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RESEARCH ARTICLE

FUNCTIONAL GENE POLYMORPHISM AS INDICATOR OF ENDOCRINE DISRUPTION AMONG OVARIAN CANCER PATIENTS

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Abstract

Introduction: Increasing evidence of hormonal disruption and incidence of female reproductive cancers has been reported in recent years. Various documents support the alteration of epigenetic mechanisms and cancer progression among females. Ovarian cancer is one of the more prevalent with high mortality cancer in females. Its 90% progression is due to exogenous materials. These environmental contaminants alter hormonal activity by disrupting the endocrine system.

Aim and Objective: The present study aimed to investigate the association between Endocrine Disrupting Chemicals and the progression of ovarian cancer.

Methodology: As a case-control study, blood samples from ovarian cancer patients and healthy volunteers were genetically analysed to determine the Tp53, CDKN2 and KRAS polymorphisms using PCR-RFLP.

Results: The patients with a mean age of 55 years and determined tumour stage, tumor grade, histological type and FIGO stage were considered for the study. The incidence of heterozygous GC genotype was high among cases (52%) followed by GG and CC genotypes at 26% and 22% respectively in determining the genotype and allele frequency of TP53 gene codon 7. The genotypic distribution of CDKN2A (p16C540G) in ovarian cancer patients the GG, GC and CC genotypes in SNP were observed in 22%, 52% and 26%, respectively (P=0.007). The frequency of the KRAS gene polymorphism (codon 12) in ovarian cancer patients was 14% (TT), 46% (TG) and 20% (GG), P=0.01.

Conclusion: A significant association between gene polymorphism and the pathogenesis of ovarian cancer was noticed in the study.

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Introduction:-

Based on the cancer registries, ovarian cancer ranks among the top 10 cancers and the second most common cancer among women (Yoneda et al., 2012; Chaturvedi et al., 2023). In 2020, it accounted for about 3.7% of cases globally, a death rate of around 4.7% and a 5-year survival rate of around 50.9%. In 2015, the World Health Organization estimated that ovarian cancer is the predominant cause of death in about 91 countries and third or fourth rank in about 22 out of 172 countries as it went unattended in most of the cases (Hamilton et al., 2009). Before the 2000s, the age-standardized incidence of ovarian cancer was high among the European and American populations. But now the incidence is declining and inclined incidence was noticed among the Asian population (Webb and Jordan, 2024). The prevalence rate of ovarian cancer is lower than breast cancer but its lethality is threefold higher (Bray et al., 2018; Gangane et al., 2023). A consistent increase in cases has been evident in the incidence of ovarian cancer in numerous registries (Gangane et al., 2023).

Numerous Clinical and molecular-level risk factors are involved in the progression of this heterogeneous cancer. The majority of ovarian cancers are epithelial (90%) which may arise from the fallopian tube, peritoneum or endometriosis, whereas about 10% of progression develops from germ cells (Bast et al., 2009). Based on the histological transformations and genetic variations, epithelial ovarian cancer is subdivided into slow-grading and high-grading tumours (Shih and Kurman, 2004). Various predisposing factors play a major role in the progression of cancer. Endocrine disruptors are one among those predisposing factors.

As per the World Health Organization, endocrine disruptors are exogenous chemical substances that mimic natural hormones or disrupt hormone regulation which in turn leads to hormonal imbalance, dysfunction (UNEP/WHO, 2013; WHO/IPCS, 2022) and alterations in the expression of functional gene (Munn and Goumenou, 2013). The altered functional gene plays a major role in carcinogenesis. Understanding the role of functional gene polymorphisms in ovarian cancer has several important clinical impacts. Hence, the present study screening the genetic mutation involved in Tp53 (the gene that regulates cell division and cell death), CDKN2 (a tumor suppressor gene that controls cell growth, division, and apoptosis) and KRAS (cell growth, maturation, and death) gene and its correlation with susceptibility towards ovarian cancer.

Materials and Methods:-

Study Subjects

After getting Institutional Ethics Committee approval, blood samples from control and ovarian cancer patients were collected. A total of 50 female controls without any cancer and 50 female ovarian cancer patients, between the age group 18 to 75 were selected on the environmental exposure towards endocrine disruptor for determining the gene polymorphism.

Blood sampling

About 5 mL peripheral blood samples were collected from patients and controls in a vacutainer and the samples were subjected to the UV visible spectrophotometry method for determining the conformational changes in haemoglobin and serum albumin and the results were recorded in the 200-800 nm range (Xie et al., 2010; Wu et al., 2019).

