RESEARCH ARTICLE

Comparison of serum Vascular Cell Adhesion Molecule (VCAM) level among epithelial ovarian cancer patients and healthy women

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Abstract

Objective: To compare serum vascular cell adhesion molecule levels among ovarian cancer patients of different standardised grades, and in relation to healthy controls.

Method: The case-control study was conducted from 6.2.2018 to 1.8.2021 after approval form the ethics review board of the University of Health Sciences, Lahore, Pakistan, and comprised females aged 20-70 years diagnosed with ovarian cancer and tentatively planned for surgical procedures in group A, and healthy controls in group B. From all the subjects, 2.5ml blood was taken in a green-top tube. The tubes were centrifuged, and the serum was separated within an hour of sample collection. The Eppendorf tubes were labelled and stored at -20°C. The samples were thawed, and, following the manual protocol, they were subjected to vascular cell adhesion molecule enzyme-linked immunosorbent assay. Cancer antigen 125 values before surgery and 6-8 months post-surgery were recorded from available laboratory reports. Also, group A was categorised in line with the International Federation of Gynaecology and Obstetrics stage classification. Data was analysed using SPSS 24.

Results: Of the 80 female subjects, 40(50%) were group A cases and 40(50%) were group B controls. The overall mean age was 48±12 years. Overall, 55(68.7%) women were aged <55 years and 25(31.3%) were aged >55 years. Within group A, 20(50%) had cancer stage I-II and 20(50%) had stage III-IV. Overall median vascular cell adhesion molecule was 72.40ug/L (interquartile range: 1857.40ug/L), with 71.15ug/L (interquartile range: 616.60ug/L) in group A and 74.10ug/L (interquartile range: 1848.70ug/L) in group B. Significant correlation was found between cancer stage and vascular cell adhesion molecule level (rho=0.73; *p*=0.003).

Conclusion: There was a decreased level of vascular cell adhesion molecule level in epithelial ovarian cancer cases compared to healthy controls. A positive association was observed between ovarian cancer stage and vascular cell adhesion molecule level.

Keywords: VCAM, FIGO, Correlation, Serum marker, Ovarian cancer. (JPMA 74: 1437; 2024)

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Introduction

Epithelial ovarian cancer tends to have adverse outcomes as its diagnosis is often missed at an early stage. This is the fourth most common cause of death in women. The five-year survival rate in ovarian cancer is <30%.[1] The recurrence rate is also high, around 80% within two years of completing the primary dose of therapeutic course.[2] In epithelial ovarian cancer, there is a lack of scientific markers for screening and monitoring response to therapy. Despite conventional tumour biomarkers, there is a clear need for smart screening tools showing specific diagnostic and prognostic potential.

Cellular adhesion molecules (CAM) are of prime physiological significance as they impart cellular interactions. An example of CAM is vascular cell adhesion molecule VCAM,[3] which is a cell surface receptor, and controls leucocyte attachment as well as extravasation in inflammatory tissues. Higher VCAM protein expression has been discovered in the mesothelium of ovarian cancer patients compared to healthy women.[4] VCAM is a soluble protein and can be detected in serum, so it is used for monitoring cancer growth. The expression of epithelial cell adhesion molecule (EpCam) is postulated to be found in normal as well as cancerous tissue sites.[5] Moreover, there is also evidence suggesting that VCAM is highly expressed in tumour tissue.[6,7] VCAM performs a function of attachment of cancerous cells, and is also responsible for enhancing the permeation through mesothelium.[8] The mesothelium is a single layer of mesothelial cells that line the peritoneal cavity. The cancer cells of the ovary metastasise by crossing the mesothelium under the effect of VCAM. Once there is peritoneal involvement by ovarian cancer cells, it affects the survival rate, with 5-year survival being <25%.[8] VCAM is also known for its functional role in angiogenesis.[9] Moreover, accelerated angiogenesis

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controlled by VCAM is also found in many other cancers, like breast cancer.[10]

VCAM plays a significant role in various immunological conditions and malignancies, and, hence, it could be targetted therapeutically by formulating VCAM action-blocking drugs11. Additionally, it is a crucial inflammatory mediator, angiogenesis marker and immunological phenomenon in numerous disease contexts.[11]

The current study was planned to compare serum VCAM levels among ovarian cancer patients of different standardised grades, and in relation to healthy controls.

