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Frequency of BRCA1 rs799917 polymorphism in ovarian cancer patients attending NCI, Gazira state – Sudan

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Abstract: *Ovarian cancer is eighth most common cancer in women globally. BRCA1 and BRCA2 genes are the two most common genes in autosomal dominant and high penetrance form of breast cancer and ovarian cancer**.** There are no studies in Sudan that specifically address BRCA1 and BRCA2 mutations in ovarian cancer patients, and despite the disease's importance for public health, the country lacks precise national epidemiologic data on it. The aim of the study evaluate the frequency of (rs799917) polymorphisms (In this study determine of one BRCA1 known mutations (rs799917) 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 rs799917-BRCA1 to evaluate the BRCA1 polymorphisms and genetic susceptibility of ovarian cancer among Sudanese women in Gezira state. All cases Ovarian cancer patients attending National Cancer Institute. For control sample take from normal women do not suffer from any type of cancer and she does not have any family history of cancer, Majority of the ovarian cancer patients were in the age group of <50 years. For all sample case and control make DNA extraction , PCR – PIRA method and gel electrophoresis , to give perfect result. In our study revealed that rs 799917 could related with ovarian cancer risk among Sudanese populations. We concluded BRCA1 mutations SNPs rs799917 in Sudanese ovarian cancer women showed high risk wth ovarian cancer**.** More studies in this mutation by high sample size and search in other mutations suspected of ovarian cancer to determine the effect of Association between genetic variants and ovarian cancer risk*

Keywords: ovarian cancer, BRCA1 gene, rs799917 , Sudan

INTRODECTION

Ovarian cancer (OC) is one of the seventh most common cancers worldwide. Ovarian cancer is a leading cause of death from gynecologic malignancy most cases are detected in advanced stages. Early detection and diagnosis of ovarian cancer would result in a 95% 5-year survival rate (1), . Little is known about ovarian cancer in the Sudan the etiology of ovarian cancer in Sudan is scarcely investigated. The availability of genetic testing is very limited in Sudan (National Institute for Public Health, and some Special coefficient. Each year, approximately 240,000 new cases are diagnosed by ovarian cancer. Age-standardized incidence rates are higher in developed countries than in developing countries, but this difference is becoming less pronounced as incidence declines in the highest incidence countries and rates rise in the lowest incidence countries. Over 80% of cases of ovarian cancer are diagnosed in women over the age of 50 year. (2) . . Approximately 90% of all OC are epithelial in origin, with the remaining OC being non-epithelial in origin. Approximately, 3% of epithelial OC are mucinous, while the rest are non-mucinous. Non-mucinous carcinomas are further classified as serous (70% of non-mucinous), endomterioid (10%), clear cell (10%), and unspecified (5%).,(3). as well as grading tumor differentiation (i.e., the extent to which the tumor resembles the normal tissue). Tumors are classified as either well-differentiated (G1), moderately differentiated (G2), poorly differentiated (G3), or undifferentiated (G4) (G4). (4). The factors associated with the increased risk of ovarian cancer are advancing age, obesity, nulliparity,, oestrogen and hormone therapy (5). The most important factor in the development of ovarian cancer is a family history of ovarian or breast cancer, although an identifiable genetic predisposition (germline *BRCA1/BRCA2* mutation) is found only in 10–15% of the patients (6,7). BRCA genes play important roles in DNA repair and recombination, cell-cycle checkpoint control, apoptosis, and transcriptional regulation. (8) . The risk of epithelial ovarian cancer in women with the *BRCA1* mutation is 39–46% while with the *BRCA2* mutation it is about 12–20% (9). Some variants of the *BRCA1* gene have been identified, and P871L (rs799917) is one of the most common variants with a minor allele (T) frequency of 32% among Caucasian cancer patients (10). *BRCA1* gene in ovarian cancer were that it is one of the most studied SNPs, though the results have been inconsistent in different populations, and little is known about the biological, functional, and clinical impact of this polymorphism. The frequency of *BRCA1* and *BRCA2* mutations has been reported in several studies in breast and ovarian cancer patients. A study on Moroccan women with hereditary breast and ovarian cancer showed the frequency to be 25.64%. Other studies conducted in neighboring populations such as Tunisia and Algeria have reported frequencies of 19.4% and 11.4% (11), respectively. No information is available on the role of variants of *BRCA1* gene in ovarian cancer in females living in sudan. This study was designed to determine frequency of *BRCA1* gene among ovarian Cancer women and normal tissues, as well

as to determine the role of *BRCA1* gene variants (rs 799917 T>C,) in the development of ovarian cancer in females attending to national cancer institute between (2020 -2023) living in Sudan.