DNA isolation and genotyping

DNA was isolated from each blood sample using QiagenDNeasy® Blood and Tissue Kit. After DNA quantification (Multiscan™ GO Spectrophotometer, Thermoscientific Inc., Wilmington, DE, USA) Single Nucleotide Polymorphism was detected using PCR-RFLP (Eppendorf Thermocycler, Germany). Primers used in this study are tabulated in Table 1. A total reaction volume of 25 µL PCR was performed with components 2x Taq DNA master mix Red (Ampliqon), 25mM MgCl2, 75 µM dNTPs, 100 µM primers and 50 ng template DNA. The PCR products were analyzed with 2% agarose gel using an Electrophoretic apparatus (BioRad).

Table 1:- Primers used for detecting SNPs.

SNP	Primer	Enzyme	Annealing
TP53 Codon 72 (Hadiand Mulakhudair, 2021)	5'-TTG CCG TCC CAA GCA ATG GAT GA-3' 5'-TCT GGG AAG GGA CAG AAG ATG AC-3'	BstUI	55^{o}C
CDKN2 p16540	5'-GATGTGCCACACATCTTTGACCT-3'	HaeIII	62 ^{o}C

(Yan et al., 2008)	5'-CTACGAAAGCGG GGTGGGTTGT-3'		
KRAS codon 12 (Dobrzycka et al., 2009)	5'- ACT GAA TAT AAA CTT GTG GTA GTT GGA CCT -3' 5'-TCA TGAAAA TGG TCA GAG AA-3'	BstOI	56 ^{o}C

Statistical analysis

Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) 25.0 version. pvalue less than 0.05 was considered as statistically significant. The odds ratios (ORs) was calculated with 80% power and 95% confidence intervals (CIs) to determine the association ofthe genotypes in progression of ovarian cancer risk.

Results:-

We included 50 ovarian cancer patients and 50 controls to determine the significance of functional gene polymorphism in the progression of cancer. All the patients and controls were selected based on the prehistory of exposure to any one of the endocrine disruptors. The patients were selected based on the structural changes in their blood components. The serum of cancer patients showed an elevated mean absorbance range of 250-300 nm whereas, control samples ranged between 360 nm to 450 nm. The mean age of patients was 55 years. Out of 50 samples, 6 patients were at Stage I, 5 were Stage II, 34 were Stage III and 5 were Stage IV. Histologically the predominant type was epithelial ovarian cancer which includes serous, Endometrioid and clear cell type tumors. Tumor grade and FIGO stage scores were recorded in Table 2.

Table 2:- Clinical and pathological features of ovarian cancer patients.

Parameter	**Incidence**
Number of patients	50
Mean age	55
Tumor Stage	
I	6
II	5
III	34
IV	5
Tumor Grade	
1	14
2	27
3	9
Histological Type	
Serous	17
Endometriod	14
Clear Cell	16
Unclassified	3
FIGO stage	
1A	13
1B	8
1C	29

In determining the distribution of genotype and allele frequency of TP53 gene codon 7, the incidence of heterozygous GC genotype was found to be high among cases (52%) followed by GG and CC genotypes as 26% and 22% respectively (95% CI =0.07-0.52). Homozygous genotype CC was significantly higher among cases than control (p-value=0.08). The genotypic incidence among control was 66% (GG), 28% (GC) and 6%(CC). The allele frequencies were significant as per the Hardy-Weinberg equilibrium (p=0.077). The results are tabulated in Table 3.

Table 3:- Frequency of TP53 Genotypes.

TP35 gene codon 7	**Case**	**Control**
GG	13 (26%)	33 (66%)
GC	26 (52%)	14 (28%)
CC	11 (22%)	3 (6%)

The genotypic distribution in CDKN2A (p16C540G) gene polymorphisms was studied. In ovarian cancer patients, the GG, GC and CC genotypes in SNP were observed in 22%, 52% and 26%, respectively (Table 4). In the case of controls, the GG, GC and CC genotypes were found 70%, 22% and 8%, respectively (P=0.007). In non-cancer control females, the incidence of GC and CC genotypes was less than in samples (95% CI =0.92-1.30, p = 0.062).

Table 4:- Frequency of CDKN2A Genotypes.

p16C540G	Case	Control
GG	11 (22%)	35 (70%)
GC	26 (52%)	11 (22%)
CC	13 (26%)	4 (8%)

The genotypic frequencies of the KRAS gene polymorphism (codon 12) in ovarian cancer patients were 14% (TT), 46% (TG) and 20% (GG) tabulated in Tabe 5. The heterozygote genotype TG and homozygote GG exhibited higher among cases, whereas the genotype TT (60%) was high among controls (95% CI: 1.8-7.8). The T allele vs. G allele frequency among the case and control agree with the Hardy-Weinberg equilibrium (P=0.01).