Patients and Methods

The case-control study was conducted from 6.2.2018 to 1.8.2021 after approval form the ethics review board of the University of Health Sciences (UHS), Lahore, Pakistan. The study was conducted in accordance with the Helsinki Declaration of Human Rights[12] and the subjects were recruited using convenience sampling technique. The cases were enrolled from the Institute of Nuclear Medicine and Oncology (INMOL) Cancer Hospital, Lahore, while the controls were enrolled from the gynaecology outpatient department (OPD) of Hijaz Hospital, Lahore. All the experimental work was carried out at the Department of Physiology and Cell Biology, UHS.

The sample size was calculated, using the formula:

$$(n = \frac{2\sigma^2 \ (Z_{1-\alpha/2} + Z_{1-\beta})^2}{(\mu_1 - \mu_1)^2}$$

The level of significance was 5, power of test was 95%, population standard deviation 1, population variance 1, test value of population mean 2,[13] anticipated population mean 1, and sample size 26.

The sample size was also calculated by taking the mean levels of micro ribonucleic acid-93 (miR-93) in the same formula, with level of significance 5, power of test 95%, population standard deviation 0.53,[14] population variance 0.2809,[14] test value of population mean 1.68,[14]anticipated population mean 2, and sample size 72.

Therefore, considering the calculated sample size on the basis of different anticipated mean values, 70 cases of ovarian cancer and as many healthy women were targeted, but, due to budget constraints, only 1 enzyme-linked immunosorbent assay (ELISA) kit was available, and the sample size had to be curtailed.

After taking written informed consent, history was obtained for the patients in group A, and the diagnosis of ovarian cancer was confirmed based on the histopathology report related to the initial visit before the patients received their first chemotherapy session. Group A cases with serous, mucinous, endometroid, clear cell and mixed forms were categorised as malignant, while the borderline category included borderline serous, cytoadeno, papillary serous, endometroid adeno, papillary adeno, borderline serous, adeno, cytology-positive and mucinous papillary forms. Cancer antigen 125 (CA125) values before surgery and 6-8 months post-surgery were recorded from available laboratory reports. Also, group A was categorised in line with the International Federation of Gynaecology and Obstetrics (FIGO) stage classification.[15]

Those included were females aged 20-70 years diagnosed with ovarian cancer and those tentatively planned for any of the following surgical procedures: unilateral or bilateral oophorectomy, salpingo-oophorectomy via laparotomy or laparoscopy, subtotal resection, or removal of tumour fragments, and hysterectomy with salpingo-oophorectomy. Patients already on chemotherapy were excluded.

The healthy controls in group B were females aged 20-70 years with no history of any cancer, and who were in good health based on self-report. Women who had previously undergone any abdominal surgery, those with a family history of any cancer, or taking any hormonal treatment for any reason were excluded.

A 2.5ml blood sample was collected from all the subjects in both groups in a green-top tube. The tube was then centrifuged to separate the plasma, which was carefully preserved in Eppendorf tubes for subsequent ELISA analysis. Each tube was appropriately labelled and stored at -20°C to maintain sample integrity.

Serum VCAM was measured by double antibody sandwich human VCAM ELISA kit (Catalogue No. PRS-01608hu; manufactured by Glory Science, United States of America). The kit was able to determine VCAM concentrations ranging 20µg/L-400µg/L in human serum, blood plasma and other biological fluids. The serum samples and standards, pre-coated with human VCAM monoclonal antibodies, were added to the wells. Biotin-conjugated anti-human VCAM antibody and horseradish peroxidase (HRP) were added. The biotin-conjugated anti-human VCAM antibody binds to the human VCAM captured by the first antibody.

Following incubation and first washing, tetramethyl-benzidine (TMB) substrate solution, which is reactive to HRP, was added to the well. A coloured product was formed in proportion to the amount of human VCAM present in the sample or standard. The reaction was terminated by adding

sulphuric acid as the stop solution. The absorbance was measured using an automated ELISA reader. The values of OD (optical density) were obtained, and the actual concentration of the samples was calculated by multiplying the concentrations with the dilution factor.[1]

Data was analysed using SPSS 24. A box plot was used to explore the data distribution (Figure), and median values with interquartile range (IQR) were determined. Qualitative variables were expressed as frequencies and percentages. Mann Whitney U test was applied to see the significance of intergroup differences. Spearman's rho correlation test was used to see the relationship between cancer stages on the basis of VCAM level. P<0.05 was considered significant.

