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Role of IL-33 and IL-1α in Ovarian Cancer Metastasis

Gulshan Parveen [a*], Aaisha Qadir [b], Abubakar Sarfraz [b], Ahmad Farooq Butt [c], Rubab Aftab [a], Qurrat-Ul-Ain Laghari [c], Faheem Hadi [a], Laila Sumreen [d], Qurban Ali [a], Awais Altaf [a], Huma Sattar [a], Anam Naz [a], Mahwish Arooj [c] and Arif Malik [a]

[a] Institute of Molecular Biology and Biotechnology, The University of Lahore, Pakistan.
[b] Pak Red Cresent Medical and Dental College, Dinanath Kasur, Pakistan.
[c] University College of Medicine and Dentistry, The University of Lahore, Pakistan.
[d] University College of Conventional Medicine, the Islamia University of Bahawalpur, Pakistan.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: The current study conducted with aims to evaluate the levels of various inflammatory cytokines in women affected by ovarian cancer.
Methods: Case-control study was conducted from 1st January 2018 to 4th March 2018 among women affected by ovarian cancer in Institute of Molecular Biology and Biotechnology, The University of Lahore, Pakistan. Ovarian cancer patients (n=50) and control individuals (n=50) were included in present study. Serum was separated from patients and control individuals from which levels of various interleukins (IL-6, IL-33, IL-8, IL-10, IL-1α) and TNF-α were measured by ELISA kit method.
Results: The results of present study explain that increased levels of IL-10, IL-6, IL-8 and TNF-α were recorded in women affected by ovarian cancer in contrary with control individuals. Elevated trend of IL-33 and IL-1α were recorded in ovarian cancer patients (9.02±2.55 pg/ml and 8.44±2.55 pg/ml) in contrary with healthy group (3.88±1.02 pg/ml and 3.56±1.32 pg/ml) with statistical significant p-value=0.000.

**Corresponding author: E-mail: gulshanparveen14@gmail.com;*

Conclusion: Study has concluded that inflammatory cytokines particularly IL-1α and IL-33 are major source of inflammatory pathway in the development of ovarian cancer invasion, proliferation and metastasis.

Keywords: Ovarian cancer; IL-33; IL-6; IL-1α; IL-10.

1. INTRODUCTION

Ovarian cancer is most lethal gynecological malignancy in USA and sixth common cause of death in worldwide. In the USA, approximately 22,321 patients with ovarian cancer were diagnosed in 2018.There are two types of ovarian cancer based on origin and phenotype of the patient. Type I ovarian tumor is classified into three groups including low grade serous ovarian cancer, malignant carcinoma and mucinous tumors. Type II ovarian tumor is categorized into high grade serous cancer and undifferentiated ovarian cancer. These are worst type of malignancy which is expressed in advance stage of disease causing 75% cases and 90% death rate in ovarian carcinomas [1].

In ovarian cancer the secretion of cytokines may influence the cellular behaviour. Cytokines as immunomodulating agents contributed in paracrine signaling, autocrine signaling, and endocrine signaling [2]. It may comprise of interferon, chemokines, tumor necrosis factor, lymphokines and interleukins. They are formed in a wide range of cells mainly immune cells (macrophages, T lymphocytes, mast cells, fibroblasts, B lymphocytes, endothelial cells and several stromal cells). Cytokines act through receptors in the immune system and modulate the balance between cell based and humoral immune responses. They are also involved in the regulation of cellular growth, maturation and receptiveness at the particular cell. There are a number of cytokines that inhibit or enhance the activity of other cytokines [3]. They are different from hormones that are also crucial cell signaling molecules. The involvement of inflammation in the progression of cancer was firstly depicted by Rudolf Virchow in 1863. According to his observation, the inflammatory cells infiltrate the tumors and cancer from the inflammatory sites [4]. Moreover, Virchow has also supported the copious evidences about the linkage of infection and chronic inflammatory diseases.