Materials and Methods

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

Methods:

Laboratory Method

After written informed consent, 3 mL venous blood sample was collected into EDTA container (whole blood) for DNA extraction.. Genomic DNA was extracted using salting out method for DNA extraction and was purified

Polymerase Chain Reaction (PCR):

PCR reactions (Mullis, 1990) 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.

Screening of BRCA1 polymorphisms (rs799917, A > G) by PCR primer introduced restriction analysis assay (PCR-PIRA):

When a mutation creates or abolishes a restriction site for specific enzyme, the cleavage with that enzyme can be used as a verification for the presence or absence of that mutation. Sometime, a PCR-mutagenesis around the polymorphic site of the mutation, using a primer carrying a single nucleotide mismatch, together with one of the two allelic form of the mutation, can be used to create a recognition site for specific restriction enzyme which can be used to type the mutation. This is a transition replacement of adenine by guanine. A mismatch in the forward primer (MutP) underline bolded together with the wild type of the polymorphism is required to incorporate the polymorphic site into an Ava II restriction site (GGWCC).

PCR amplification around BRCA1 (rs799917, T > C) 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 3 minute, 56°C as annealing temp for 45 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 15µl as described above using primer pair:

Primer	Sequance
MutP	AAGGTTTCATAGCGCCAGTCATTTGGTC
ComP	GTCTGTACAGGCTTGATATTAG

Digestion with Restriction Enzyme

In a total volume of 15 µl, 2 µl PCR product was digested overnight at 37°C with 2.5 U Ava II, 1.5 µl 10x Buffer Y+/Tango™ (330 mMTris acetate (pH 7.9), 660 mM potassium acetate, 100mM magnesium acetate, 1 gm/ml BSA (MBI Fermentas) and deionized water.

Electrophoresis on 10% polyacrylamide Non denaturing gel:

The Digested DNA was mixed with 5 µl loading dye before being loaded on a 10 % Non-denaturing polyacrylamide gel and electrophoresed at 100 V for two hours. Then the gel was stained in 0.1 µg/ml ethidium bromide solution for 10-15 minutes and visualized under UV light in Gel Documentation System (GDS).

Interpretation of digestion reaction electrophoresis profiles and genotyping:

The complete cleavage of 182 bp to 154, 28bp fragment characterized the profile of homozygote wild type (CC), incomplete cleavage into182bp, 154bp and 28bp is of heterozygote (CT) where absolute absence of digestion characterized of homozygote muted type (TT).

PCR mix: -

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

snp	MM	R1	CP	DNA	H2O	Total v.
	7	1	1	3	3	15

PCR Conditions: -

The number of thermal cycles was 35 , with initial denaturation temperature of 95◦C for 3 minutes, Annealing temperature 56◦C for 45 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

RESULTS

Overall ,85 Ovarian cancer patients attending National Cancer Institute. Majority of the ovarian cancer patients were in the age group of <50 years representing 63%, and 33% in age group >50 years. The mean and median age was with age means (50.3) and (53) respectively.

BRCA1 mutations (rs799917)

The study observed that the majority of ovarian cancer patients, constituting 66% of the total, were under the age of 50, while the remaining 34% were aged over 50. To provide a measure of central tendency, the study calculated both the mean and median ages, which were found to be 50.3 and 53, respectively. This information offers insight into the age demographics of the ovarian cancer patient population under investigation.

