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Gene polymorphisms of Interleukin 6 (−174 G/C) and transforming growth factor β-1(+915 G/C) in ovarian cancer patients

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Abstract

Background: In the study on hand, we investigated the effect of IL-6 (−174 G/C; rs 1800795) and TGF-β1 (+915G/C; rs 1800471) gene polymorphisms on the susceptibility to Ovarian Cancer and their effect on plasma levels. IL-6 (−174 G/C) SNP was analyzed using mutagenically separated polymerase chain reaction (MS-PCR) while TGF-β1 +915G/C (codon 25) SNP was investigated by the sequence-specific primer polymerase chain reaction (SSP-PCR). An enzyme-linked immunosorbent assay (ELISA) was used to quantify IL-6 and TGF-β1 plasma levels in 48 ovarian cancer patients and 48 normal controls.

Results: Regarding IL 6 (−174 G/C), a significant increase in CC and GC+CC genotypes parallel with the C allele was considered as risk factors for ovarian cancer; on the other hand, the G allele was considered as a protective factor for ovarian cancer. TGF-β1 (+915G/C) investigations showed a significant elevation in GC and GC+CC genotypes which can be considered as a risk factor for ovarian cancer. Plasma IL-6 and TGF-β1 were higher in ovarian cancer patients compared with controls. No specific genotype or allele could be responsible for the elevation of TGF-β1 in ovarian cancer patients' plasma, while the highest significant value for IL6 in subjects carrying GG and CC genotypes in comparison with GC genotype.

Conclusions: This study supports an association of IL6 (−174G/C) and TGF-β1 (+915G/C) gene polymorphisms with the susceptibility to ovarian cancer.

Keywords: Cytokine, IL-6, Ovarian cancer, Polymorphism, TGF-β1

1 Background

Ovarian cancer is the most common cause of mortality associated with gynecologic cancer in women [1]. Ovarian cancer has a global incidence of 3.4% and a mortality rate of 4.4% in females every year [2]. In the USA, the American Cancer Society predicts 21,750 cases and 13,940 deaths in 2020 [3]. ASR affects 9.2 per 100,000 women in more developed areas and 5 per 100,000 women in less developed areas. Central and Eastern Europe have the highest ASR (11.4 per 100,000 women), while the lowest ASR, 3.0 per 100,000 women, was found in Micronesia [4]. This significant incidence and mortality rate of ovarian cancer can be explained by the early symptoms, which were usually few and lead to an advanced stage before diagnosis and an increasing rate of metastasis at the time of diagnosis [5, 6]. Epithelial ovarian cancer (EOC) comprises the majority of ovarian neoplasms (about 80%) [7].

Cytokines are multifunctional and low molecular weight proteins that affect the inflammatory and immune

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responses and have an effective impact on the pathogenesis of multiple aggressive malignancies, involving ovarian cancer [8, 9]. Alteration of cytokines indeed may have prognostic and diagnostic value; multiple cytokines are secreted by ovarian cancer cells [10].

Inflammation has a substantial link with cancer growth because inflammatory cytokines in a microenvironment enable malignant cells to be highly proliferative [11]. Interleukin-6 (IL-6) is a general inflammatory marker that acts as a pro-inflammatory and anti-inflammatory mediator in the regulation of immune response, inflammation, and many pathophysiologic processes [12]. IL-6 is found in a wide variety of cell types, including B cells, T cells, fibroblasts, macrophages, and adipose cells [13]; most ovarian cancer cells release IL-6 [14–16]. Several studies have found that IL-6 is linked to a variety of malignancies, including breast carcinoma, colorectal cancer, lung cancer, and ovarian cancer [17–21]. IL-6 has pro-angiogenic properties [22] and regulates immune cell infiltration, stromal reaction, and the tumor-promoting actions of Th17 lymphocytes [23]. IL-6 inhibits the apoptosis of ovarian cancer cells contributing to tumor growth and induces vascular endothelial growth factor (VEGF) mediated angiogenesis [24]. IL-6 and other pro-inflammatory cytokines of this family, including oncostatin M, directly stimulate the invasion of cancer cells through basement membrane degradation due to matrix metalloproteinase (MMP) overexpression, induce the epithelial-mesenchymal transition of ovarian epithelial cells, and increase resistance to chemotherapy [15]. IL-6 inhibits the differentiation and maturation of dendritic cells [25]. IL-6 is known to stimulate tumor infiltration by macrophages in ovarian tissue, and this phenomenon is associated with a worse prognosis in OC patients [22]. It is known that they are linked to immunological and metabolic changes that eventually contribute to cancer cachexia; one of the most common causes of cancer-related death in OC patients. In OC cells, IL-6 has been shown to increase their ability to release MMP-9 [26], thus promoting angiogenesis. IL-6 also inhibits immunological cells by inhibiting the expression of IL-2, reducing T cell activation, and accelerating lymphocyte death, which might predict immune surveillance of cancer cells [27].

In the endeavor to improve the survival rate of ovarian cancer, surgical procedures and chemotherapy have been extensively investigated [15]; however, in recent decades, the five-year survival rate has not increased significantly [11, 15]. As a result, new therapeutic approaches with lower side effects are desperately needed, and targeted therapy is a possible option [28]. In addition to being a cytoprotective cytokine in normal physiological settings, IL-6 also protects tumor cells against radiation and chemotherapy drugs, posing a serious barrier in cancer treatment therapeutic gain [29]. IL-6 may promote carcinogenesis and protects cells from therapeutic stress-induced cell death by promoting a variety of pro-survival signaling, including apoptosis inhibition, survival, and proliferation [30, 31]. The radio resistance created by IL-6 can potentially be used to protect normal tissues from radiation [32, 33]. In ovarian cancer patients, IL-6 levels beyond a particular threshold can develop chemo-resistance and are a predictor of poor prognosis [29, 34, 35]. As a result, the Food and Drug Administration (FDA) has approved the IL-6 inhibitor as a novel targeted therapy for ovarian cancer. Six months treated patients showed a significant reduction in blood IL-6 [36, 37].

The IL-6 gene is located on the short arm of human chromosome 7 (7p21). Four polymorphisms have been found in the promoter region of IL-6, at positions −174 (G/C), −373 (A/G), −572 (G/C), and −597 (G/A). In particular, the functional SNP rs1800795 (−174G>C) is associated with the intrinsic IL-6 transcription rate, which may influence IL-6 levels [38]. The IL-6-174G>C is linked to vulnerability to a variety of cancers [39, 40]. This SNP has also been linked to the prognosis of cancer, including non-small cell lung cancer, bladder cancer, neuroblastoma, and breast cancer [39, 41–43].

Angiogenesis, cell differentiation, cell proliferation, extracellular matrix formation, and apoptosis are all regulated by transforming growth factor-beta 1 (TGF-β1). As a female reproductive hormone, it plays a vital role in the development and function of the ovary [44]. TGF-β1 is produced in large amounts near tumors and suppresses effector cell proliferation and function while promoting the differentiation of certain suppressive T-cells [45]. TGF-β1 suppresses the growth, proliferation, and activation of T cells, NK cells, and macrophages [46]. TGF-β1 signaling dysregulation is associated with a variety of pathological events, including tumor development and fibrosis [47]. TGF-β1 signaling has both tumor suppressor and oncogenic properties throughout tumor development [48]. Ovarian cancer cells lose their ability to respond to TGF-β1 because of alterations in TRI quality. About 27% of human ovarian malignancies carry the TRI 6*A allele, suggesting that it functions as a key entering tumor marker in the progression of OC [49]. Diminished expression or function of the Sma- and Mad-related protein 4 (SMAD4) protein causes a decreased capacity to connect DNA [50]. Smad4 and Smad3 have been implicated in the metastatic potential of ovarian malignancies, according to a study by Singh et al. [51]. TGF-β1 aided metastatic migration in ovarian cancer cells in the tiniest way possible by activating MMPs [52]. During ovarian carcinogenesis, deregulation of TGF-β1/SMAD4 signaling leads to epigenetic silence of a potential tumor

suppressor, RunX1T1 [53]. Recently, genome-wide screening of TGF-β1 induced SMAD4 activation in EOC using ChIP-seq revealed that the SMAD4-dependent administrative network in ovarian cancer was dramatically different from that in normal cells and was predictive of patient survival [54].

As a result, TGF-β1 inhibitors have a lot of potential as a supplement to checkpoint blockade therapy [45]. To prevent TGF-β1 production and block TGF-β1 signaling pathways, there are different reports for inhibitors that have been explored in tumor carcinogenesis in preclinical or clinical trials [55]. As a result, TGF-β1 plays a critical role in tumor therapy and may open the door to new antitumor strategies in cancer patients [55]. An immuno-oncology designed TGF-β1 inhibitor has been approved by the FDA [56].

TGF-β1 gene is located on human chromosome 19 s long arm (19q13) and contains seven exons and six introns. There are at slightest seven polymorphisms within the gene of TGF-β1. Among them, the first exon has a higher gene mutation rate and single-nucleotide polymorphism (SNP), which is associated with inter-individual variation within the level of its generation and was confirmed to be closely related to the occurrence of a variety of malignant tumors, such as esophageal, breast, prostate, liver cancer, and others [57, 58]. This polymorphism is located at position +915G/C, which changes codon 25 (G → C substitution) resulting in arginine → proline (rs1800471) [59]. Hereditary variations within the coding area of the TGF-β1 gene may affect translation and protein synthesis [44]. These IL-6 (−174 G/C) and TGF-β1 (+915C/G) polymorphisms have been studied in ovarian cancer patients in this case–control study. For both ovarian cancer patients and control groups, we analyzed IL-6 and TGF-β1 plasma levels to conclude the possible correlation between fluctuations in their levels and the genetic changes at these polymorphic locations.

