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# Determine Frequency of BRCA1 rs1799950 Polymorphism among Sudanese Ovarian Cancer Women

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***Abstract:*** *Ovarian cancer (OC) occurs infrequently, but a subset of cases is linked to BRCA1 gene mutations. The ovarian cancer susceptibility gene (BRCA) plays important role in tumor susceptibility. BRCA1 mutations in the germline significantly increase the risk of ovarian cancer and other cancers in women. (1) BRCA1 is a tumor suppressor gene, and its most important action in DNA repair. The aim of the study evaluate the frequency of ( rs1799950) polymorphisms ( In this study determine of one BRCA1 known mutations ( rs1799950 ) in Sudanese women patients with ovarian cancer because suspect effect on risk of cancer). we conducted a case-control population study and spotted that occurrence of rs1799950 -BRCA1 to evaluate the BRCA1 polymorphisms and genetic susceptibility of ovarian cancer among Sudanese women in Gezira state. Overall,85 Ovarian cancer patients attendin g National Cancer Institute. For control used 65 sample take from normal women do not suffer from any type of cancer and she does not ha ve any family history of cancer, Majority of the ovarian cancer patients were in the age group of <50 years representin g 66%, and 34% in age group >50 years. The mean and median age was with age means ( 55.44 ) and ( 56 ) respectively. For all sample case and control make DNA extraction , PCR – CTPP method and gel electrophoresis , to give perfect result. In our study revealed that rs1799950 could related with ovarian cancer risk among Sudanese populations. In this study determine frequency of one BRCA1 known mutations ( rs1799950 ) in Sudanese women patients with ovarian cancer because suspect effect on risk of cancer r are. And, missense variants were detected were found within the study, ( rs1799950) variants were heterozygousone One patient from case group have mutation in BRCAI gene and 4 mutations in control group were detected within postmenopausal patients, with n o family history of ovarian cancer.*

**Keywords: ovarian cancer, BRCA1 gene, rs1799950 , Sudan**

## Introduction

Ovarian cancer (OC) is the most offensive gynecological malignancy and leading cause of death among women . According to the latest Global Cancer Observatory (GLOBOCAN) report, OC accounted for 1.6% of all cancers and 2.1% of all cancer deaths worldwide in 2020 .(1)

It is the second most common gynecological cancer in developing countries and the fourth most common cancer in women overall. (2)

According to GLOBOCAN 2020, female age-standardised incidence rates per 100,000 in Sudan were 41.2, 8.7 and 6.7 for breast, cervix and ovary cancer, respectively . (3)A range of genetic factors are related with an increased risk of developing ovarian cancer Germ line BRCA1 and BRCA2 mutations are the most significant known genetic risk factors for ovarian and either mutation is found in up to 17% of patients with ovarian cancer. ( 4) BRCA1/2 mutations are associated with better short-term survival, but this advantage decreases over time and in BRCA1 carriers is eventually reversed. This may have important implications for therapy of both primary and relapsed disease and for analysis of long-term survival in clinical trials of new agents, particularly those that are effective in BRCA1/2 mutation carriers, ( 5)

BRCA1 and BRCA2 are tumor suppressor genes implicated in the maintenance of genomic stability and thus the control of cell growth. The BRCA1 and BRCA2 proteins are primarily implicated in the homologous recombination (HR) pathway in the repair of DNA double-strand breaks (DSBs). In humans, the BRCA1 gene is located on chromosome 17, position 17q21. It has 24 exons spread across 81 kb of DNA, 22 of which are coding exons. These exons encode a 7000-bp transcript that is translated into an 1863-aa protein. . ( 6) BRCA 1 and 2 genes were first identified as breast cancer susceptibility genes in 1994 and 1995, respectively (7). The BRCA1 gene is expressed in a variety of tissues, including the breast and the ovary. The BRCA1 gene is a large gene that spans approximately 100 kb of genomic DNA. It has 24 exons, with the first and fourth exons being non-coding. The eleventh exon is the largest exon, accounting for more than 60% of the total coding sequence of the BRCA1 gene. (8). one in every 1000 women is BRCA positive, with the incidence reaching 2.5% in certain ethnic groups. According to lifetime risk estimates, 15%-40% of women