Inflammatory responses and inflammation-associated diseases are stimulated by pathogenic agents and tissue injury. If inflammatory process is dysregulated, then it turns into chronic and stimulating malignant cell transformation in adjacent tissues. Inflammatory response contributes in several molecular pathways and cell signaling events, including angiogenesis and apoptosis. Other literature has suggested that the use of nonsteroidal anti-inflammatory drugs (NSAIDs) may reduce the mortality rate of various cancers [5-6]. The acute inflammation is due to the result of modification induced by pathogens, chemical or physical stress. The main reason of inflammation is to trigger cell deterioration and restore homeostasis in the damaged tissues. Chronic inflammation induces carcinogenesis and malignant cell transformation. Various inflammatory mediators, including IL-6, TGF-β and TNF-α, have been reported to contribute in ovarian cancer initiation and progression [7]. The main objective of the study is to determine the levels of various interleukins (IL-1α, IL-33, IL-8, IL-6, IL-10 and TNF-α) in Pakistani women affected by ovarian cancer and to find out the difference among all interleukins in affected females which progressively involved in the progression and metastasis of ovarian cancer. Among all interleukins, to find the specific interleukins that progressively involved in the development of ovarian carcinoma.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Fifty female ovarian cancer patients were monitored at INMOL Cancer Hospital Lahore, Pakistan along with fifty health individuals as control. The patients with the age group of 20-50 were included in the present study. All the history and medical prognosis of patients were collected from the hospital medical records. The patients with ovarian cancer presented wide range of vague and nonspecific symptoms such as constipation, shortness of breath, bloating, feeling pain during sex, indigestion, vaginal bleeding, swollen tummy, discomfort in pelvic area, feeling tired all the time, back pain and unintentional weight loss. During this condition, incessant ovulation and unexpected vaginal bleeding may affect the hematological profile and hormonal levels in ovarian cancer patients. It

have been confirmed that the experimental sample collection, complied with relevant institutional, national, and international guidelines and legislation with appropriate permissions from authorities of Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Lahore, Pakistan and the study has been reported in accordance with ARRIVE guidelines. Blood sample (5 mL) of blood sample was drawn in gel tube from patients and control individuals than centrifuge the whole blood sample for two hours at 2000rpm to separated serum. The serum was stored at -60°C for further biochemical analysis. During this study, various chemicals and reagents were used and purchased from chemical industry. The patients of this study were receiving neoadjuvant chemotherapy which was used to shrink the tumors to make them easier to remove. Before each chemotherapy session, the patients had blood tests to ensure their body's healthy cells become recovered or not.

2.2 Biochemical Examination

The complete blood count was determined by automated analyzer and levels of various interleukins including IL-1α, IL-33, IL-8, IL-6, IL-10 and TNF-α were measured by human available diagnostic ELISA kit method.

2.3 Statistical Examination

The statistical analysis was achieved by SPSS statistics (version 10) and the results of all biomarkers were analyzed by independent sample t-test.

3. RESULTS

3.1 Blood Profile of Female Affected by Ovarian Cancer Versus Control

The data expressed in Fig. 1 suggests the blood of women affected by ovarian cancer as compared to control group. The mean values of RBCs and platelets were significantly reduced in ovarian cancer women (3.32**±**0.12 million/$mm^3$and 224.12±121.21 10^9 /L) in contrary to control group (10.11**±**2.12million/$mm^3$and 399.33±40.55 10^9 /L) respectively. Increase trend of WBCs (9.21**±**1.21 million/mm^3 vs. 3.33±0.44 million/mm^3) and neutrophils (8.87±2.33% vs. 4.13±0.28%) were recorded in ovarian cancer women in contrary to control group correspondingly. Low levels of Hct, Hb and creatinine were measured in patient group (21.23±2.55%, 7.22±1.22 g/dl and 1.23±0.21 mg/dl) in contrast to healthy group (45.11±3.43%, 13.21±3.54 g/dl and 2.32±0.99 mg/dl) respectively. Apart from that, the mean values of lymphocytes (3.33±1.09% vs. 2.32±0.22%) and monocytes (2.21±0.55% vs. 1.44±0.24%) were significantly increased in ovarian cancer women as compared to healthy individuals.