2 Methods

2.1 Subjects population

This was a descriptive retrospective cross-sectional study that involved 48 Egyptian women with histologically confirmed epithelial ovarian cancer recruited consecutively at the obstetrics—gynecology Department, Faculty of Medicine, Cairo University, Cairo, Egypt, in the period between (December 2017 and December 2019). The results of histopathological examination of the tissue taken during the surgery detected the final clinical diagnosis, following the International Federation of Gynecology and Obstetrics (www.figo.org) instructions and guidelines; the 48 ovarian cancer was classified into (stage I, II, III, or IV), tumor grade (G1: well-differentiated tumor, G2: moderately differentiated tumor, and G3: undifferentiated tumor) and histological types (endometrioid, mucinous, serous-papillary and clear cell carcinoma), and 48 Egyptian healthy women were included in the study as healthy controls. Controls were disease-free individuals without cancer and age-matched to the ovarian cancer patients.

According to Helsinki's (1975) declaration and clinical research practices, the protocol of the current study was authorized, and the verbal informed consent of all study subjects was approved by the ethics committee.

The eligible patients for the study were chosen according to the following criteria:

2.1.1 Inclusion criteria

1) Females aged between 45 and 80 years old.
2) Females were newly histologically confirmed as primary EOC including several subtypes (endometrioid, serous, mucinous, clear cell carcinoma)

2.1.2 Exclusion criteria

1) Age below 45 or over 80 years.
2) Non-neoplastic ovarian masses cases.
3) Lactation or pregnancy.
4) Pneumonia or diabetes mellitus type I patients (acute disease).
5) Not having EOC as a final diagnosis in patients in whom there was only a suspicion of the disease.
6) Borderline tumors because they are a different entity from ovarian tumors.
7) Previous or other concomitant malignancies.
8) Inflammatory, infectious, autoimmune, thyroid, or vascular diseases.
9) Carcinoma patients with a smoking history.
10) Carcinoma patients with any other ovarian disease.

2.1.3 Experimental design

The enrolled patients and healthy subjects were classified into two main groups.

1) Healthy controls ($N=48$), mean age = 45.25 years.
2) Ovarian cancer patients included several subtypes (serous, mucinous, clear cell, endometrioid carcinoma) ($N=48$), mean age = 50.59 years.

2.2 DNA isolation

The blood of the samples was collected using tubes of EDTA.K3 (Tri-potassium ethylene diamine tetraacetic acid) by vein puncture from 48 women with ovarian cancer and 48 healthy controls. We centrifuged the tubes for 10 min at 1500 rpm. For measuring the cytokine level, we isolated, aliquoted, and held plasma at − 80 °C concurring with the manufacturer's instructions. To extract genomic DNA from patients' blood, GeneJET™ Purification Column (Fermentas Life Science, Thermo Fisher Scientific Inc., MA, USA) was used.

2.2.1 Sample lysis with Proteinase K

Two hundred µl of whole blood sample was added to the bottom of 1.5 ml microcentrifuge tubes. Twenty µl of Proteinase K and 400 µl of Lysis Solution were added to the blood tube and mixed by vortexing for 15 s to obtain a uniform suspension. The sample homogenate was incubated at 56 °C for 20 min. Two hundred µl of absolute ethanol (Sigma) was added to the samples and mixed by pipetting.

2.2.2 Absorption to the column silica membrane

The sample lysate was loaded onto GeneJET™ Purification Column which was in a collection tube and then centrifuged for 2 min at 6000 × g. DNA was absorbed onto the column's silica-gel membrane during centrifugation. Salt and pH conditions in the lysate ensure that protein and other contaminations, which can inhibit PCR, were not retained on the silica membrane.

2.2.3 Removal of the residual contaminants

The flow-through collection tube was discarded after centrifugation, and the column was moved to a new collection tube. DNA bound to the column membrane was washed in 2 centrifugations. After adding 500 µl of Wash Buffer I to the column, it was centrifuged for 2 min at 8000 × g, and the flow-through was discarded. The column was filled with 500 µl of Wash Buffer II, centrifuged for 3 min at 12,000 × g, and the collection tube holding the flow-through was discarded. The use of two separate wash buffers increases the purity of the eluted DNA substantially. The washing conditions ensure that any remaining contaminants are removed completely without interfering with DNA binding.

2.2.4 Elution of pure DNA

The column was transferred into a new 1.5 DNase-RNase free microcentrifuge tube, 200 µl of Elution Buffer was added, and then the column was incubated for 2 min, and after that centrifuged for 1 min at 8000 × g. The DNA would be in the flow-through. This step was done to elute the DNA from the column. The DNA was stored at − 20 °C for further application. The quality of DNA was tested using 1% agarose gel electrophoresis.

2.3 Genotyping

For IL-6 (−174 G/C) SNP analysis, the mutagenically separated polymerase chain reaction (MS-PCR) method was used. Based on Schotte et al.'s study [60], three primers combinations were utilized (Table 1). As previously indicated, the reaction was carried out in a single tube with a final reaction volume of 25 µl [51]. We used Dream Taq Green PCR Master Mix (2X) (Fermentas), 10 Pmoles of each forward primer (Primer G) and reverse primer (Primer C), and 40 ng of DNA. All reactions were carried out using a Biometra thermal cycler (Biometra GmbH, Germany).

Table 1 PCR primers used to detect polymorphisms of TGF-β1 (+915G/C) and IL6 (−174 G/C)

IL-6 (−174 G/C)	
Forward primer (G)	5′-GCACTTTTCCCCCTAGTTGTGTCTTACG-3′
Forward primer (C)	5′-GACGACCTAAGCTTTACTTTTCCCCCT AGTTGTGTCTTGAC-3′
Reverse primer	5′-ATAAATCTTTGTTGGAGGGTGAGG-3′
TGF-β1 +915 G/C	
Primer G (sense)	5′-GTG CTG ACG CCT GGC CG-3′
Primer C (sense)	5′-GTG CTG ACG CCT GGC CC-3′
Generic primer (antisense)	5′-GGC TCC GGT TCT GCA CTC-3′
Internal control (forward and reverse)	5′-GCC TTC CCA ACC ATT CCC TTA-3′
	5′-TCA CGG ATT TCT GTT GTG TTT C-3′

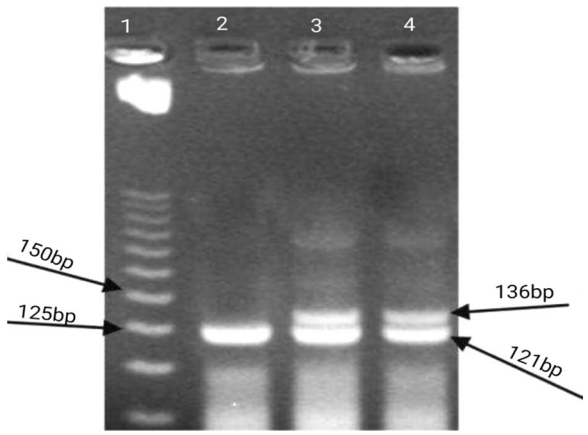

Fig. 1 IL-6 (−174 G/C) PCR products of three samples. Lane (1) 25 bp ladder, sample 1 in lane (2) GG genotype, sample 2 in lane (3) GC genotype and sample 3 in lane (4) GC genotype

The cycling conditions of PCR were as follows: 1 cycle was in 95 °C for 10 min, then 40 cycles were in 94 °C for 30 s, 66 °C for 45 s, and 72 °C for 45 s, then 1 cycle was 72 °C for 7 min. The PCR products were 121 bp (for G allele) and 136 bp (for C allele) (Fig. 1). To assess the size of the PCR products, 4% agarose gels in 0.5% Tris–acetate-EDTA buffer with ethidium bromide staining (10 mg/ml) and the migration of a 25 bp step ladder (Promega Co. WI, USA) were used.

TGF-β1 codon 25 SNP was analyzed by (SSP-PCR) using four primer mixtures (Table 1). A 25 µl final reaction volume in the two tubes used in the PCR reaction. The PCR reaction was performed in two tubes with 25 µl final reaction volume [61]. Additionally, each tube has a generic primer as well as a forward primer that is specific to one allele. Mixtures of PCR involved Dream Taq Green PCR Master Mix (2X) (Fermentas), 50 ng of DNA, and 10 Pmoles of each primer. All reactions were carried out in a Biometra thermal cycler (Biometra GmbH, Germany). The migration patterns of a 100 bp DNA ladder (Promega Co. WI, USA) on a 2% agarose gel in 0.5X Tris–acetate-EDTA (TAE) buffer with ethidium bromide staining (10 mg/ml) determined the size of the PCR products.

The cycling conditions of PCR were as follows: 1 cycle was at 95 °C for 1 min then 10 cycles were in 95 °C for 15 s, 65 °C for 50 s, and 72 °C for 40 s, then 20 cycles were 95 °C for 20 s, 56 °C for 50 s, and 72 °C for 50 s. PCR products were at 233 bp and 429 bp for codon 25 and internal control, respectively (Fig. 2).

2.4 Measurement of plasma IL6 and TGF-β1

Total concentrations of plasma IL6 and TGF-β1 were measured in 48 ovarian cancer women and 48 healthy women by a sandwich ELISA, Minneapolis, USA, according to the manufacturer's instructions. The computerized information of crude absorbance value was prepared into a standard curve by utilizing the ELISA reader-controlled with a computer program (Softmax), and cytokines concentrations were expressed as pg/ml.

