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Identification of Genetic Polymorphism of Centrosomal Protein 290 to Assess Its Role as A Novel Diagnostic and Prognostic Biomarker for Ovarian Cancer

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ABSTRACT

Ovarian cancer (OC) is one of the most lethal gynecological malignancies, with the dilemma of diagnosing at an advanced stage due to the lack of sensitive early detection markers. Centrosomal protein 290 (CEP290) is important for various functions within cellular processes, and its genetic changes may contribute to tumor development and progression. Identifying polymorphisms in the CEP290 gene could provide valuable insights into its potential as a novel diagnostic and prognostic biomarker for OC. **Objective:** To identify genetic polymorphism of CEP 290 in ovarian cancer and to determine its role as a novel prognostic and diagnostic biomarker for OC. **Methods:** A genetic profiling methodology was utilized to detect single-nucleotide polymorphisms (SNPs) within the CEP290 gene in patients diagnosed with ovarian cancer. Techniques such as polymerase chain reaction (PCR) and DNA sequencing were implemented to ascertain these genetic variations, followed by statistical analysis to evaluate their association with disease susceptibility and prognosis. **Results:** Two potential SNPs, CXCR2 C+785T and VEGF C+936T, were identified in the CEP290 gene.These polymorphisms may be linked to ovarian cancer pathogenesis, influencing tumor growth, angiogenesis, and immune response mechanisms. **Conclusions:** It was concluded that this study highlights CXCR2 C+785T and VEGF C+936T as potential SNPs within the CEP290 gene, suggesting their role in ovarian cancer diagnosis and prognosis. Further research with larger cohorts is necessary to validate these findings and discover their clinical utility as predictive biomarkers.

INTRODUCTION

Ovarian cancer, often referred to as a silent killer, ranks as the fifth most prevalent cancer among women globally. In Pakistan, it is classified among the top three most common malignancies affecting females, with a rising incidence rate [1]. The challenge posed by OC lies in its detection, as over 70% of ovarian cancer cases are identified at their later stages, leading to a high mortality rate. This underscores the urgent necessity for specific biomarkers to facilitate early and accurate diagnosis. CA-125, though currently accepted and widely used serum biomarker, it is nonspecific in advanced stages and neither sensitive nor specific in early-stage OC diagnosis [2]. In our previous study, by employing 2-DE and Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) Mass spectrometry, comparison of proteomic maps and profiles of differential proteins of human OC and healthy ovarian tissues was conducted to explore the potential proteomic biomarker of OC [3]. In this regard, an upregulated expression of a novel protein, Centrosomal protein of 290 kDa (CEP290), was identified and validated by ELISA as a

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potential candidate biomarker for early diagnosis of OC. This protein is involved as an integral part of the centrosome and cilia and cellular signal transduction, but its genetic role in OC was not elucidated before. It has been shown that CEP290 controls the molecular maintenance of the primary cilium and results in various multi-organ disorders known as ciliopathies (like Leber congenital amaurosis and Joubert syndrome), if a mutation occurs via disruption of key cellular processes. Moreover, the genetic mutations related to renal and retinal diseases have been linked to the presence of antibodies targeting this protein, showing an association with different types of cancer. While CEP290 mutations have been implicated in various ciliopathies, their role in tumorigenesis, including ovarian cancer, remains underexplored [4]. Centrosomes and centrioles play a role in cell polarity, migration, proliferation, signalling in cell transition phases and in all processes which may affect tumorigenesis. Since cancer is a condition where unchecked cell proliferation occurs [5, 6]. So, CEP290 may have a crucial involvement in the unregulated growth of cells, which can lead to the onset of cancer. Previous studies determined its role in pancreatic ductal adenocarcinoma by suppression of ciliogenesis for centrosome amplification in cancer [7]. It was also suggested that genome instability and centrosomal alterations are significant characteristics in developing cancer. Moreover, it was proposed that the amplification of centrosomes has been identified as a potential initiator of cancer. Following this groundbreaking study, centrosomal proteins have once more garnered significant interest in the context of carcinogenesis. Single-nucleotide polymorphisms (SNPs) are germline genetic variations present from birth and detectable before the onset of malignancy. Identifying SNPs associated with elevated CEP290 expression can genetically validate proteomic observations, thereby reinforcing the biological significance of CEP290 as a biomarker in ovarian cancer. This genotype-phenotype correlation supports its involvement in tumorigenesis and its potential utility in early detection and prognostication. Protein markers that reflect dynamic tumor states, SNPs are stable and constitutive throughout life, rendering them ideal for assessing cancer susceptibility in asymptomatic individuals [8]. Currently, there is no specific and sensitive marker which can detect OC at the preliminary stage to reduce the high mortality and morbidity associated with this disease. CEP290 will be used as a minimally invasive novel biomarker for early diagnosis in OC. This tool can serve as an effective screening method for the early detection of OC in high-risk populations, particularly those with a positive family history of breast and ovarian cancer. Early and prompt disease diagnosis leads to the development of more targeted diagnostic tests, better prognosis and ultimately a healthy outcome by decreasing mortality and morbidity of OC patients. Early detection of this deadly disease will be highly beneficial in reducing the financial burden of chemotherapy on individuals and the government, which is otherwise given in the advanced stages only. Keeping in view the unexplored genetic role of CEP290 in OC, the present study is a continuation of the previous study to identify the genetic mutation of CEP290 in OC predisposition and its further validation as a potential novel biomarker for early diagnosis and prognosis of OC [3]. This study aims to identify genetic polymorphism of CEP 290 in ovarian cancer and to determine its role as a novel prognostic and diagnostic biomarker for OC.