with a BRCA1 or BRCA2 mutation will be diagnosed with ovarian cancer, compared to 1.4% of women in the general population.(9). According to Abdulrashid et al. it has been shown that HBOC patients in Arab countries have BRCA mutations and the prevalence of BRCA2 mutations were more common (17%) than BRCA1 mutations (11%) among the HBOC patients in the Arab region (10), (11). Mutations in BRCA genes cause faulty DNA repair mechanisms, which are related with an increased risk of developing breast and/or ovarian cancer. Changes in BRCA genes occur throughout the entire gene. There have been reports of mutations in BRCA1 and BRCA2 that exceed 600 and 400 mutations, respectively. (12). rs1799950 is located in the region of exon 11 that binds Rad50, which is part of the DNA damage repair complex . rs1799950 can be found in many families who are at a high-risk for BC and prostate cancer. In sudan in 2018 Aabdein etal discover tow mutation related with BRCA1 mutation in breast cancer patients Q356R (rs1799950).( 13) , but no study about SNP rs1799950 in ovarian cancer.

**Material and method**

This is hospital-based case-control study was conducted to evaluate the BRCA1 polymorphisms and genetic susceptibility of ovarian cancer among Sudanese women in Gezira state. The study was conducted in National Cancer Institute (NCI), Gezira University Wad Medani, Sudan.

More than 50 % from Patients come from Gazira state and the rest of patients from neighboring stats, The study has been carri ed out over five months from February 2022 to july 2022. The patients were received from Gezira Hospital for Obstetrics and gynecology and Patients who recruit to NCI during the study period and have been diagnosed with Ovarian Cancer will be selected for the study after taking consent . Special questionnaire containing personal and demographic characteristics will recor ded at time of admission.

**.Inclusion Criteria:**

**_ cases**

all women diagnosed with ovarian cancer using histopathology techniques and attending National Cancer Institute during study period

_Healthy donation with match cases age were included as control

In case and control agreement to joined study

**Exclusion Criteria:**

**_ for cases**

excluded from this study non ovarian cancer patients, benign lesions, cases of metastatic tumors from other primary sites .

_ **for control**, no diagnosis by any type of cancer , no family history of any type of cancer

**Methods:**

**Laboratory Method**

After written informed consent, 3 mL venous blood sample was collected into EDTA container (whole blood) for DNA extraction.

**DNA extraction method**

Genomic DNA was extracted by The G-spinTM Total DNA Extraction Mini Kit from iNtODEWORLD, Inc. USA was used for DNA extraction from blood samples

**Polymerase Chain Reaction (PCR):**

PCR reactions were performed in a total volume of 30 µl containing 200 ng genomic DNA, 15-20 picomoles of each primer, 200 µM dNTP (dATP, dTTP, dCTP and dGTP), 3µl from 10 x Taq Gold Buffer (100mM TrisHCl, pH 8.3, 500 mMKCl, 15 mM MgCl2 and 0.01 % (w/v) gelatin (Perkin Elmer Cetus), 1.5 U AmpliTaqGoldTM polymerase (Perkin Elmer Cetus) and completed to final volume with deionized water. The efficiency of the amplification was tested by electrophoresis in 1.5-2.5% agarose (SeaKemÒ GTGÒ agarose, FMC BioProducts) in 1.5x TBE at 100-140 Volts for about 30minutes and visualized under gel documentation

system (GDS). Both The agarose concentration in gel electrophoresis and the current in the gel depend on the length of the electrophoresed DNA fragment.

**Screening of BRCA1 polymorphisms (rs 1799950, A > G) by polymerase chain reaction with confronting two-pair primers (PCR-CTPP):**

The principle of PCR-CTPP is based on the fact that: the efficiency of PCR amplification depends on the complete matching of primer sequence at it's 3`end. Thus, any mismatch at this end of the primer will affect the PCR amplification system. Accordingly, PCR-CTPP genotyping system needs four primers; two common primers flanking the polymorphic site one at each side, but with different length from the polymorphic site24. The other two, are allele specific primers and each primer had be design to be absolutely complementary to one allele sequence specially at the 3` end of the primer. PCR-CTPP is an accurate method for genotyping of SNPs. It is time-saving and inexpensive to perform. It can be used for genotyping of all types of mutation. This system will be used to screen BRCA1 polymorphisms (rs 1799950, A > G) in study subjects and controls. Four primers are designed for BRCA1 polymorphisms (rs 1799950, A > G) PCR-CTPP genotyping system.