The study focused on identifying specific mutations within the BRCA1 gene, particularly the rs799917 variant. Among the case group (women with ovarian cancer), homozygous variants (TT) of rs799917 were detected in nineteen patients. Additionally, three mutations were identified in the control group. Interestingly, only one patient in the entire study population carried the homozygous (CC) variant of rs799917. Notably, the majority of these mutation findings were observed among postmenopausal patients with no familial history of ovarian cancer.

The study assessed the distribution of genotypes and evaluated whether they complied with Hardy-Weinberg Equilibrium, a principle in population genetics. The analysis revealed a p-value of 1, indicating adherence to Hardy-Weinberg Equilibrium. The odds ratio was calculated as 1.00. This information suggests that the observed genotype frequencies in the study population were consistent with what would be expected under Hardy-Weinberg Equilibrium, providing additional context for the genetic findings.

Percentage of typed samples: 109/109 (100%)

SNP3 genotype frequencies (n=109)						
	All subjects		**CASE.CONTROL=Case**		**CASE.CONTROL=control**	
Genotype	**Count**	**Proportion**	**Count**	**Proportion**	**Count**	**Proportion**
C/C	**1**	**0.01**	**1**	**0.01**	**0**	**0**
T/C	**23**	**0.21**	**19**	**0.24**	**4**	**0.13**
T/T	**85**	**0.78**	**58**	**0.74**	**27**	**0.87**

Table 1: SNP genotype frequencies

SNP3 exact test for Hardy-Weinberg equilibrium (n=109)						
	N11	N12	N22	N1	N2	P-value
All subjects	85	23	1	193	25	1
CASE.CONTROL=Case	58	19	1	135	21	1
CASE.CONTROL=control	27	4	0	58	4	1