Table 5: Frequency of KRAS Genotypes

codon 12	Case	Control
TT	7 (14%)	30 (60%)
TG	23 (46%)	18 (36)
GG	10 (20%)	2 (4%)

Discussion:-

Ovarian cancer is one of the most fatal female cancers. In this research, we explored the impact of endocrine disruption leading to functional gene polymorphism as a risk factor in the progression of ovarian cancer. In analysing the endocrine disruption, the blood analysis of patients showed the structural changes in their blood components. The serum of cancer patients showed an elevated mean absorbance range of 250-300 nm whereas, control samples ranged between 360 nm to 450 nm. This result coincides with the early reports (Wu et al., 2019; Keshavarz-Maleki et al., 2021). Further gene polymorphism among the functional genes is studied to determine the susceptibility of patients towards endocrine disruptors.

The p53 protein of the TP53 gene regulates cell cycle, metabolism, DNA repair and apoptosis (Rivlin et al., 2011). A somatic TP53 gene mutation is the most common genetic predisposition factor among human cancers (Olivier et al., 2010). This mutation leads to altered and uncontrolled cell division resulting in tumor growth (He et al., 2017). TP53 mutations account for about 60-70% of cases in both early and advanced-stage ovarian cancer (Havrilesky et al., 2003; Leitao et al., 2004) which increases the risk of metastasis and reduces the chance of survival rate and progression-free survival (Jiang and Mansel, 2006; Bernardini et al., 2010). There are limited studies relating TP53 codon 72 polymorphism with the risk of development of ovarian cancer (Malisic et al., 2013). Few contradictory results link this mutation with the development of certain cancers (Pietsch et al., 2006; Shao et al., 2008; Zhang et al., 2010). Some research indicates the prevalence of Arg/Arg genotype is likely a predominant risk factor in the development of ovarian cancer (Pegoraro et al., 2003; Agorastos et al., 2004), whereas the results of] Wang et al. (2004) and Santos et al. (2006) concords with the present study. The direct relationship between ovarian cancer progression and 72 codons (GG) was proposed in a few studies (Hadi and Mulakhudair, 2021). The study suggests the presence of Pro allele positively correlates with the development of ovarian cancer.

CDKN2A (p16) is an essential tumor suppressor gene. The mutation in CDKN2A affects the increasing cell proliferation which leads to malignancy. The deletion mutation and lack of expression of CDKN2A in the study population range between 11 to 37 % was reported in various studies (Marchini et al., 1997; Fujita et al., 1997; Langosch et al., 1998). Havrilesky et al.(2001) reported the progression of ovarian cancer occurred due to loss of expression of this tumor suppressor. As in the present study, few previous studies also reported the association of the development of ovarian cancer occurs due to CDKN2A polymorphism (Debniak et al., 2005; McCloud et al., 2004; Yan et al., 2008).

Numerous studies were conducted to assess the role of KRAS mutation in ovarian cancer. KRAS gene plays a vital role in the cell signalling pathway which controls cell growth, maturation and cell death. In the present study, we intended to explore the correlation between exposure to endocrine disruption and KRAS gene polymorphism. Few previous studies have reported the affirmative role of KRAS alteration in ovarian cancer as a prognostic factor

(Singer et al., 2003; Jumaa, 2022), whereas few studies report a lack of correlation between KRAS gene polymorphism and cancer and survival (Cuatrecasas et al., 1998; Varras et al., 1999). Dobrzycka et al. (2009) reported that the mutation frequency correlates with the histological type and is significantly higher among mucinous and borderline tumor than serous tumor. Various multivariant studies also conclude that the KRAS gene mutation and ovarian cancer were correlated negatively (Scambia et al., 1997). However the trend in controversies changed as in the present study, KRAS mutation among endocrine-disrupted patients shows positive affirmation in the development of ovarian cancer.

Conclusion:-

The positive correlation between functional gene polymorphism and the pathogenesis of ovarian cancer was noticed in the study. The polymorphism in a functional gene regulating hormonal signalling and metabolism plays a significant role in an individual's susceptibility towards endocrine disruption. Understanding the relationship between functional gene polymorphism and endocrine disruption provides us insight into the pathogenesis of ovarian cancer. Continued research should be performed to explicate the role of genetic predisposing factors in the development of cancer.

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Conflicts of Interest

There are no conflicts of interest to disclose regarding this study.

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