METHODS

This study was carried out for one year, starting from 23 March 2023 to 23 March 2024, following the approval from the institutional Ethical Review Board (Case# 999/ERC/CMH/LMC). Ovarian tissue samples (n=40), including both malignant and healthy, were collected by using a purposive sampling technique, after getting their informed consent and recording of all demographic data on a predesigned proforma. Similarly, the study population was divided into two distinct groups, with Group 1 comprising patients who had been diagnosed with ovarian cancer, aged between 40 and 65 years. Group 2 included women within the same age range who were scheduled to undergo a hysterectomy accompanied by bilateral salpingo-oophorectomy for indications other than OC. The sample size was calculated by the World Health Organization (WHO) calculator using the following formula: $n=2\sigma^2$ (z 1- α/2 + z 1- β) 2. (μ1 - μ2)2. Where: μ1 is the anticipated mean in the population, μ2 is the anticipated mean in cases, Z 1- β is the desired power of the study = 90 %, Z 1- α /2 was the desired level of significance = 1.96 41, and n is the calculated sample size. Therefore, sample size is calculated by taking the difference between 2 means of expressed proteins in each group (control and ovarian cancer). Population standard deviation = 6.5. Population variance = 42.25. Difference between the two population means = 6.9. Sample size (n) = 20 for each group. The histopathological analysis for each malignant ovarian tissue sample had been compiled, confirming the cancer staging and the presence of 80-90% cancer cells within the resected tissues. Similarly, the analysis for each healthy ovarian tissue sample confirmed the absence of tumor cells. DNA was extracted from ovarian tissues for each of the 40 patients (malignant & healthy). Samples were thawed on ice before extraction to prevent DNA degradation. Tissue lysis was done by mincing approximately 20-30 mg of tissue and homogenized manually in a sterile mortar and pestle to increase surface area for efficient lysis. Transferred the homogenized tissue to a micro-centrifuge tube and added 300 µL of tissue lysis buffer. Proteinase K (20 mg/ml) was added to the sample to

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aid in protein digestion, followed by RNA removal. Added 150 µL of protein precipitation solution to the lysate after cooling the lysate and mixing, as it clears digested RNA fragments and proteins together, leaving behind purer DNA. Centrifuged at 12,000 x g for 10 minutes at 4°C and transferred the clear supernatant to a new tube, avoiding the protein pellet. For DNA precipitation, added 500 µL of cold isopropanol was added to the supernatant, and the tube was gently centrifuged 5-10 times until DNA appeared as visible threads, leaving the DNA pellet intact. Finally, the DNA pellet was re-suspended in 30-50 µL of nuclease-free water to protect the DNA from degradation. DNA concentration was quantified by using a spectrophotometer (260/280 nm) to confirm adequate yield and purity for PCR. Following extraction, all DNA samples were stored at −20°C. The quality and concentration of the DNA were assessed using photometric methods, with an optical density ratio (OD260/OD280). Nucleotide sequence of the CEP290 gene (GenBank accession no DQ109808.1) was retrieved from the NCBI database. Primers of the CEP290 genes were designed using Primer 3 software. Properties of primers such as melting temperature, GC content, and self-complementarity were checked by using Oligo-Calc software.The sequence of primers is given in the table below. Primer Name Sequence Length (bp). Forward primer 5' ATG CCA CCT AAT ATA AAC TGG AAA 3'24 bp.Reverse primer5' TTA ACA GGA CTT TCT TCT TCA TCT TCA 3'27bp.