Table 2: SNP exact test for Hardy-Weinberg equilibrium

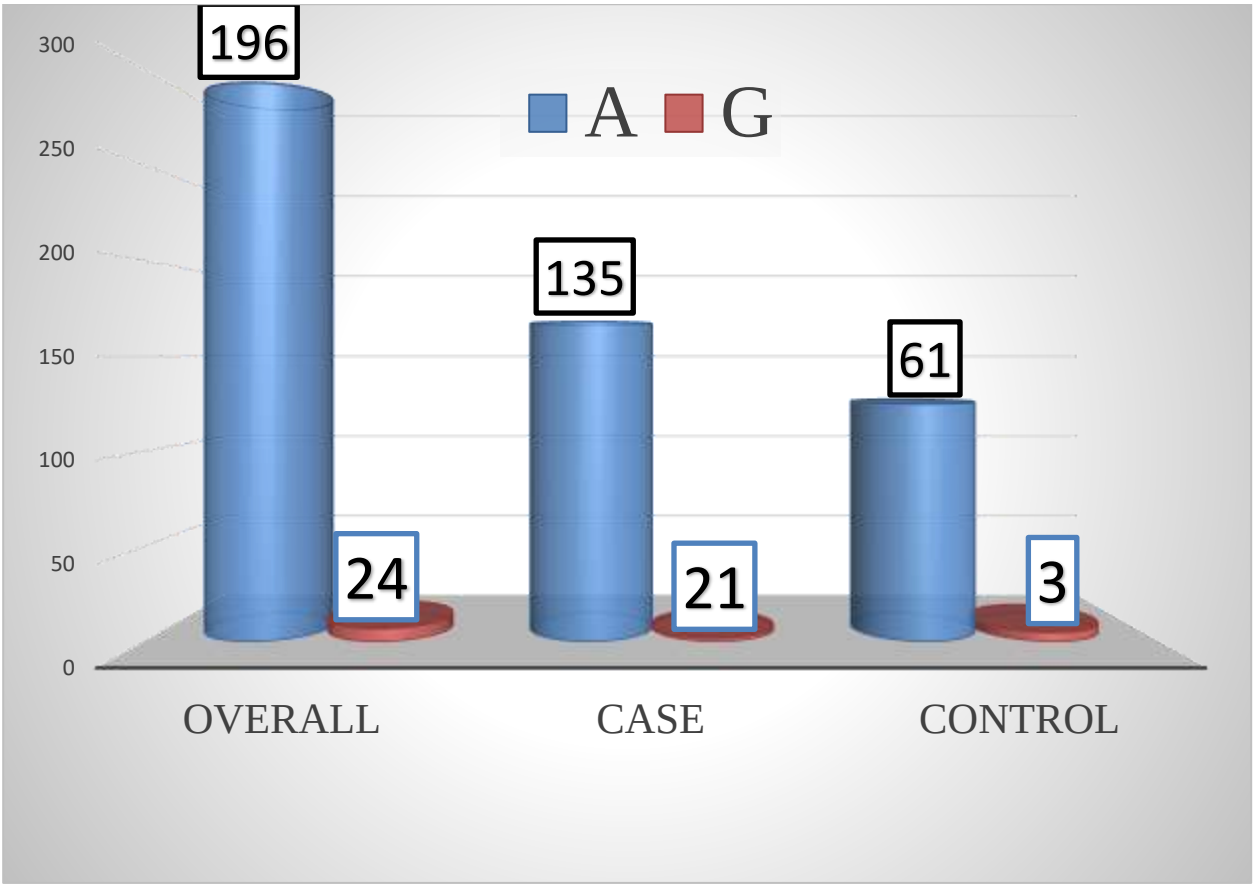

Figure 1: SNP allele for BRCA (rs 799917)

Parameter	Class	Mutation		P. value
		Absent	Present	
Age group	< 50	16	9	0.209
	> 50	53	11	
Marital status	Non married	21	5	0.507
	Married	37	15	

Oral contraceptive	yes	13	1	0.08
	no	45	19	
Tribes	Arab	38	3	0.212
	African	20	17	
Family history	No	54	15	0.043
	Yes	4	5	
Breast feeding	No	11	3	0.690
	Yes	47	17	
Hormonal drug	No	50	19	0.289
	Yes	8	1	
Type of O.C	Serous .C	50	19	0.524
	Musinous .C	6	1	
	C.C.C	2	0	
Stage	I	9	6	0.578
	II	16	6	
	III	41	8	
Grade	I	17	7	0.841
	II	19	6	
	III	21	6	

Table 3: Association between rs799917 and ovarian cancer risk factors:

Causal Factor relationship

The SNP rs799917 was not found to be significantly associated with ovarian cancer across various genetic models, including co-dominant, dominant, recessive, and log additive models. Specifically, odds ratios (ORs) of 1 were observed for the C/T genotype, with 95% confidence intervals (CIs) spanning from 0 to 1 and p-values of 1. For the T/T genotype, the ORs were 1.10 and 1.17 for the co-dominant and dominant models, respectively, with 95% CIs of 0 and p-values of 1. Additionally, no significant association was found for the recessive model (OR 95% CI: 1, p=1) and the log additive model (OR=1.24, 95% CI = 0, p=1).

Regarding socio-demographic characteristics, there were no significant differences observed in age group, marital status, breastfeeding history, oral contraceptive use, family history of ovarian cancer, hormonal drug use, type of ovarian cancer tumor, stage, or grading system among ovarian cancer patients. The respective p-values for these characteristics were 0.209, 0.507, 0.690, 0.08, 0.043, 0.289, 0.524, 0.578, and 0.841

Case Control

SNP3 association with response CASE.CONTROL (n=109, adjusted by NO)					
Model	**Genotype**	**CASE.CONTROL**	**Case CASE.CONTROL**	**control OR (95% CI)**	**P-value**
Codominant	**T/T**	**58 (74.4%)**	**27 (87.1%)**	**1.00**	**1**
	T/C	**19 (24.4%)**	**4(12.9%)**	**1.10 (0.00NA)**	
	C/C	**1 (1.3%)**	**0 (0%)**	**NA (0.00-NA)**	
Dominant	**T/T**	**58 (74.4%)**	**27 (87.1%)**	**1.00**	**1**
	T/C-C/C	**20 (25.6%)**	**4(12.9%)**	**1.17 (0.00-NA)**	
Recessive	**T/T-T/C**	**77 (98.7%)**	**31(100%)**	**1.00**	**1**
	C/C	**1 (1.3%)**	**0 (0%)**	**NA (0.00-NA)**	
Over dominant	**T/T-C/C**	**59 (75%)**	**27 (87.1%)**	**1.00**	**1**
	T/C	**19(24.4%)**	**4(12.9%)**	**1.08 (0.00NA)**	
Log-additive				**1.24 (0.00-NA)**	**1**