2.4.1 Measurement of plasma IL-6 levels

2.4.1.1 Plate preparation The diluted capture antibody (monoclonal) in phosphate-buffered saline was used to measure IL6 using a human Duo ELISA package (PBS—137 mM NaCl, 2.7 mM KCl, 8.1 mM Na2HPO4, 1.5 mM KH2PO4, pH 7.2–7.4, 0.2 µm filtered) was coated on 96-well microplates, 100 µl/well, and the plates were incubated at 1 h at 37 °C then overnight at 4 °C. Plates were washed three times with 200 µl/well washing buffer (0.05% Tween-20 in PBS), blocked with 200 µl/well-blocking buffer (1% BSA in PBS, pH 7.2–7.4), and incubated at room temperature for 1 h. The plates were then washed three times with 200 µl/well washing buffer.

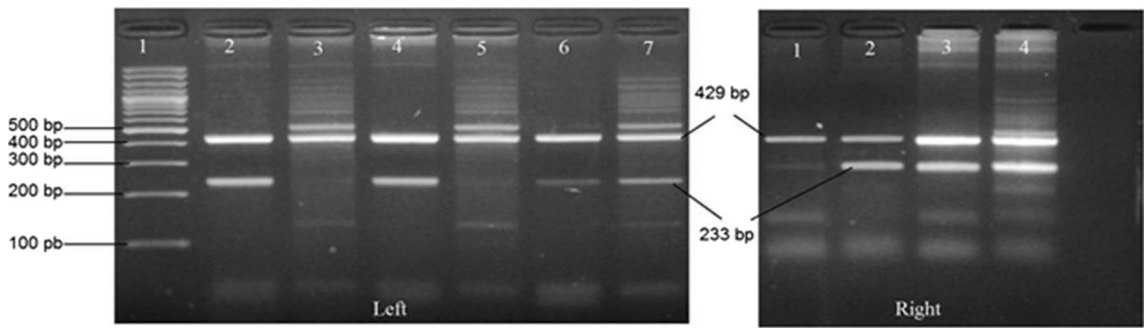

Fig. 2 TGF-β1 (+915G/C) PCR product. Left picture: lane 1 showed 100 bp DNA ladder, lanes 2, 3 and lane 4, 5 showed GG genotype whereas the size of allele band is 233 bp and the control band was 429 bp, Lane 6, 7 showed GC genotype. Right picture: lane 1, 2 showed CC genotype while lanes 3, 4 showed GC genotype

2.4.1.2 Procedure The plates were incubated for 2 h at room temperature after adding 100 µl of plasma sample or normal in Reagent Diluent (1% BSA in PBS). At the end of the incubation period, the plates were washed three times with 200 µl/well washing buffer, then 100 µl/well of biotin-labeled detection antibody, diluted in reagent diluent were added followed by 2 h incubation at room temperature.

With 200 µl/well washing buffer, the plates were washed three times. Streptavidin-HRP was added (100 µl/well) and then the plates were incubated for 1 h. at room temperature. Three washes with 200 µl/well washing buffer are performed on the plates. The enzyme reaction was carried out by adding 100 µl/well Substrate Solution (1:1 mixture H_2O_2 and TMB), and the plates were incubated for 20 min at room temperature. Color development was stopped by the addition of 50 µl/well of stopping solution (1 M HCl). Using a microplate reader (SUNRISE Remote Control, TECAN, Austria), the strength of the formed color was determined by reading optical absorbance at 450 nm. The ELISA reader-controlling software (Softmax) converts raw absorbance data into a standard curve from which the IL-6 concentration of unknown samples can be calculated directly.

2.4.2 Measurement of plasma TGF-β1 levels

2.4.2.1 Plate preparation Mouse anti-human TGF-β1 capture monoclonal antibody was diluted to the working concentration; 2 µg /1 ml PBS. 100 µl of diluted capture antibody was applied to each well of the 96-well microplates. The plates were sealed and incubated for 2 h at 37° C; then, each plate was aspirated well and washed with the washing buffer. The plates were blocked by the addition of 300 µl blocking buffer (5% Tween 20 in PBS with 0.05% Tween-20) to each well, and they were incubated at room temperature for 1.5 h. The plates were aspirated and washed three times by 200 µl washing buffer.

2.4.2.2 TGF-β1 sample activation This step was done to activate the latent TGF-β1 to the immunoreactive form of TGF-β1 which was achieved by the addition of 20 µl 1 N HCl to 40 µl plasma, and they were mixed well together and incubated for 10 min at room temperature. The acidified samples were neutralized by the addition of 20 µl of 1.2 N NaOH/0.5 M HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid). The activated samples were diluted 20 fold by addition of 10 µl of the activated samples to 190 µl reagent diluent (1.4% BSA, 0.05% Tween 20 in PBS).

2.4.2.3 Procedure One hundred microliters of each sample or standard in reagent diluents were applied to each well, and then, the plates were covered and incubated for 2 h at room temperature. After that, the plates were aspirated and washed with washing buffer, then 100 µl of the diluted detection antibody (Biotinylated chicken anti-human TGF-β1 at 150 ng/ml) was added to each well and the plates were sealed and incubated at room temperature for 2 h. After washing, 100 µl of working dilution of Streptavidin-HPR (1:250) was added to each well and incubated for 1 h at room temperature. At the end of the incubation period, 100 µl of TMP + H_2O_2 substrate were added into the wells to trigger the enzymatic reaction, and then, the plates were covered and incubated for 20 min at room temperature. Finally, 50 µl of the stopping solution (1 M HCl) was added to each well. At a wavelength of 450 nm, the optical density of each well was calculated using a microplate reader (SUNRISE Remote Control, TECAN, Austria). To determine TGF-β1 concentrations, the raw data from the microplate reader was applied to ELISA analysis software (SoftMax, Molecular Devices Corp., USA), which converts the absorbance data into a standard curve.

2.5 Statistical analysis

All measurable investigations were analyzed utilizing version 25 of the SPSS statistical package for the social sciences (SPSS, IBM, USA). An independent *t* test was used for performing the comparisons between patients and control groups, and we displayed the data as means and standard deviation (SD). The Chi-square test was used to analyze the variations in allele and genotype distribution between groups. A one-way ANOVA test was utilized to compare genotype and ELISA information within the same group. If the difference between groups was significant, we carried out a post hoc investigation by applying the LSD. We utilized Spearman's correlation test for correlation between variables. DeFinetti program (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl) investigated the genotype distribution for Hardy–Weinberg equilibrium, and the chi-square test evaluated it with one degree of freedom. To assess the risk associated with a particular allele or genotype, the online tool SNPstats (http://bioinfo.iconcologia.net/SNPstats; Sole' et al. 2006) calculated LD parameters (D0 and r2), odds ratio (OR), 95 percent confidence interval (CI), and *P* values. To be statistically significant, *P* values less than 0.05 had to be two-tailed.

3 Results

3.1 Pathological and clinical characteristics of patient population

This study included 48 women with ovarian cancer and 48 women without cancer. A statistically significant difference in age between the two groups was not observed because the study was designed to match the age and gender of cases and controls, without gender difference

Table 2 Clinical data of ovarian cancer patients

Characteristics	Study group (*N* = 48) (*n*, %)
Histological type	
Papillary Serous cystadenocarcinoma	27 (56.25%)
Mucinous cystadenocarcinoma	15 (31.25%)
Endometrioid carcinoma	4 (8.33%)
Clear cell carcinoma	2 (4.16%)
Grading	
G1	6 (12.5%)
G2	28 (58.33%)
G3	14 (29.16%)
FIGO stage	
I	9 (18.75%)
II	5 (10.41%)
III	15 (31.25%)
IV	19 (39.58%)

FIGO International Federation of Gynecology and Obstetrics

which indicates that the matching according to these two variables was adequate.

The detailed clinicopathologic characteristics of the cancer patients are shown in Table 2, and the detailed biochemical characteristics of the cancer patients and controls are presented in Table 3. Surface epithelial carcinomas were responsible for 100% of all malignant lesions. The majority of patients were diagnosed with an advanced FIGO at stage III and IV accounted for 34 (70.83%), stage I (18.75%), and II (10.41%). Papillary serous cystadenocarcinoma was the most common malignant tumor constituting 56.25% (27/48) cases.

The ovarian cancer group showed a significant increase in AST and ALT ($P<0.001$), CA125, and BUN levels ($P<0.01$) compared to the healthy control. While white blood cells count showed a significant decline in ovarian cancer patients compared to healthy women ($P<0.01$). Concerning healthy controls, the ovarian cancer group showed a substantial decrease in albumin levels ($P<0.01$). Pearson correlation analyses were performed between ovarian cancer and demographic and biochemical parameters. Ovarian cancer has a statistically significant positive correlation with plasma levels of CA125, AST, ALT ($P<0.001$), and BUN ($P<0.01$). A statistically significant inverse association was observed between the disease and albumin production ($P<0.001$), and between the disease and the number of white blood cells ($P<0.01$) was recorded.