The PCR tubes were placed on a 96-well plate. Allowing 0.2 ml of cold water to be added with PCR reagents. In a PCR tube, pipette the PCR reagents like MgCl2, primers, template DNA, sterile water, 10x PCR buffer, master mix and dNTPs. On completion of the procedure, the tubes were taken out, followed by loading of aliquots into the wells of an agarose gel, and after electrophoresis, DNA that had migrated into the gel was stained with ethidium bromide, enabling bands to be seen using a UV illuminator. PCR was conducted by using 20 µM of primers, and the amplification was carried out under specific thermal cycling parameters. An initial denaturation step was followed by denaturation, annealing and extension.The PCR products were then analyzed by electrophoresis on a 1.0% agarose gel, with visualization performed under UV light.The resulting gels were documented using a UV gel imaging system.This was followed by DNA sequencing to identify any single-nucleotide polymorphism related to the CEP 290 gene.

RESULTS

DNA sequencing revealed two potential single-nucleotide polymorphisms, i.e. CXCR2 C+785T, VEGF C+936T (Table 1).

Table 1: Ovarian Cancer Polymorphism

Gene	Properties	Location of Polymorphism	Role in OC
VEGF	Blood and LymphaticVessel Regulation and Control	5'UTR C+405G/C-634G3'UTR C+936T	Over-expresses and potential role in the facilitation the progression of OC
CXCR2 C+785T	Facilitates Angiogenic Potential of IL-8	C+785T	Strong expression in OC cells was found

Whereas the association of identified SNPs with developing ovarian cancer risk was found to be statistically significant (Table 2).

Table 2: Association of SNPs with Ovarian Cancer Risk

SNP	Allele	Ovarian Cancer (n=20)	Control (n=20)	OR (95% CI)	χ^2	p-value
CXCR2 C+785T	T	18	8	3.27 (1.21–8.84)	4.615	0.0317*
	C	22	32			
VEGF C+936T	T	15	5	4.20 (1.35–13.06)	5.400	0.0201*
	C	25	35			

OR: Odds Ratio; CI: Confidence Interval. *Statistical significance considered at p<0.05. χ^2: Chi-square test for allele frequency differences between groups.

The PCR products were then analyzed by electrophoresis on a 1.0% agarose gel, with visualization performed under UV light. The resulting gels were documented using a UV gel imaging system (Figure 1).

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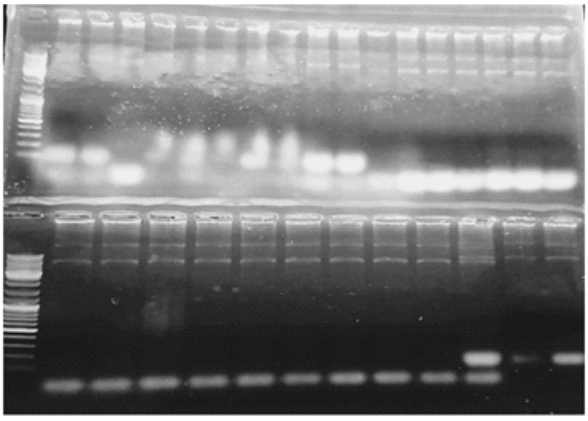

Figure 1: 1% Agarose Gel Electrophoresis Showing Isolated DNA in Healthy and Ovarian Cancer Groups