3.2 Levels of Several Interleukins in Women Affected by Ovarian Cancer Versus Control

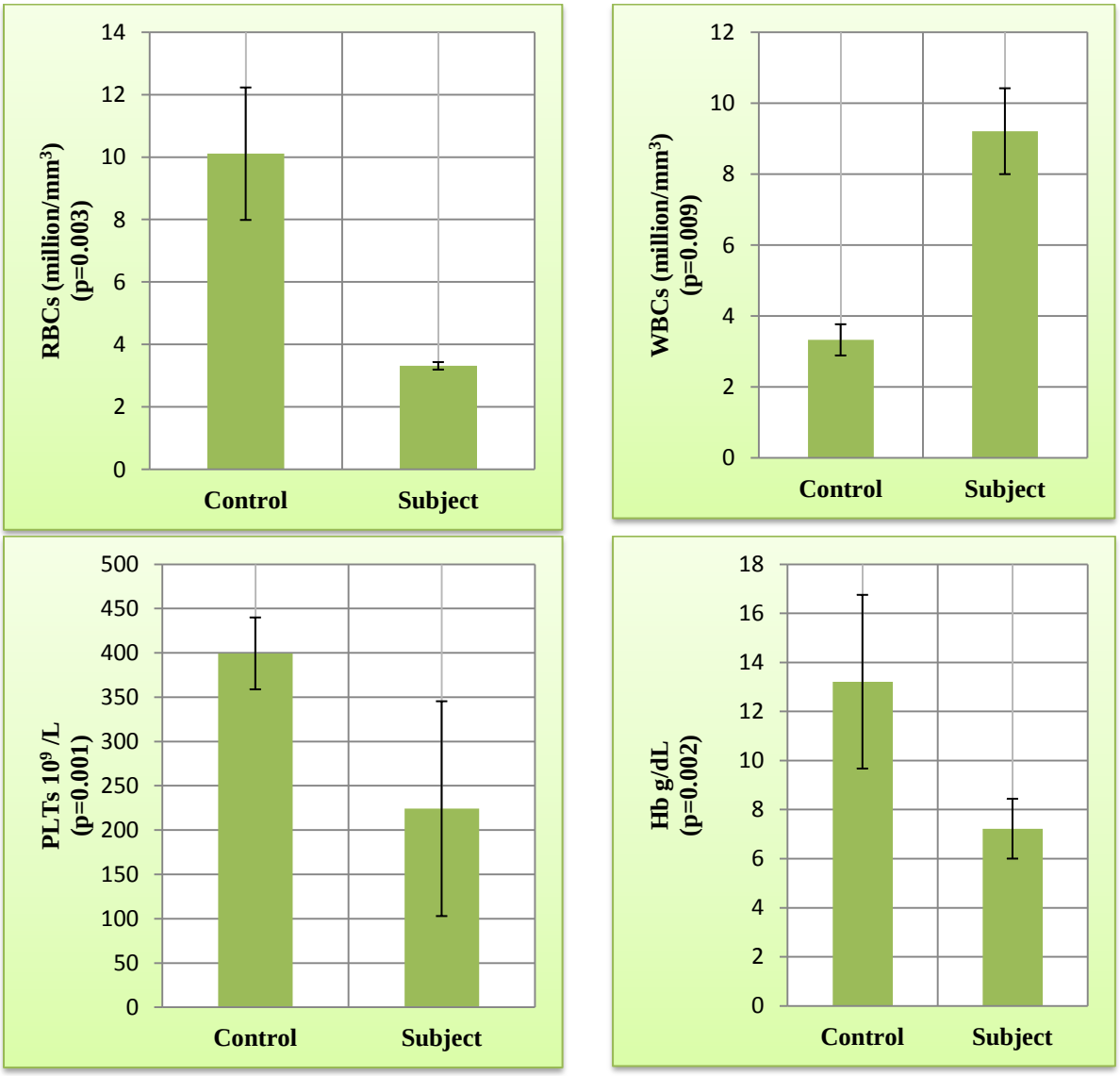
Data depicted in Fig. 1 represented the differential expression of interleukins in women affected by ovarian cancer as compared to control group. The mean values TNF-α and IL-10 were significantly increased in ovarian cancer women (9.32±1.21 pg/ml and 8.56±1.32 pg/ml) in contrary with control group (2.45±0.23 pg/ml and 2.22±0.10 pg/ml) respectively. Increased level of IL-6 (9.10±0.22 pg/ml vs. 2.48±0.19 pg/ml), IL-8 (9.23±1.40 pg/ml vs. 2.21±0.12 pg/ml) were recorded in these affected women by ovarian cancer in contrary with control group correspondingly. Highly statistically significant p-values of IL-33 and IL-1α were recorded among all interleukins in ovarian cancer patients. The mean values of IL-33 and IL-1α were significantly increased in ovarian cancer patients (9.02±2.55 pg/ml and 8.44±2.55 pg/ml) in contrary with healthy group (3.88±1.02 pg/ml and 3.56±1.32 pg/ml) with statistically significant p-value (i.e., 0.000).