3.2 Association between IL-6 (−174 G/C) and TGF-β1 (+915G/C) polymorphisms and ovarian cancer patients

3.2.1 Genotyping of IL-6 −174G/C

The genotypes and allele frequencies of IL-6 −174 in ovarian cancer and healthy controls were demonstrated (Table 4). The genotype distribution of IL-6 (−174 G>C) gene polymorphisms frequencies among both groups was in agreement with the predicted Hardy–Weinberg equilibrium, where the deviation testing of their genotypes from the equilibrium did not show any significant difference, in the controls ($\chi^2=0.0218$, $P=0.864$), in ovarian cancer ($\chi^2=44.08$, $P=0.489$). Therefore, we considered this polymorphism for comparison between cases and controls.

Table 3 Demographic and biochemical characteristics of control and ovarian cancer patients

Laboratory investigations	Control (*N* = 48) (mean ± SD)	Ovarian cancer (*N* = 48) (mean ± SD)	*P* value	Correlation with OC
Age (years)	45.25 ± 15.31	50.59 ± 11.93	NS	$r=0.239$*
CA125 (U/ml)	12.92 ± 7.29	176.30 ± 244.91	$P<0.01$**	$r=0.430$***
TLC (1000/mm3)	7.50 ± 1.83	6.18 ± 2.20	$P<0.01$**	$r=-0.311$**
HB (g/dl)	11.71 ± 1.36	11.25 ± 2.06	NS	$r=-0.168$
PLT (1000/mm^3)	269.52 ± 68.22	276.91 ± 142.88	NS	$r=0.033$
AST (IU/L)	20.77 ± 6.12	32.50 ± 18.77	$P<0.001$***	$r=0.391$***
ALT (IU/L)	18.95 ± 5.49	34.95 ± 18.61	$P<0.001$***	$r=0.507$***
ALB (g/dL)	4.25 ± 0.42	3.16 ± 0.56	$P<0.001$**	$r=-0.737$***
T.Bili (mg/dl)	0.65 ± 0.17	0.55 ± 0.38	NS	$r=-0.157$***
Cr. (mg/dl)	0.88 ± 0.1	0.91 ± 0.3	NS	$r=0.066$
BUN (mg/dl)	9.60 ± 2.04	11.35 ± 3.64	$P<0.01$**	$r=0.286$**

PLT Platelet, *AST* Aspartate aminotransferase, *ALT* Alanine aminotransferase, *ALB* Albumin, *TLC* Total leucocytic count, *Cr* Creatinine, *BUN* Blood urea nitrogen, *CA125* Cancer antigen 125, *NS* not significant, *HB* Hemoglobin, *T.Bili.* Total bilirubin

* $P<0.05$; ** $P<0.01$; *** $P<0.001$

Table 4 Genotype distribution and allelic frequency of IL-6 (−174G/C) in controls and patients with ovarian cancer

Cytokine gene	Control (*N* = 48)	Ovarian cancer (*N* = 48)	*P* value	OR (95% CI)	AAIC	BBIC
IL-6 (− 174 G > C) Genotype (*N*, %)						
Codominant model (GC, CC vs. GG) GG	34 (70.8%)	23 (47.9%)	*P* < 0.001	1.00	107.3	112.4
G/C	13 (27.1%)	3 (6.2%)	*P* < 0.001	0.34 (0.09–1.33)		
C/C	1 (2.1%)	22 (45.8%)	*P* < 0.001	32.52 (4.09–258.36)		
Dominant model (GC+CC vs. GG) GC+CC	14 (29.2%)	25 (52.1%)	*P* < 0.05	2.64 (1.14–6.12)		
Recessive model (CC vs. GG+GC) GG+GC	47 (97.9%)	26 (54.2%)	*P* < 0.001	1.00		
C/C	1 (2.1%)	22 (45.8%)	*P* < 0.001	39.77 (5.07–312.10)		
Allele frequency (*N*, %)						
G	81 (84.0%)	49 (51.0%)	*P* < 0.001	0.193 (0.098–0.381)		
C	15 (16.0%)	47 (49.0%)	*P* < 0.001	5.180 (2.622–10.233)		

IL interleukin, *OR* odds ratio, *CI* confidence interval, *AIC* Akaike information criterion, *BIC* Bayesian information criterion; *P* value and OR were calculated using SNP Stats software program

IL6 −174G>C homozygous genotype (GG) was used as a reference to estimate the risk for dominant (GC+CC vs. GG), recessive (CC vs. GC+GG) and codominant models 1 and 2 (GC vs. GG, CC vs. GG, respectively).

In the codominant model, GC (heterozygous mutant type) versus GG (homozygous wild-type) ($P < 0.001$; OR 0.34; 95% CI 0.09–1.33), and CC (homozygous mutant type) versus GG ($P < 0.001$; OR 32.52; 95% CI 4.09–258.36), a significant increase ($P < 0.001$) was observed in CC genotype distribution in ovarian cancer compared with controls suggested that the substitution was considered as a risk factor for ovarian cancer in the homozygous forms but protective in the heterozygous forms. On the other hand, it revealed that GG genotype was found more frequently in both ovarian cancer and controls.

In the dominant model (GC+CC vs. GG), the substitution of IL6 (−174G>C) had a significant increase in ovarian cancer ($P < 0.05$; OR 2.640; 95% CI 1.14–6.12) (Table 4).

Further analysis using a recessive model (CC vs. GG+GC), ($P < 0.001$; OR 39.77; 95% CI 5.07–312.10) suggested that G>C substitution might be a risk factor for ovarian cancer and that the recessive model is the inheritance best fit model (according to AIC and BIC) (Table 4).

Allele C showed a significant increase ($P < 0.001$) in ovarian cancer compared with controls and could be considered a risk factor for ovarian cancer (OR 5.180 with 95% CI 2.622–10.233). On the other hand, allele G was more frequent in both ovarian cancer and controls and had a significant decrease ($P < 0.001$) in ovarian cancer compared with the healthy group (OR 0.193 with 95% CI 0.098–0.381) (Table 4).

The possible effect modification of the association between IL-6 −174G/C SNP genotypes and ovarian cancer risk by age are summarized in Table 5. This involved studying the effect exerted on the association between (GG, GC, CC, the dominant model, and the recessive model) and the ovarian cancer risk by age. In 45–55 y, an increased ovarian cancer risk was observed in CC genotype (OR 24.29 (95% CI 2.89–204.22); $P < 0.05$), C allele [OR 4.457 (95% CI 2.077–9.565; $P < 0.05$), and CC vs. GG+GC (OR 34.00 (95% CI 4.15–278.81); $P < 0.05$]. A decreased ovarian cancer risk was observed in GC genotype (OR 0.24 (95% CI 0.05–1.23); $P < 0.05$) and G allele (OR 0.224 (95% CI 0.105–0.481); $P < 0.05$). In 56–65 y, increased ovarian cancer risk was observed in C allele (OR 1.3 (95% CI 1.005–1.769); $P < 0.05$); a decreased ovarian cancer risk was observed in G allele (OR 1.0 (95% CI 0.9–1.769); $P < 0.05$). In 66–80 y, an increased ovarian cancer risk was observed in C allele (OR 11.0 (95% CI 1.103–109.67); $P < 0.05$); a decreased ovarian cancer risk was observed in G allele (OR 0.09 (95% CI 0.009–0.906); $P < 0.05$). We analyzed the correlation of the IL-6 −174G/C promoter SNP did not have any significant association with age ($\chi^2 = 3.698$; P value = 0.448).

3.2.2 Genotyping of TGF-β1 (+915G/C)

The TGF-β1 +915G>C genotype and allele frequencies in ovarian cancer patients and healthy controls are shown in Table 6. Genotype distribution frequencies of TGF-β1 +915G>C gene polymorphisms among both groups were in agreement with the predicted Hardy–Weinberg equilibrium, where deviation testing of their genotypes from the equilibrium showed no significance, in the controls ($\chi^2 = 1.586$, $P = 0.916$), in ovarian cancer ($\chi^2 = 2.222$, $P = 0.822$). Therefore, we considered this polymorphism for comparison between cases and controls.

Table 5 Genotype distribution and allelic frequency of IL-6 (−174G/C) in controls and patients with ovarian cancer according to ages subtypes

Cytokine gene	Control (*N* = 48)	OC (*N* = 48)	*P* value	OR (95% CI)
IL-6 (−174G/C) Genotype (*N*, %)				
Age	45–55 y			
G/G	20 (60.6%)	14 (42.4%)	*P* < 0.05	1.0
G/C	12 (36.4%)	2 (6.1%)	*P* < 0.05	0.24 (0.05–1.23)
C/C	1 (3%)	17 (51.5%)	*P* < 0.05	24.29 (2.89–204.22)
G	52 (79%)	30 (45%)	*P* < 0.05	0.224 (0.105–0.481)
C	14 (21%)	36 (55%)	*P* < 0.05	4.457 (2.077–9.565)
Dominant model (GC+CC vs. GG) GC+CC	13 (39.4%)	19 (57.6%)	NS	2.09 (0.78–5.57)
Recessive model (CC vs. GG+GC) GG+GC	32 (97%)	16 (48.5%)	*P* < 0.05	1.0
CC	1 (3%)	17 (51.5%)	*P* < 0.05	34.00 (4.15–278.81)
Age	56–65 y			
G/G	9 (100%)	6 (75%)	NS	1.0
G/C	0	0	–	–
C/C	0 (0%)	2 (25%)	NS	0.368 (0.204–0.664)
G	18 (100%)	12 (75%)	*P* < 0.05	1.0 (0.9–1.769)
C	0 (0%)	4 (25%)	*P* < 0.05	1.3 (1.005–1.769)
Dominant model (GC+CC vs. GG) GC+CC	0 (0%)	2 (25%)	NS	0.4 (0.215–0.743)
Recessive model (CC vs. GG+GC) GG+GC	9 (100%)	6 (75%)	NS	1.0
CC	0 (0%)	2 (25%)	NS	0.4 (0.215–0.743)
Age	66–80y			
G/G	5 (83.3%)	3 (42.9%)	NS	1
G/C	1 (16.7%)	1 (14.3%)	NS	1.67 (0.07–37.73)
C/C	0 (0%)	3 (42.9%)	NS	2.00 (0.899–4.452)
G	11 (92%)	7 (50%)	*P* < 0.05	0.09 (0.009–0.906)
C	1 (8%)	7 (50%)	*P* < 0.05	11 (1.103–109.67)
Dominant model (GC+CC vs. GG) GC+CC	1 (16.7%)	4 (57.1%)	NS	6.67 (0.49–91.33)
Recessive model (CC vs. GG+GC) GG+GC	6 (100%)	4 (57.1%)	*P* < 0.05	1.00
CC	0 (0%)	3 (42.9%)	NS	1.75 (0.721–3.324)