DISCUSSION

Ovarian cancer has one of the worst stages among gynecologic oncological diseases, owing to the stage of diagnosis and life expectancy after treatment [9]. There is an urgent need for reliable prognosis and diagnostic biomarkers to enhance detection as well as the overall outcomes for patients. CEP290, a critical player in centrosomal and ciliary function, has emerged as a candidate for genetic polymorphism studies in cancer. As a result, it was found that CXCR2 C+785T and VEGF C+936T SNPs were found, which are putative markers for disease susceptibility and progression [10]. A few studies have pointed towards the relevance of CEP290 with cellular maintenance and the consequences of its mutations in some disease conditions like ciliopathies and cancers. The presence of the CXCR2 C+785T SNP in our study corroborates the previous work of some researchers who indicated that it is involved in inflammation and tumorigenesis. High-grade serous ovarian carcinomas with CXCR2 overexpression are poor prognostic factors for early disease recurrence. It has been reported that CXCR2 markedly facilitated cell cycle advancement by influencing several regulatory proteins of the cell cycle [11]. It promotes apoptosis along with elevating p53 phosphorylation and stimulating multiple pro-angiogenetic factors, which may collectively explain the enhanced cell death. This phenomenon is likely brought about by increased p53 phosphorylation and elevated stimuli of pro-apoptotic factors like Puma and Bcl-xs, plus weakened anti-apoptotic factors Bcl-xl and Bcl-2. These observations suggest that CXCR2 enhances the mitotic activity of cells by influencing several proteins which control the cell cycle, including D1 cyclin and its enzyme, CDK-6. If CXCR2 is inhibited in xenograft tumor tissues from ovarian cancer models, there is a decrease in the overall density of vascular structures in addition to increased quantities of VEGF and diminished TSP-1. This evidence serves to underline a role for CXCR2 in angiogenesis in ovarian tumor [12]. Research has suggested that the activation of these pathways could be significant in the CXCR2 signalling network among various cell types [13, 14]. VEGF plays a significant part in the development of blood and lymphatic vessels. In a nonmalignant state, it is mainly synthesized by endothelial, hematopoietic, and stromal cells when there is a lack of oxygen or are stimulated by some growth factors like TGF, interleukin, and PDGF. The isoform VEGF165, for instance, is a prominent mediator of angiogenesis in the tumors, enhancing growth, invasiveness, and metastatic spread of the tumors [15]. In solid and hematological cancers, the expression of VEGF in cancer cells relates directly to tumor tissue quantity, metastases, and negative prognostic factors [16]. Likewise, the C+936T polymorphism in VEGF was suggested to modify the angiogenic potential and, therefore, have an impact on the tumors' microenvironment and progression. These polymorphisms overall are predisposed to ovarian cancer by altering pathways of cell proliferation, migration, and angiogenesis [17]. On the contrary, some studies could not find an explicit link between these SNP's and their subsequent risk of ovarian cancer. For example, one study found a large cohort study with no significant correlation between susceptibility to ovarian cancer and the polymorphisms of VEGF C+936 T. Similarly, there have been studies with CXCR2 polymorphisms in various ethnic groups, which cannot consistently support the existence of a genetic risk for ovarian cancer. Such differences could stem from the combination of environmental, genetic diversity, and changes in sample sizes [17-19]. Current results imply that CEP290 polymorphisms, along with CXCR2 and VEGF variations, may function as potential biomarkers for ovarian cancer [20]. To clarify the molecular involvement of these SNPs in the pathophysiology of ovarian cancer and their therapeutic value in risk stratification and individualized therapy, further extensive, multi-ethnic investigations with functional validation are required. Comprehending the genetic terrain of ovarian cancer will aid in the creation of focused therapies, ultimately enhancing therapy results and patient prognosis.

CONCLUSIONS

It was concluded that this study explores two single-nucleotide polymorphisms, CXCR2 C+785T and VEGF C+936T, in association with CEP290, to evaluate their potential as prognostic and diagnostic biomarkers for ovarian cancer. This helps uncover the underlying molecular mechanisms of OC progression and the identification of CEP290 mutations linked to therapeutic resistance. This could also support the development of targeted therapies and improve early diagnosis, treatment outcomes, and overall survival, while reducing healthcare costs.

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Authors Contribution

Conceptualization: AT
Methodology: AT, SB
Formal analysis: ZA, UZ
Writing review and editing: AT

All authors have read and agreed to the published version of the manuscript

Conflicts of Interest

All the authors declare no conflict of interest.

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