**PCR amplification around BRCA1 (rs 1799950, A > G) in study subject and controls**

In PCR conditions as an initial denaturation 95°C for 10 minutes, followed by 35 cycles of 95°C as melting temp for 2 minute, 52°C as annealing temp for 30 seconds and 72°C as a prolongation temp for 45 seconds, then a final prolongation step at temperature 72°C for 2 minutes. A 182 bps DNA fragment flanking the polymorphic site was PCR amplified in a total volume of 12µl as described above using primer pair:

| Primer | sequance |
|---|---|
| Asp950-G: | TTCTCTGAGCATGGCAGTTACC |
| CP950F: | ACAGATGGGCTGGAAGTAAG |
| Asp950-A | GAGAGAAAAGAATGGAATACGCA |
| CP950R | TGTCTTCAATATTACTCTCTACT |

In the presence of the G allele, Asp950-G and 133 bp will work to amplify with the primer CP950F to give a PCR fragment of 133 bp while in the presence of the A allele, the primer Asp950-A will work to amplify with the primer CP950R to give a PCR fragment of 331bp.

PCR product will be electrophoresis in 2% agarose gel and the gels will be stained in 1µg/ml ethidium bromide solution for 10-15 minutes and visualized with UV light under GDS. The genotypes will be assigned according to obtained profile. Two bands of 420 and 331 bp will be assigned as homozygote AA, three bands 420, 331 and 133 bp as heterozygote AG while two bands of 420 and 133 bp will be assigned as homozygote GG

**PCR mix: -**

The concentration of master mix was 2X, the total volume of PCR mix was 12 µL

| snp | MM | CPA | CPG | APA | APG | DNA | H2O | Total v. |
|---|---|---|---|---|---|---|---|---|
| | 5 | 0,3 | 0,6 | 0,2 | 0,4 | 3 | 3 | 12 |

**PCR Conditions: -**

The number of thermal cycles was 35 , with initial denaturation temperature of 95◦C for 2 minutes, Annealing temperature 52◦C for 30 secound and final extension at 72◦C for 2 minutes.

The rest temperatures as follow: -

| snp | denaturation/ time | Annealing/ time | extension/ time |
|---|---|---|---|
| | 95◦C / 2 min | 52◦C / 30 sec | 72◦C / 45 sec |

**Result**

**Clinical data of study population**.

Overall ,85 Ovarian cancer patients attending National Cancer Institute and For control used 65 sample take from normal women do not suffer from any type of cancer and she does not have any family history of cancer . The mean and median age was with age means ( 55.44 ) and ( 56 ) respectively, and Std. Deviation 12.942. The sample population was divided into four aged groups including (5.9%) for age group (20 to 35 years), (28.2%) for age group (36 to 50 years), (37.6%) for age group (51 to 65 years), and (28.2%) for age group (66 to 80 years). Ninety percent of ovarian cancer patients were diagnosed with serous carcinoma , 8 percent were mucinous carcinoma and 2 percent were clear cell carcinoma. Majority of the ovarian cancer patients were at FIGO Stage between ( I to III ) which account fivty one percent; while the remaining 28 percent and 21 percent were in II and 1 FIGO Stage respectively. 35% from patients diagnosed in grad III , 33% in grad II and 32% in grad I. This is Socio-demographic and clinical data of study population explanie in table 1.

| Characteristics | | Frequency | Percent % |
|---|---|---|---|
| Type of O.C tumer | Serous carcinoma | 76 | 89.4 |
| | Musinous carcinoma | 7 | 8.2 |
| | Clear cell carcinoma | 2 | 2.4 |
| | Total | 85 | 100.0 |
| stage | Stage I | 18 | 21.2 |
| | Stage II | 24 | 28.1 |
| | Stage III | 43 | 50.7 |
| | Total | 85 | 100 |
| grade | Grade I | 27 | 31.8 |
| | Grade II | 28 | 32.9 |
| | Grade III | 30 | 35.3 |
| | Total | 85 | 100 |
| Age/years | 20 - 35 | 5 | 5.9 |
| | 36 - 50 | 24 | 28.2 |
| | 51 - 65 | 32 | 37.6 |
| | 66 - 80 | 24 | 28.2 |
| | total | 85 | 100 |

Table 1 ; This is clinical data of study population

**BRCA1 mutations of study population**

In this study determine frequency of one BRCA1 known mutations ( rs1799950 ) in Sudanese women patients with ovarian cancer because suspect effect on risk of cancer rare. And, missense variants were detected were found within the study, ( rs1799950) variants were heterozygousone One patient from case group have mutation in BRCAI gene and 4 mutations in control group were detected within postmenopausal patients, with no family history of ovarian cancer.

**Disscuion**

Over 14.1 million new cases of cancer and 8.2 million deaths were reported globally in 2012, according to estimates from GLOBO CAN.