Table 6 Genotype distribution and allelic frequency of TGF-β1 (+915G>C) in controls and patients with ovarian cancer

Cytokine gene	Control (*N* = 48)	Ovarian cancer (*N* = 48)	*P* value	OR (95% CI)	AAIC	BBIC
TGF-β1 (+915G>C) Genotype (*N*, %)						
G/G	41 (85.4%)	31 (64.6%)	*P* < 0.05	1.00		
G/C	6 (12.5%)	17 (35.4%)	*P* < 0.05	3.75 (1.32–10.61)	131.4	136.5
C/C	1 (2.1%)	0 (0.0%)	NS	0.976 (0.931–1.023)		
Dominant model (GC+CC vs. GG) GC+CC	7 (14.6%)	17 (35.4%)	*P* < 0.05	3.21 (1.19–8.70)		
Recessive model (CC vs. GG+GC) GG+GC	47 (97.9%)	48 (100%)	NS	1.00		
C/C	1 (2.1%)	0 (0.0%)	NS	0.979 (0.940–1.020)		
Allele frequency (*N*, %)						
G	88 (92.0%)	79 (82.0%)	NS	0.422 (0.173–1.032)		
C	8 (8.0%)	17 (18.0%)	NS	2.367 (0.969–5.785)		

IL interleukin, *OR* odds ratio, *CI* confidence interval, *AIC* Akaike information criterion, *BIC* Bayesian information criterion, *P* value and OR were calculated using SNP Stats software program

TGF-β1 +915 homozygous genotype (GG) was used as a reference to estimate the risk for recessive (CC vs. GC+GG), dominant (GC+CC vs. GG), and codominant models 1 and 2 (GC vs. GG, CC vs. GG, respectively).

In the codominant model, GC (heterozygous mutant type) versus GG (homozygous wild-type) ($P<0.05$; OR 3.75; 95% CI 1.32–10.61). A significant increase ($P<0.05$) in GC genotype in ovarian cancer patients compared with controls proposed that GC genotype may be a risk factor for ovarian cancer. CC (homozygous mutant type) vs GG ($P>0.05$; OR 1.024; 95% CI 0.977–1.074), showed that GG genotype was the more frequent in both ovarian cancer and controls.

In the dominant model (GC+CC vs. GG), we found that TGF-β1 (+915G>C) substitution was significantly higher in ovarian cancer ($P<0.05$; OR 3.212; 95% CI 1.19–8.70) (Table 6).

In the analysis using the recessive model, an insignificant difference between cases and controls ($P>0.05$; OR 1.021; 95% CI 0.980–1.064). We concluded that the dominant model is the inheritance best fit model of (according to AIC and BIC) (Table 6). An insignificant increase in the C allele, on the other hand, Allele G, was more frequent in both ovarian cancer and controls (Table 6).

The possible effect modification of the association between TGF-β1 +915G/C SNP genotypes and ovarian cancer risk by age is summarized in Table 7. This involved studying the effect exerted on the association between (GG, GC, CC, the dominant model, and the recessive model) and the ovarian cancer risk by age. In 45–55 y, an increased ovarian cancer risk was observed in GC genotype (OR 3.15 (95% CI 0.87–11.36); $P<0.05$) and GC+CC (OR 3.15 (95% CI 0.87–11.36). In 56–65 y, an increased ovarian cancer risk was observed in GC genotype (OR 2.667 (95% CI 1.090–6.524); $P<0.05$) and GC+CC genotype (OR 13.33 (95% CI 1.07–166.38).

We analyzed the correlation of the TGF-β1 +915G/C SNP with the different ages of the case group subjects.

Table 7 Genotype distribution and allelic frequency of TGF-β1 (+915G>C) in controls and patients with ovarian cancer according to ages subtypes

Cytokine gene	Control ($N=48$)	OC ($N=48$)	*P* value	OR (95% CI)
TGF-β1 (+915G>C) genotype (*N*, %)				
Age	45–55 y			
G/G	29 (87.9%)	23 (69.7%)	$P<0.05$	1
G/C	4 (12.1%)	10 (30.3%)	$P<0.05$	3.15 (0.87–11.36)
C/C	0	0	–	–
G	62 (94%)	56 (85%)	NS	0.361 (0.107–1.217)
C	4 (6%)	10 (15%)	NS	2.768 (0.822–9.324)
Dominant model (GC + CC vs. GG) GC + CC	4 (12.1%)	10 (30.3%)	$P<0.05$	3.152 (0.875–11.362)
Recessive model (CC vs. GG + GC) GG + GC	33 (100%)	33 (100%)	–	1.0
CC	0	0	–	–
Age	56–65 y			
G/G	8 (88.9%)	3 (37.5%)	$P<0.05$	1
G/C	0 (0%)	5 (62.5%)	$P<0.05$	2.667 (1.090–6.524)
C/C	1 (11.1%)	0 (0%)	NS	0.889 (0.706–1.120)
G	16 (89%)	11 (69%)	NS	0.275 (0.045–1.681)
C	2 (11%)	5 (31%)	NS	3.636 (0.595–22.234)
Dominant model (GC+CC vs. GG) GC+CC	1 (11.1%)	5 (62.5%)	$P<0.05$	13.33 (1.07–166.38)
Recessive model (CC vs. GG+GC) GG+GC	8 (88.9%)	8 (100%)	NS	1.0
CC	1 (11.1%)	0 (0%)	NS	0.500 (0.306–0.816)
Age	66–80 y			
G/G	4 (66.7%)	5 (71.4%)	NS	1
G/C	2 (33.3%)	2 (28.6%)	NS	0.80 (0.08–8.47)
C/C	0	0	–	–
G	10 (83%)	12 (86%)	NS	1.2 (0.142–10.119)
C	2 (17%)	2 (14%)	NS	0.833 (0.099–7.027)
Dominant model (GC+CC vs. GG) GC+CC	2 (33.3%)	2 (28.6%)	NS	0.8 (0.076–8.474)
Recessive model (CC vs. GG+GC) GG+GC	6 (100%)	7 (100%)		1.0
CC	0	0	–	–

The TGF-β1 +915 G/C SNP did not had any significantly associated with age ($\chi^2 = 3.086$; $P = 0.214$).

3.2.3 Correlations between IL-6 −174 G>C and TGF-β1 +915G>C SNPs and ovarian cancer

Spearman correlation analyses were performed for IL-6 and TGF-β1genotypes and alleles among ovarian cancer patients. In IL6 −174 G>C, the CC genotype in addition to C allele is positively correlated with ovarian cancer disease ($r = 0.513$; $P < 0.001$ and $r = 0.233$; $P < 0.05$ respectively), while GG, GC genotypes and G allele were negatively associated with disease ($r = -0.233$; $P < 0.05$, $r = -0.280$; $P < 0.01$ and $r = -0.513$; $P < 0.01$, respectively).

In TGF-β1 +915G>C, the GC genotype and C allele are positively correlated with ovarian cancer disease ($r = 0.268$; $P < 0.01$ and $r = 0.241$; $P < 0.05$ respectively), while GG genotype is negatively correlated with disease ($r = -0.241$; $P < 0.05$).