Results

Of the 80 female subjects, 40(50%) were group A cases and 40(50%) were group B controls. The mean age of the sample was 48±12 years. Overall, 55(68.7%) women were aged <55 years and 25(31.3%) were aged >55 years. Median VCAM level was low in group A compared to group B (Table 1).

Table-1: Median values of serum VCAM.

Overall VCAM	
Median	72.40
Range	1857.40
Minimum	26.00
Maximum	1883.40
Cases only	
Median	71.15
Range	616.60
Minimum	26.00
Maximum	642.60
Controls only	
Median	74.10
Range	1848.70
Minimum	34.70
Maximum	1883.40

VCAM: Vascular cell adhesion molecule.

Table-2: Distribution of VCAM cases.

Factors	n (%)
Age (years)	
<55	26 (65.0)
≥55	14 (35.0)
FIGO Stage	
Stage I	17 (42.5)
Stage II	3 (7.5)
Stage III	15 (37.5)
Stage IV	5 (12.5)
Tumour Type	
Malignant	33 (82.5)
Borderline	7 (17.5)
CA125 Baseline	
CA125 Follow-up	
High	40 (100)
Normal	11 (27.5)
High	29 (72.5)

VCAM: Vascular cell adhesion molecule, FIGO: International Federation of Gynaecology and Obstetrics, CA125: Cancer antigen125.

Table-3: Inter-group comparison on the basis of VCAM values.

Group Name	n	Median	Mann Whitney U Test	*p*-value
Cases	40	71.15	787.5	0.90
Controls	40	74.10		
Age <55 Years	55	75	507.0	0.06
Age ≥55 Years	25	67.1		

VCAM: Vascular cell adhesion molecule.

Table-4: Relationship between FIGO stage and VCAM values.

FIGO Stage of Cancer	n	Minimum	Maximum	Median	Spearman's rho	*p*-value
Stage I & II	20	45.60	642.60	71.55	0.739	0.003
Stage III & IV	20	26.00	271.50	69.35		

FIGO: International Federation of Gynaecology and Obstetrics, VCAM: Vascular cell adhesion molecule.

Within group A, 20(50%) had cancer stage I-II and 20(50%) had stage III-IV (Table 2).

The difference in median VCAM levels between the groups was non-significant, but VCAM level was higher in those aged ≥55 years (Table 3).

Significant correlation was found between cancer stage and VCAM level (rho=0.73; *p*=0.003) (Table 4).

Discussion

The mean age of the current sample was 48±12 years, which was in contrast with the median age 56.5 years (IQR: 22-83 years) reported earlier.[16] The current study had equal distribution of FIGO stage I-II and III-IV, whereas another study reported fewer FIGO stage I-II cases compared to stage III-IV cases.[12] The media VCAM level in the current sample was 72.40ug/L compared to 302.2ng/ml[16] reported earlier. Serum concentrations were found to be higher in ovarian cancer in another study[6] than the current study. The reason for inconsistent result might be explained on the basis of non-normally distributed data of the current study. Also, certain factors, like environmental, dietary habits, stress, tumour biology and disease behaviour could be the cause of varied results.

Another study showed serum VCAM in epithelial ovarian cancer 897±54g/L[17] and also found VCAM level higher in FIGO stage II-IV cases than FIGO stage I cases. The current study fund non-significant difference between FIGO stages 1-II and III-IV.

A study reported no correlation between serum VCAM level and histological types of cancer, but showed a correlation

with stage (metastatic vs non-metastatic) (p=0.03). In the current study, a significant positive correlation was noted with FIGO stage. In the current study, there were 11(27.5%) cases with normal CA125 (<35U/mL), while in 29(72.5%) cases, the level was ≥35U/Ml. Another study16 reported normal CA125 in 10 and high CA125 in 40 cases.

The current study has limitations as the ovarian cancer cases and healthy controls were not age-matched and belonged to different histological subtypes. Moreover, an unequal distribution of FIGO stages and financial constraints were also among the limitations.

Conclusion

Monitoring VCAM concentration, both in early and advanced stages, could serve as a promising marker in epithelial ovarian cancer. There was an association between FIGO stage and VCAM levels, indicating VCAM's potential as a non-invasive diagnostic and prognostic serum biomarker for ovarian cancer patients.

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Author Contribution:

RH: Conceived, design, statistical analysis, editing, data collection and writing.

SK: Review and final approval.