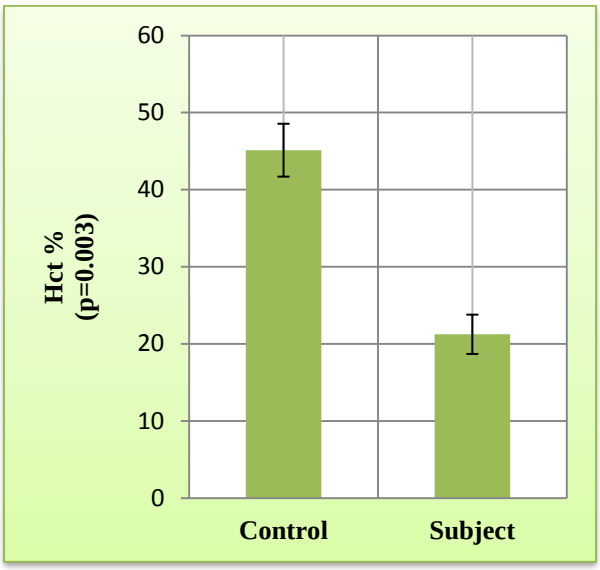
4. DISCUSSION

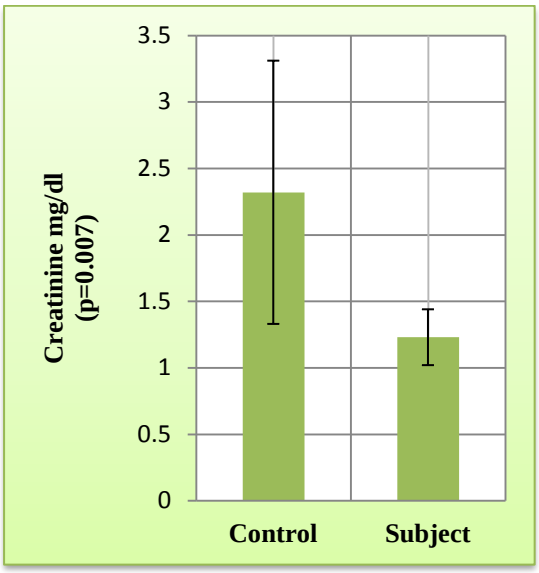
The importance of different interleukins and tumor microenvironment of ovarian cancer patients has been demonstrated in recent study. IL-33 and IL-1α is humoral factor and strongly interlinked with ovarian cancer. The overexpression of IL-33 facilitates tumorigenesis and significantly prolonged tumor metastasis in ovarian cancer. The immunological response consists of a series of events that induce recognition of tissue damage and pathogens. A number of soluble mediators, including cytokines of adaptive and innate immune response are involved during this process. One the main importance of the inflammatory response is to eliminate foreign particles that disturb cellular homeostasis [8]. During the physiological context, after the removal of pathogens and

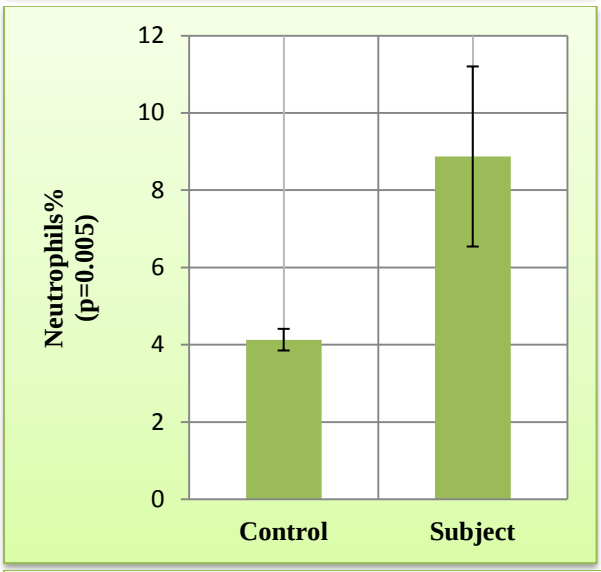
tissue repair, the homeostatic condition and inflammation is recovered [9]. Mounting evidence reported that inappropriately resolved of chronic inflammation may enhance the risk factor of cancer progression. Various pathologies demonstrated this association, such as chronic prostatitis, endometriosis, inflammatory bowel disease (IBD), ovarian cancer, primary sclerosing cholangitis (PSC), and gastritis. Bioactive molecules (*i.e*., cytokines, chemokines, growth factors) can be activated by inflammation that are responsible to enhance the risk of various cancers. These bioactive molecules maintain the cell survival, proliferative rate, apoptosis, several proangiogenic factors and ECM degrading enzymes, including matrix metalloproteinases which trigger epithelial mesenchymal transition (EMT) and promote other carcinogenic program, including immune evasion, reprogrammaing of energy metabolism, and genomic instability [10].