3.2.4 IL-6 and TGF-β1 gene polymorphisms and their plasma levels correlation

Plasma IL-6 and TGF-β1 had a significant increase ($P < 0.001$) in the ovarian cancer group compared to normal controls, IL-6 (97.83 ± 26.94 pg/ml versus 28.71 ± 19.42 pg/ml for patients and controls, respectively) (Fig. 3A). Plasma IL-6 and TGF-β1 had a significant increase ($P < 0.001$) in the ovarian cancer group compared to normal controls, IL-6 (97.83 ± 26.94 pg/ml versus 28.71 ± 19.42 pg/ml for patients and controls, respectively) (Fig. 3A), while TGF-β1 (139.18 ± 31.86 versus 70.52 ± 54.27 for patients and controls, respectively) (Fig. 4A). Ovarian cancer had a significant positive correlation with the IL-6 plasma level ($r = 0.830$;

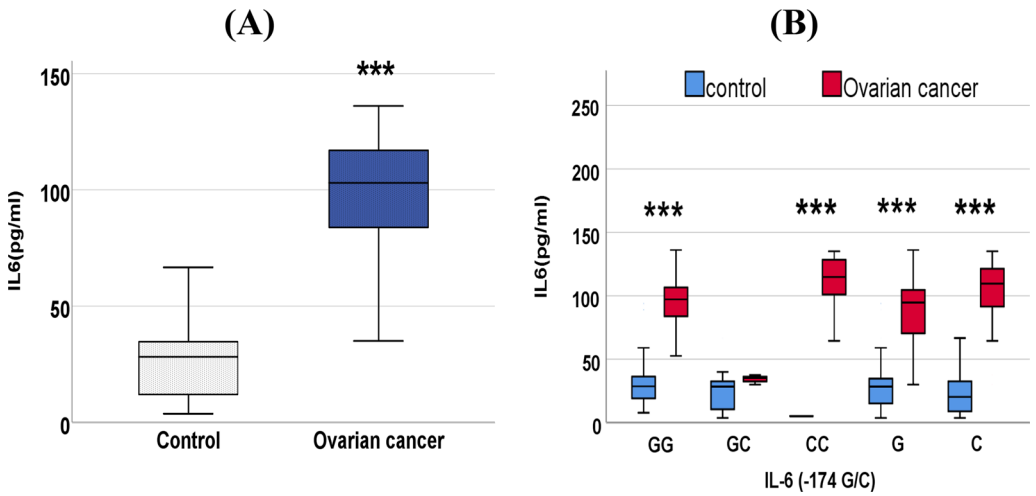

Fig. 3 IL6 concentration (pg/ml) in relation to **A** control and OC groups, **B** genotypes and alleles of IL6 (−174 G/C) polymorphism in control and ovarian cancer groups. Results are expressed as mean ± standard error. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

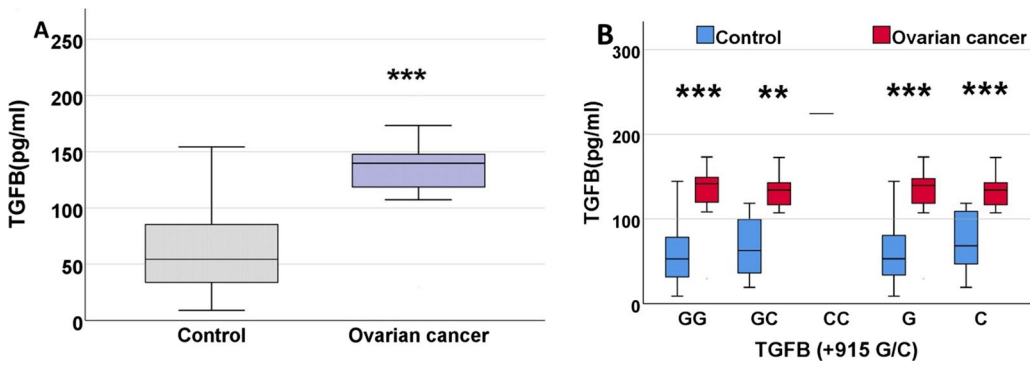

Fig. 4 TGF-β1 concentration (pg/ml) in relation to **A** control and ovarian cancer groups, **B** genotypes and alleles of TGF-β (+915 G/C) polymorphism in control and ovarian cancer groups. Results are expressed as mean ± standard error. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

Table 8 Mean plasma concentrations of TGF-β1 according to (+915 G/C) genotypes and IL-6 according to (−174 G/C) genotypes in controls and ovarian cancer patients

Genotype	Control group (*N* = 48) (Mean ± SE)	Ovarian cancer (*N* = 48) (Mean ± SE)	*P* value
IL-6 (−174 G/C) (pg/ml) (*N*)			
G/G (34, 23)	31.03 ± 3.40	95.16 ± 4.27	*P* < 0.001***
G/C (13, 3)	24.45 ± 4.88	34.16 ± 2.20	NS
C/C (1, 22)	5.20 ± 0.0	109.31 ± 4.55	*P* < 0.001***
G (47, 26)	29.21 ± 2.81	88.12. ± 5.42	*P* < 0.001***
C (14, 25)	23.07 ± 4.73	100.30 ± 6.39	*P* < 0.001***
TGF-β1 (+915G/C) (Pg/ml) (*N*)			
G/G (41, 31)	67.34 ± 8.08	142.57 ± 6.72	*P* < 0.001***
G/C (6, 17)	66.56 ± 15.30	133.01 ± 4.13	*P* < 0.01**
C/C (1, 0)	–	–	–
G (47, 48)	67.24 ± 7.26	139.18 ± 4.59	*P* < 0.001***
C (7, 17)	89.11 ± 25.99	133.01 ± 4.13	*P* < 0.001***

SE standard error

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Data were analyzed using Students *t* test

$P < 0.001$) and plasma TGF-β1 ($r = 0.615$; $P < 0.001$). IL-6 and TGF-1 plasma concentrations in different genotypes of control and ovarian cancer patients are compared in (Table 8; Figs. 3B, 4B).

Plasma IL-6 and TGF-β1 had increased in age 56–65 y compared to age 45–55y and age 66–80y in ovarian cancer patients but not reach to be significant difference, IL6 (56–65 y: 107.56 ± 14.73; 45–55y: 101.47 ± 20.84; 66–80y: 87.20 ± 38.72, $P > 0.05$), TGF-β1 (56–65y: 146.92 ± 36.05; 45–55y: 135.51 ± 35.22; 66–80y 134.21 ± 17.75, $P > 0.05$).

In IL-6 (−174 G>C), IL-6 concentration in ovarian cancer patients with GG and CC genotypes was increased significantly ($P < 0.001$) in ovarian cancer patients compared to controls. Moreover, the plasma level of IL-6 in ovarian cancer patients with G and C alleles was also increased significantly ($P < 0.001$) compared to healthy control. The plasma level of IL-6 was higher in ovarian cancer patients with GC genotype but without a statistical significance (Table 8; Fig. 3B).

A substantial difference in IL6 plasma levels was observed between the genotypes of the IL6 gene polymorphism in the ovarian cancer group. The post hoc comparison test showed that participants with GG and CC genotypes had significantly ($P < 0.001$) higher mean + SE values for IL 6 than those with GC genotypes.

In TGF-β1 (+915 G>C), the mean plasma levels of TGF-β1 in ovarian cancer patients with GG and GC genotypes had a significant increase ($P < 0.001$ and $P < 0.01$, respectively) compared to controls. The plasma level of TGF-β1 in ovarian cancer patients with G and C alleles was also increased significantly ($P < 0.001$) (Table 8; Fig. 4B). Plasma TGF-β1 levels did not differ significantly between genotypes of TGF-β1gene polymorphism. These results indicated that no specific genotype or allele could be responsible for the elevation of plasma TGF-β1 in ovarian cancer patients.

Plasma TGF-β1 and IL6 levels did not differ significantly between all subgroup ages for GG, GC, and CC genotypes in ovarian cancer patients. These results indicated that no specific age could be responsible for the elevation of plasma TGF-β1 or IL6 in any genotype of two SNPs in ovarian cancer patients.

3.2.5 Association between different biochemical parameters and cytokine gene polymorphisms

The subgroup analysis of IL-6 and TGF-β1 genotypes for the biochemical parameters showed that none of the genotypes of IL-6 (−174) and TGF-β1 (+915) gene polymorphisms were significantly different for any biochemical parameters for patients and control groups.

3.2.6 Plasma IL-6 and TGF-β1 with biochemical characteristics of patients and controls correlations

Upon analysis for correlation of IL-6 with TGF-β1 serum levels with biochemical characteristics of patients and healthy control, there was a statistically significant positive association of IL-6 with CA125, AST, and ALT ($P < 0.01$) and with a BUN ($P < 0.05$). A significant negative correlation of IL-6 was found with Albumin and TLC ($P < 0.01$) (Fig. 5). Whereas for the correlation of TGF-β1 plasma level with biochemical characteristics of patients and controls, there was a significant positive correlation of TGF-β1 was found with CA125, AST, and ALT ($P < 0.05$) and BUN and IL6 ($P < 0.01$) and a statistically significant negative correlation with albumin ($P < 0.01$) (Fig. 6).

4 Discussion

Uncertainty surrounds the pathophysiology of ovarian cancer and there is a lack of early detection. It is therefore unlikely that ovarian cancer will survive five years [62]. According to tumor stages distribution rates, about a quarter of ovarian cancer patients are diagnosed as stage I [63]. With some variations, consistent with our analysis that showed FIGO III and FIGO IV was 70.83% of the ovarian cancer patients, Braicu et al. [64] study found FIGO III and FIGO IV in 76.4% of ovarian cancer patients, while FIGO III and FIGO IV were found in only 40–50% of the patients in the Bushley et al. [65] study.