Hereditary and environmental variables interact to cause cancer; familial cancer is predominantly brought on by hereditary causes, whereas sporadic cancer is primarily brought on by environmental ones.

In order to better understand cancer, both hereditary and environmental components must be considered.

Hollis et al. 2018 (14), About 10–15% case of ovarian carcinomas (OC) are attributed to inherited susceptibility, the majority of which are due to mutations in BRCA1 or BRCA2 (BRCA1/2).
Different research groups have studied cancer risk association with SNPs located in same genes, We selected SNPs that were been studied to be associated with OC risk in one or more.

Majority of studies have showed BRCA1 polymorphisms are associated with cancer risk, this study evaluate the frequency of BRCA1 polymorphisms (rs1799950) in ovarian cancer patients.

Single nucleotide polymorphisms (SNPs) associated with different disorders such as ovarian cancer, SNPs is a widespread mechanism relevant to cancer susceptibility. To evaluate whether target SNPs are implicated in OC susceptibility, SNPs can affect protein function by changing the amino acid sequences (non synonymous SNP) or by perturbing their regulation (e.g. affecting promoter activity , splicing process , and DNA and pre-mRNA conformation).(15)

High-risk pathogenic alleles make a significant effect to cancer morbidity worldwide, accounting for approximately 5%–10% of breast cancer (BC) and 15%–20% of ovarian cancer (OC) incidence, respectively.

However, BC-and OC-predisposing mutations across populations are, by definition, far from being apportioned evenly. Therefore, communities, , are usually characterized by distinct patterns of hereditary diseases and pathogenic variants. Many reports demonstrated that consecutive series of high-grade serous OC (HGSOC) were characterized by a high frequency of BRCA1/2 mutations .(16)

Acorreding to Aabdein etal, 2018 in Sudan (rs1799950) this variant was found in patients with a family history of ovarian cancer, suggesting that this variant may increase ovarian cancer risk.

Acorreding to Smolarz etal, 2019 (17), correlation was found of Q356R BRCA1 gene polymorphism. with ovarian cancer in the examined patients. Based on the obtained results, it was demonstrated that 356R allele predisposed to cancer development. The R/R genotype may increase the risk of cancer ( Smolarz etal, 2019)

Janezic SA etal 1999 (18) and Aabdein etal, 2018 ,The rare form of the Q356R polymorphism was significantly (P= 0.03) associated with a family history of ovarian cancer, suggesting that this polymorphism may influence ovarian cancer risk.

The control group that had the mutation showed were people who carry the mutation and are at risk of developing ovarian cancer Although they do not have a family history of ovarian cancer.

Hollis et al. 2018, data demonstrate that Furthermore characterisation of rs1799950 is now warranted in relation with chemosensitivity and susceptibility to developing ovarian carcinoma, Among the 111 successfully sequenced PLD-treated patients, 11 instances of the missense-causing SNP rs1799950, conferring a Gln356Arg amino-acid change and predicted to be detrimental to BRCA1 function.
In present study explain frequency of BRCA1 mutations SNPs rs1799950 in Sudanese ovarian cancer patients the mutation present but rare agree with Janezic SA etal 1999 and Aabdein etal, 2018 Smolarz etal, 2019.
Agree with Janezic SA etal 1999 the control group appeared were people who carry the mutation and are at risk of developing ovarian cancer Although they do not have a family history of ovarian cancer.

limitation of this study was the small sample size and the functional assessment facilities available to assessment and Financial constraints also limited the study. Some patients do not accept the nature and importance of scientific researchTherefore.

**Conclusion**

In present study explain frequency of BRCA1 mutations SNPs rs1799950 in Sudanese ovarian cancer patients the mutation present but rare. All the statistical tests were carried at the level of significance $\alpha = 0.05$. In order to verify the hypothesis about the significance of age, hormonal replacement therapy, and family cancer history in the studied group. P-values <0.05 were considered significant. No relation was clear between the polymorphisms studied and the cancer stage of OC with FIGO classification. The polymorphisms studied not associated with other risk factors, such as the, menarche, number of pregnancy and hormonal.

the study has highlighted a need for further research of these identified variants amongst a larger population (including patients and controls), This will aid the understanding of a variant's frequency and clinical significance

**Recomondation**

We require more studies on the relation between rs799917 polymorphism and risk of ovarian cancer to verify these conclusions, and the biological function of the polymorphism should be investigated as well

Further research among larger groups is warranted to determine the effect of Association between genetic variants and ovarian cancer risk and determine frequency of genetic mutation

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