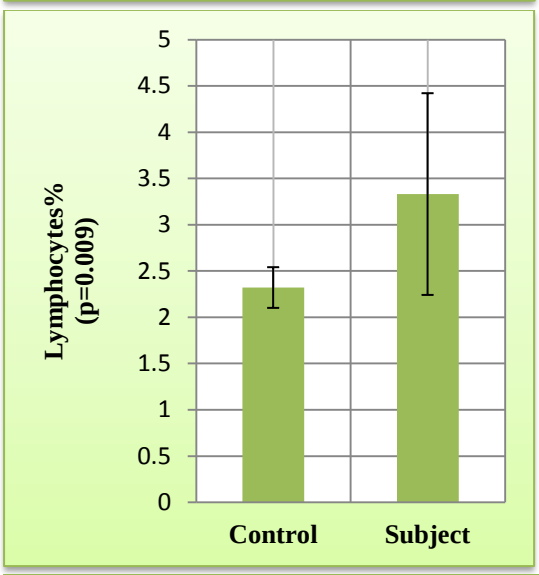
In this study, the patients with ovarian cancer had been observed with unexpected vaginal bleeding which is the major reason for the fluctuation of blood profile and anemia. Moreover, pro-inflammatory cytokines induce modification in the proliferation of erythroid progenitors, survival of erythrocytes and erythropoietin (EPO) [11]. In cancerous patients, overproduction of pro-inflammatory cytokines is linked with increased production of reactive oxygen species (ROS). These reactive species may inhibit erythropoiesis. Inflammation may also interfere with nutritional status, which in turn cause anemia in cancerous patients [12-13].