In this study, we investigated the role of functional IL-6 −174G/C SNP in the promoter region of IL-6 gene and TGF-β1 +915 in exon 1 as a potential ovarian cancer risk

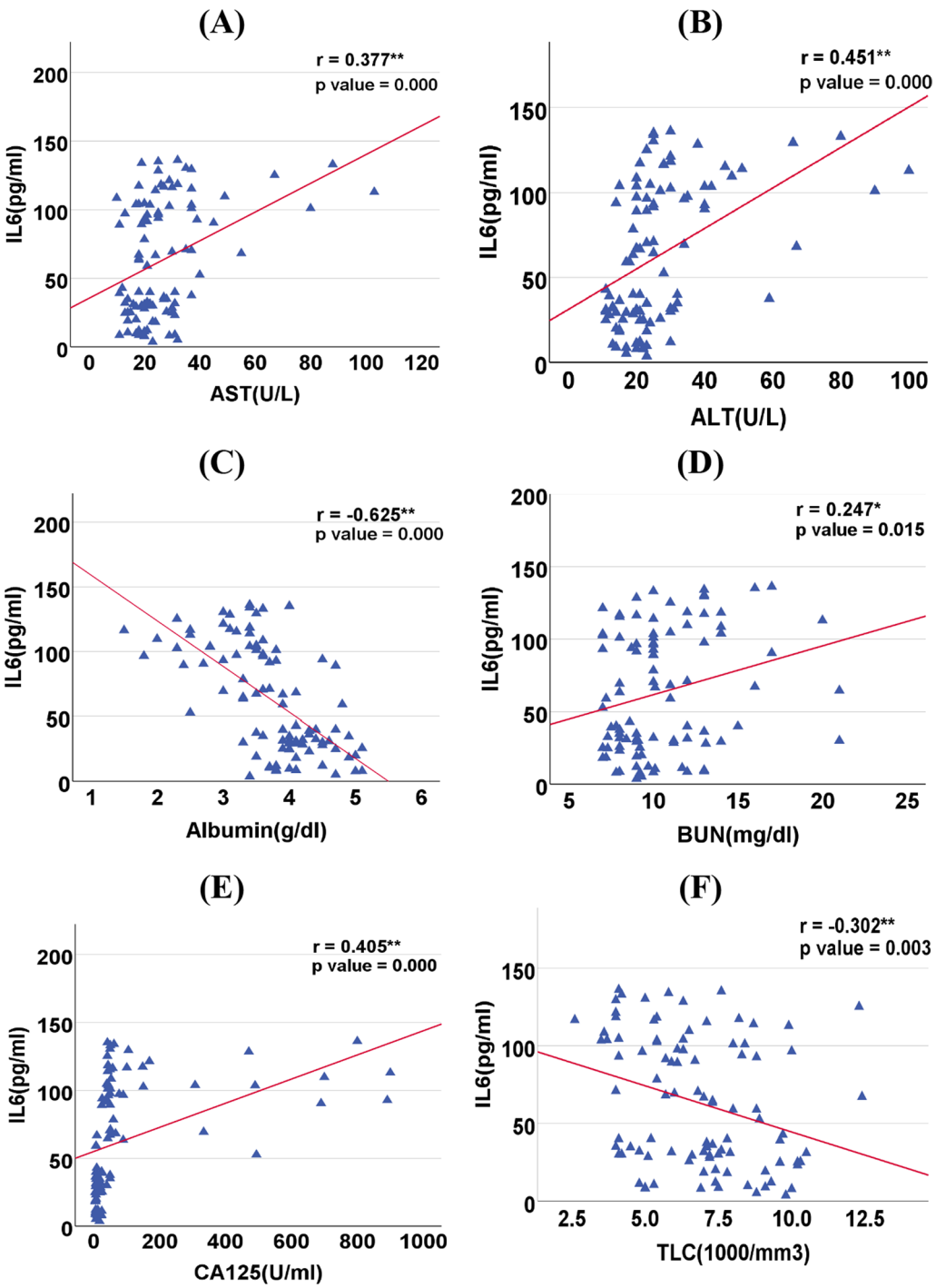

Fig. 5 Correlation between IL6 (pg/ml) with **A** AST, **B** ALT, **C** Albumin, **D** BUN, **E** CA125 and **F** TLC among ovarian cancer and control groups.r, Pearson's correlation coefficient. *$P < 0.05$; **$P < 0.01$ and ***$P < 0.001$. Abbreviations: *IL* Interleukin, *AST* Aspartate aminotransferase, *ALT* Alanine aminotransferase, *BUN* Blood urea nitrogen, *CA125* Cancer antigen 125, *TLC* Total leucocytic count

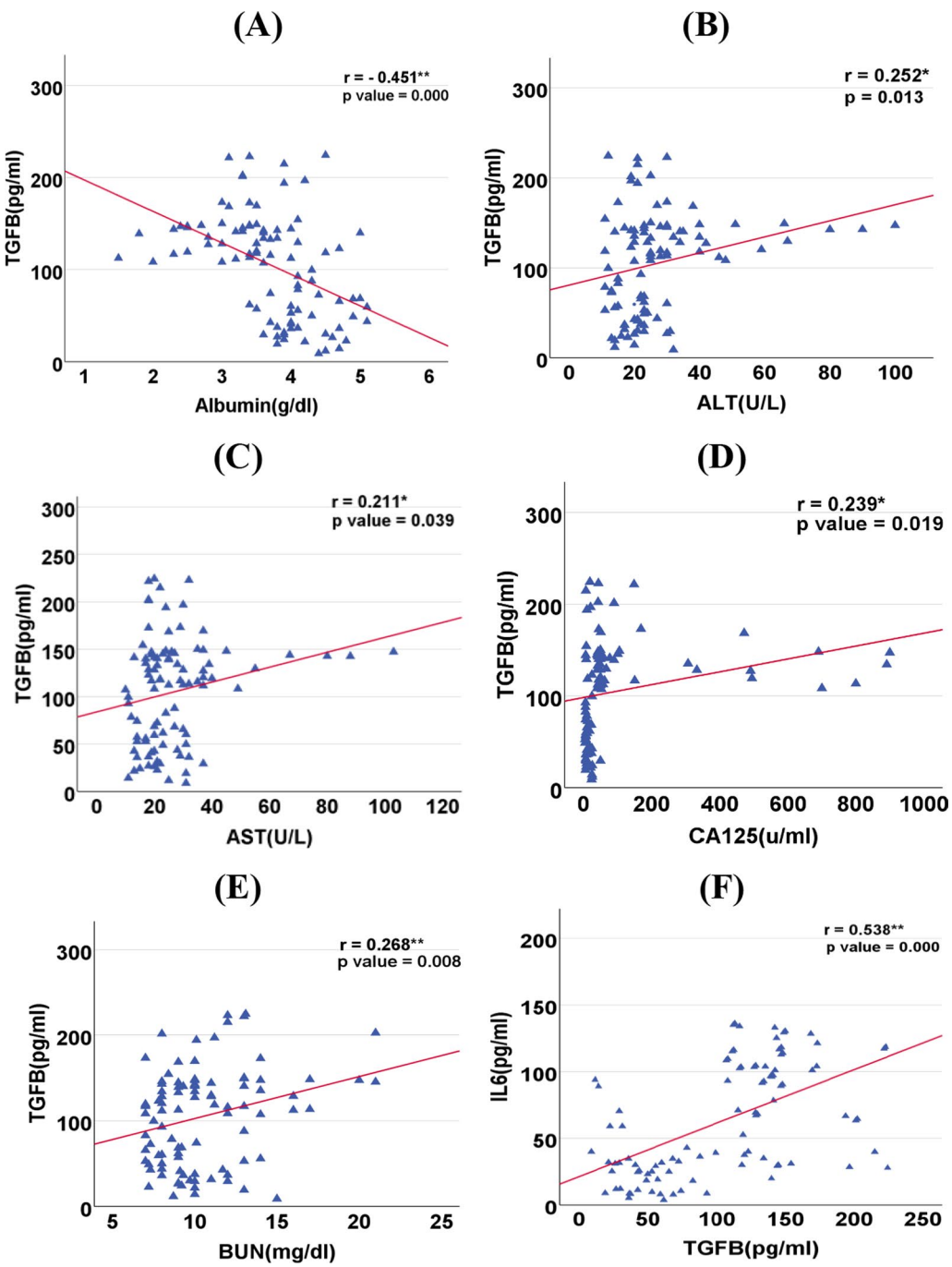

Fig. 6 Correlation between TGF-β1 (pg/ml) with **A** Albumin, **B** ALT, **C** AST, **D** CA125, **E** BUN and **F** IL 6 levels among ovarian cancer and control groups.r, Pearson's correlation coefficient. **P* < 0.05; ***P* < 0.01; ****P* < 0.001. Abbreviations: *IL* interleukin, *ALT* Alanine aminotransferase, *AST* Aspartate aminotransferase; *CA125* Cancer antigen 125, *BUN* blood urea nitrogen, *TGFB* transforming growth factor beta

factor in a case–control study design with 48 case subjects and 48 control subjects.

In the tumor microenvironment, interleukin-6 (IL-6) is a critical cytokine that has been found in significant levels and is known to be uncontrolled in cancer [29]. Due to IL-6 s ability to regulate all hallmarks of cancer and multiple signaling pathways, including apoptosis, survival and proliferative processes, invasiveness, angiogenesis, and metastasis in addition to metabolism, high

levels of IL-6 in the tumor microenvironment reflect the association between inflammation and cancer [29].

The results of IL6 concentration in ovarian cancer are controversial. As a result of our investigation, the plasma IL-6 concentration in ovarian cancer patients was significantly greater than in healthy controls. All of these outcomes were in line with earlier research findings [62, 66, 67]. Conversely, an earlier work by Ziltener et al. [68] showed that IL-6 expression was reduced in cultures of immortalized and malignant ovarian cells compared to epithelial cells of the normal ovarian surface.

In our study, ovarian cancer was significantly positively correlated with plasma IL-6. Consistent with our results, Clendenen et al. [67] found a positive association between IL-6 and ovarian cancer risk, while the results of Poole et al. [69] did not observe any correlation between IL-6 and ovarian cancer risk.

In the genotyping of IL6 (−174G>C), in our results, there was a significant increase in CC, GC+CC genotypes in ovarian cancer patients compared to healthy controls, and the C allele was considered susceptible to ovarian cancer while allele GC genotype and G allele was considered a protective factor for ovarian cancer.

