et al.* [77] did not observe any association between Arg399Gln and UL in Taiwan. However, some reports showed that the 399Gln allele might be associated with more DNA adduct and sister chromatid exchange, as well as mutations. Such findings may, at least in theory, be associated with higher risk of malignancy [78-79].

In this study, the data showed that Egyptian women with the genotype Arg/Gln were found to have an elevated risk of UL compared with those with the Arg/Arg genotype (OR 3.34; 95% CI 1.45–7.7; P, 0.004). These results are in accordance with the results obtained by Jeon *et al*. [80] in Korean women population indicated that the Arg/Gln genotype is associated with higher risk of UL than the Arg/Arg genotype and, they observed that the incidence of UL was 6.8 fold higher in individuals with the Arg/Gln genotype compared to the Arg/Arg genotype.

Codon 399 was thought to be associated with increased cancer risk because it is located in the vicinity of poly (ADP-ribose) polymerase (PARP) binding domain [81]. Furthermore, the 399Gln mutation might affect the XRCC1 repair function through micronuclei assay in vitro [82]. Divine *et al*. [83] found that 399Arg is a risk factor for lung adenocarcinoma. Qu and Morimoto [82] had a conclusion that the interaction of 399Gln/Gln genotype and smoking might be associated with a three-fold increased cancer risk. In the study of Kiran *et al.* [84], genotypes Arg194Trp increased the risk of hepatocellular carcinoma of Indian population. There are also studies that showed that no association between the codon 399 and 194 and bladder cancer or breast cancer ([85-86]).

Many studies have been done and the results of the effects of XRCC1 polymorphisms on cancer risks were different, even conflicting in various case–control studies. The reason for this situation is complicated. It may be due to different experimental design, sample size, inclusion criteria of analyzed individuals, and types of cancers, etc. [76].

Limitations of the study

The current research suffers from some limitations; for example low sample size that might affect the findings.

Conclusion

The levels of serum concentrations of PRL and E2 hormones, oxidative stress and antioxidants may be useful in the diagnosis of UL disease and in researching therapies. This study also indicates that the Arg/Gln and Gln/Gln genotypes are associated with higher risk of UL than the Arg/Arg genotype and can be considered as a risk factor and genetic marker for UL disease. Further studies are recommended for other related parameters to clarify the role of female sex hormones.

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