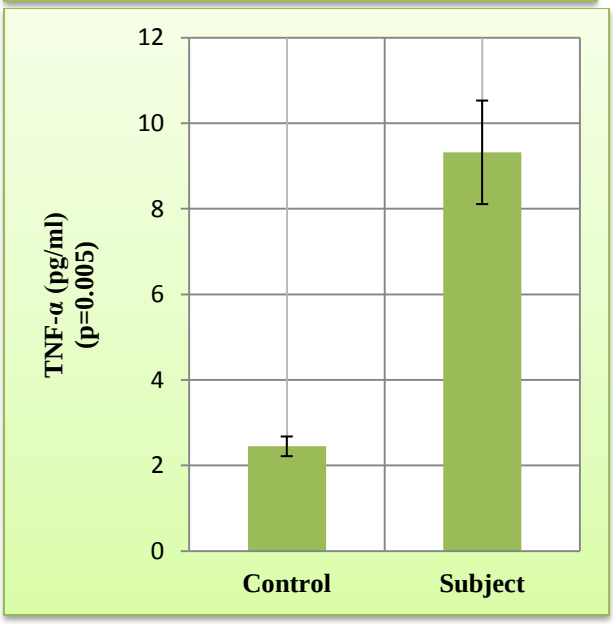


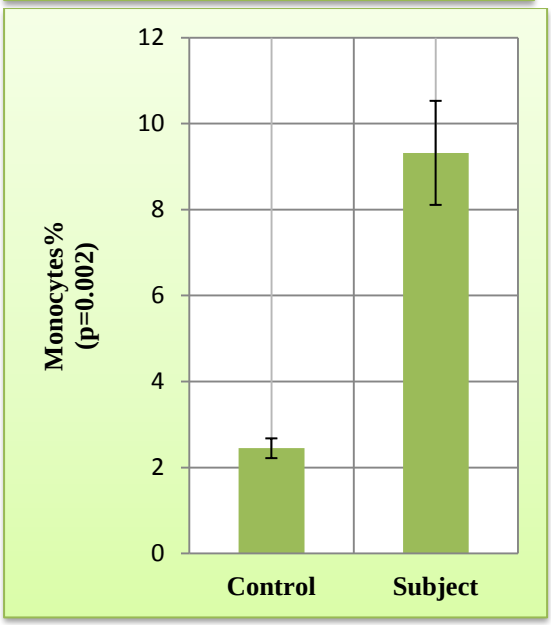

Hct %
(p=0.003)
60
50
40
30
20
10
0
Control
Subject


Creatinine mg/dl
(p=0.007)
3.5
3
2.5
2
1.5
1
0.5
0
Control
Subject


Neutrophils%
(p=0.005)
12
10
8
6
4
2
0
Control
Subject


Lymphocytes%
(p=0.009)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Control
Subject