In contrary to our results, the studies of Lu et al. [70] and Bushley et al. [65] showed that genotypic distribution of the IL-6 (−174) polymorphisms did not show any significant difference between ovarian cancer patients and the control group, the results of Liu et al. [71] study showed also no association between IL6 (−174 G/C) and a few common sorts of cancer, inverse associations have been reported in the study of Cozen et al. [72] and Banday et al. [73] between the −174 C allele and the risk of young adult Hodgkin lymphoma (HL) and colorectal cancer, respectively, IL6 −174 CC genotype was associated with a significant risk decrease in colorectal cancer relative to the GG genotype [72–75], and Banday et al. [73] reported the combined variant genotype (GC+CC) was significantly associated with a decreased risk of colorectal cancer. Hefler et al. [76] reported the CC genotype was significantly higher among breast cancer Caucasian women, consistent with our results.

There are conflicting results in the studies that analyzed the association between IL-6 plasma concentration and IL-6 genotypes. In our study, the mean plasma concentrations of IL-6 in ovarian cancer patients with GG and CC genotypes and G and C alleles showed a significant increase in ovarian cancer patients compared to controls. Besides, the highest value for IL 6 in subjects carrying CC genotypes in comparison with GC and GG genotypes, and C allele compared with the G allele.

Furthermore, this effect is possibly due to the increased IL-6 activity associated with the CC genotype or C allele which results in an increase or stimulation of the pro-tumorigenic activity of this cytokine [62], which may reflect through inter-individual differences in susceptibility to ovarian cancer associated with this SNP. Consistent with our study, it has been shown that the −174 C allele is related to high levels of unstimulated IL-6 [77].

Inconsistent with our findings, genotyping GG resulted in a larger production of interleukin-6 (IL-6), according to Fishman et al. [78] and Cardellini et al. [79], Fishman et al. [78] reported the −174 C allele was related to low levels of IL-6 in the unstimulated plasma, Hegedus et al. [80] and Talaat et al. [51] confirmed the absence of any correlation between the IL-6 (−174G/C) genotypes and plasma IL-6 levels, and many disorders have been linked to greater IL6 levels when the −174 G allele is present [81]. IL-6 expression is greater in −174GG/GC genotypes carriers than in −174CC genotype carriers [38].

The TGF-β family of proteins is involved in a variety of biological processes, including tissue homeostasis, development, growth, and immune system modulation [82]. As it turns out, the Smad4 (Mad-related protein 4) binding region shares some nucleotide similarity with the −174G/C polymorphism. Sma- and Mad-related protein 4 (Smad4) is a transcription factor that inhibits the expression of proinflammatory molecules by participating in the signal transduction cascade of TGF-β and activin [83].

Patients with ovarian cancer had significantly higher plasma TGF-β1 levels than healthy controls in our study, which was in line with previously documented data [84]. Other studies have found that hepatocellular carcinoma, colon, lung, and prostate cancer patients also have been reported to have elevated plasma levels of TGF-β1 in cancer patients and have a poor prognosis [85]. Two studies also have been reported to have elevated plasma levels of TGF-β1 in schizophrenia patients [86, 87]. Inconsistent with these data, El-Etreby et al. [88] demonstrated a statistically insignificant decrease in their levels with the progression of ovarian cancer. The secretion of TGF-β1 from activated mononuclear cells within the circulation causes elevation of TGF-β1 in the plasma of ovarian cancer patients. Therefore, patients with ovarian cancer may have significant thrombocytosis [89], and increases in plasma TGF-β1 concentration in cancer cases have been correlated with the count of platelets, proposing that TGF-β1 originated in platelets [90]. This result was inconsistent with our study where we reported a nonsignificant negative correlation between TGF-β1 and platelet count.

Our study revealed that GG was more frequent in both ovarian cancer and controls, similar to the study of Farahbakhsh et al. [91] in pancreatic cancer. In our results, there was a statically significant increase in GC and GC+CC in ovarian cancer cases compared to healthy

controls and considered as risk factors for ovarian cancer. Similar to our study, Hsu et al. [92] concluded that TGF-β1 +915 GC genotype was significantly higher in frequency in oral cancer patients compared with healthy control; also, Tang, et al. [93] study on esophageal squamous cell carcinoma (ESCC) cases reported that patients carrying GC/CC of TGF-β1 +915 polymorphism had an increased risk of ESCC and shorter overall survival. Di et al. [94] suggested that the TGF-β1 +915 CC genotype and C allele were significantly higher than that in the control group, inconsistent with our results. Another study by Guan et al. on gastric patients showed that patients with TGF-β1 +915 CG and CC genotypes had a poorer 2-year survival than patients with the GG genotype [95]. Inconsistent with our results, Wei et al. [96] in HNC, Gaur et al. [97] in tobacco-related oral carcinoma, Farahbakhsh et al. [91] in pancreatic cancer, and Niu et al. [98] in gastric cancer reported that no significant association between TGF-β1 +915G/C polymorphism and cancer risk.

Genetic variations in the coding region of the TGF-β1 gene may affect protein synthesis and transcription [44]. In our study, the mean plasma levels of TGF-β1 in ovarian cancer cases with GG and GC genotypes and G and C alleles were increased significantly in ovarian cancer cases compared to healthy controls. Besides, there was no statistically significant difference in plasma TGF-β1 between the genotypes of TGF-β1gene polymorphism. In the line of our study, Anna Liberek et al. [99] did not observe any significant correlation between TGF-β1 +915G>C genotypes and plasma level in children with inflammatory bowel disease. Dunning et al. [100] showed that the wild-type G allele of codon 25 is associated with increased production of TGF-β1 and Di et al. [94] reported the TGF-β1 +915 CC genotype is associated with increased production of TGF-β1 in lung cancer patients, which is different from our study. Interestingly, the TGF-β1 can change its function from a tumor suppressor to an oncogenic status in the later stages of cancer development [91].

In general, the EOC is a postmenopausal disease [101]. Consistent with our results, some studies have shown that the median age of diagnosis is 50–79 y [102, 103]; however, there is no definitive link between age and the result of ovarian cancer. In this study, we evaluated the possible effect modification of the association between IL-6 −174G/C SNP and TGF-β1 +915 G/C genotypes and ovarian cancer risk by age. We did not have any significant association between the IL-6 −174G/C SNP or TGF-β1 +915 G/C with age in ovarian cancer patients. In contrast to our findings, Banday et al. [73] found a significant association between the IL-6 −174G/C SNP with age in colorectal carcinoma (CRC), and they found that the risk of developing CRC in subjects who carried the variant genotype (CC) increased with age.

An important issue in the current study is the sample size because genetic variations are population-specific. Only tentative inferences may be drawn based on these results due to the relatively small sample size. To confirm this research, as well as to thoroughly understand the putative association between cytokine gene polymorphisms and ovarian cancer, and their interplay with other genetic and environmental factors, the results should be replicated in a larger population with different polymorphic sites of the IL-6 and TGF-β1 genes. Finally, ovarian cancer cells secrete IL6 to support its development and metastasis, and TGF-β1 elevation is associated with increased tumor dissemination. So, both should be considered as potential targets for ovarian cancer diagnosis and treatment.

5 Conclusions

This preliminary data indicated that IL-6 −174 CC and GC+CC genotypes and IL6 −174C allele may be risk genetic components to the susceptibility to ovarian cancer. On the other hand, the IL6 −174G allele is considered a protective factor for ovarian cancer. TGF-β1 +915GC and GC+CC genotypes may be a risk genetic component to the susceptibility to ovarian cancer. Plasma IL6 and TGF-β1 levels were significantly increased in ovarian cancer patients. Our results indicated that no specific genotype allele could be responsible for the elevation of TGF-β1 in the plasma of ovarian cancer patients, while the highest significant value for IL 6 in subjects carrying GG and CC genotypes compared to GC genotype and C allele compared with the G allele. Collectively, IL-6 (−174 G/C) and TGF-β1 (+915 G/C) gene polymorphisms may be associated with the susceptibility to ovarian cancer patients.

Abbreviations

TGF-β1: Transforming growth factor β-1; IL-6: Interleukin 6; MS-PCR: Mutagenically separated polymerase chain reaction; ARMS-PCR: Amplification mutation refractory system; FIGO: International Federation of Gynecology and Obstetrics; PCR-SSP: Polymerase chain reaction sequence-specific primer; EOC: Epithelial ovarian cancer; LD: Linkage disequilibrium; Smad4: Sma- and Mad-related protein 4; BRCA: Breast Cancer Gene; FDA: U.S. Food and Drug and Drug Administration; ASR: Age-standardized rate; PBS: Phosphate buffer saline; BSA: Bovine serum albumin; HPR: The horseradish peroxidase; TMP: Thermo-mechanical pulp; TRI: TGFβ receptor I; MMP: Matrix metalloproteinase; VEGF: Vascular endothelial growth factor; Smad4: Sma- and Mad-related protein 4.

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Authors' contributions

ZMA and AN designed the research. ZMA, KAE and EHMH performed the research. ZMA analyzed data. ZMA and RE designed the figures. ZMA, AN, EHMH, KAE, and RE wrote the text. All authors discussed the data and contributed to the final version. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
This study was approved by the ethical committee of Department of Biotechnology and Life Sciences, Faculty of Postgraduate Studies for Advanced Sciences, Beni-Suef University, Beni-Suef, Egypt, and the verbal Informed consent was approved by the ethical committee from all the study subjects.

Consent for publication
Not applicable.

Competing interests
The authors declare that there are no conflicts of interest or personal relationships with other people or organizations that could have appeared to influence the work reported in this paper.

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