TNF-α (pg/ml)
(p=0.005)
12
10
8
6
4
2
0
Control
Subject


Monocytes%
(p=0.002)
12
10
8
6
4
2
0
Control
Subject

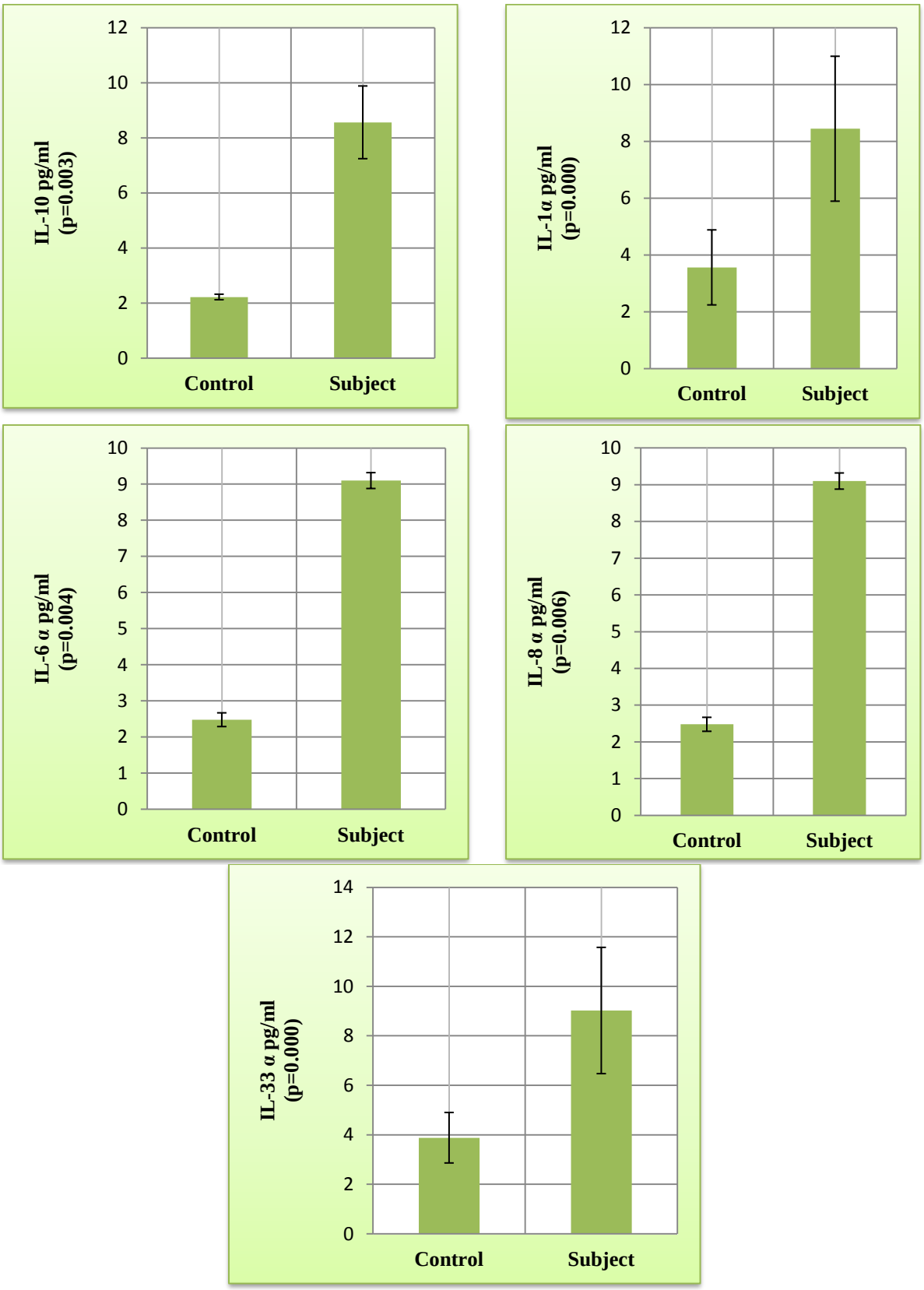


Fig. 1. Profile of CBC and different interleukins in the patients suffering from ovarian cancer versus control

Interleukins are secretary and low molecular weight proteins that induce cell to cell communication. Stromal and immune cells, including endothelial cells and fibroblasts may produce them and stimulate cell survival, proliferation, immune cell activation, differentiation, cell migration and cell death. In the tumor microenviroment, cytokines can promote an anti-tumor response, while in case of chronic inflammation, they can modulate cell malignancy and cell transformation in ovarian cancer [14]. There are basically two ways in which interleukins can promote the growth of tumor cells. They can promote the development of tumor cells directly by growth factors that trigger the metastasis by enhancing tumor angiogenesis and cell adhesiveness and act as influential mediatorof the immune system, which increase tumor growth byhampering cell-mediated mechanism that destroy and identify tumor cell. Through induction of JAK/STAT pathways, IL-6 is significantly involved in ovarian cancer cell proliferation, survival and migration. IL-6 is a major group of pro-inflammatory cytokine that plays an important role in ovarian tumor metastasis [15].In the current study, it has been reported that IL-6 protein in theca cell and granulosa cells were overexpressed due to elevated expression of IL-1α. It suggested that IL-1α not only has regulatory role on ovarian cells but also involved in various biological activities by producing other inflammatory cytokines. Moreover, it may also regulate ovulation gene expression through paracrine and autocrine manner in granulosa cells. IL-6 induces cytokine gene expression through mediating NF-κB and MAPK signaling mechanism. These pathways play important role in the stimulation of various activities in tumor microenvironment. NF-κB is signaling mechanism in IL-1α for the stimulation of cytokine gene expression. During this pathway, phosphorylation of Iκ-Bα was higher due to the stimulation of IL-1α [16].

In the present study, the levels of IL-8 are significantly increased in women affected by ovarian cancer as compared to control group. These high expressions of IL-8 reported to induce cell invasion, migration and epithelial mesenchymal transition to facilitate ovarian survival and growth. IL-8 is secreted from ovarian tumor that stimulate NF-κB/TAK-1 signaling pathway resultantly induce ovarian cancer growth and seeding in peritoneal cavity through intracellular protein (CXCR-2). Thereby, targeting CXCR-2 by various pharmaceutical or genetic approaches might be provide promising for suppressing ovarian cancer metastasis [17]. In the present study, IL-10 considered to be a crucial cytokine that repeatedly overexpressed in ovarian tumors and destruction of ovarian cell. In this study, IL-10 has positive correlation with advanced stage and poor prognosis of disease. Increased levels of IL-33 were also observed in ovarian cancer women in this study. IL-33 along with its receptor (ST2) might involve in ovarian cancer progression and development. On the other hand, ST2 has the ability to be suppressed by inhibitor which resultantly block ovarian cancer cell invasion, viability, migration. Moreover, IL-33 attaches with ST2 that stimulates downstream signaling mechanisms by MAPK and NF-κB mechanism which is the key player of cancer cell invasion and metastasis. NF-κB is also major inducer of inflammation and proliferation of ovarian cancerous cell [18-22].

5. CONCLUSION

Study has concluded that high levels of inflammatory cytokines are expressed in ovarian cancer tumors, particularly at metastatic sites. IL-33 and IL-1α promote ovarian cancer development and metastasis by facilitating JNK and ERK signaling cascades. Targeting these inflammatory cytokines and their related signaling mechanism may act as novel strategy to overcome ovarian cancer metastasis.

CONSENT AND ETHICAL APPROVAL

As per international standard or university standard guideline Patient's consent and ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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