Table 4 :Haplotype association with ovarian Cancer

Picture 1 : BRCA 1 rs 799917 band after gel electrophoresis

DISCUSSION

BRCA1 mutations (rs799917)

Ovarian cancer is the seventh most common cancer, and it is the most common cause of high mortality from gynecological cancers worldwide.

Data on ovarian cancer about demographic data of patients most tranded in previous study mean age at diagnosis , histological type of ovarian cancer stage and grade of ovarian cancer . Our study agree with most of studies about ovarian cancer cases .

Similar to findings from other regions, our study observed a trend of higher OC risk with increasing age. In Sudan, as reported by Wisal et al. (2017), two-thirds of diagnosed women are 55 or older. This aligns with data from Schildkraut et al. (2014) in the US, where the average age for African American women at diagnosis is 57.4 years. However, some variations exist. Studies by Rambau et al. (2020) in East Africa and North America report a mean age of 50.5 years, while Machida et al. (2019) in Japan observed the highest prevalence in the 50-59 age group. These findings suggest a potential influence of regional factors. An important finding in our study is the presence of younger Sudanese patients with OC. This highlights the limitations of current screening methods, as ovarian cancer is often perceived as a disease primarily affecting older women.

The majority were diagnosed at FIGO stages III, reflecting a prevalence of advanced-stage disease, consistent with findings by Abuidris et al. (2016). Serous carcinoma was the most common type (90%), followed by mucinous and clear cell carcinomas, aligning with global trends reported by Schildkraut et al. (2014) and others.

Inheritable genetic mutations, known as high-risk pathogenic alleles, significantly impact global cancer rates. While these mutations account for an estimated 15-20% of ovarian cancer cases, their distribution varies considerably across different populations. This means that communities tend to have unique patterns of hereditary diseases and specific genetic variations associated with increased risk. Research has consistently shown a high frequency of mutations in the BRCA1 and BRCA2 genes among women diagnosed with high-grade serous ovarian cancer (HGSOC).

The T allele frequencies was the most frequent overall study population (TT) , furthermore TC genotype frequency was the high frequent in population (our study showed high proportion of mutant genotype frequencies (TC).

Previous reports have examined the relationship between polymorphisms in genes Common polymorphisms in BRCA1 are high priority breast/ovarian cancer susceptibility candidates because germ line mutations in these genes strikingly increase risk. In this regard, ovarian cancer risk is significantly higher in BRCA1 carriers.

Our study not agree with Dunning et al., Dunning examined the P871L polymorphism in three case control studies of breast cancer (572 total controls and 801 total breast cancers) and a hospital-based series of 237 consecutive ovarian cancers in the United Kingdom. There was no relationship between P871L genotype and risk of either breast or ovarian cancer. The frequency of the L allele was 0.32 in controls and 0.33 in ovarian cancer cases. Although no relationship with ovarian cancer risk was seen.. We did not find an association between P871L genotype and ovarian cancer risk. The frequency of LL homozygotes was slightly lower in cases relative to controls, African-Americans have a lower incidence of ovarian cancer (ages 40–59:17/100,000; ages 60: 24.5/100,000) relative to Caucasians (ages 40–59: 26/100,000; ages 60: 38.4/100,000;). One possible explanation for the racial difference in ovarian cancer incidence may be differences in frequencies of susceptibility alleles. In this regard, we observed a striking racial difference in BRCA1 P871 allele frequencies. In Caucasians, the P allele was more common (0.64), whereas in African- Americans, the L allele predominates (0.76). This polymorphism was not associated with ovarian cancer risk in either race, however.

Previous studies of BRCA1 polymorphisms have been performed predominantly in Caucasians, Racial variation in allele frequencies of P871L probably explains the higher L allele frequency in ovarian cancer cases relative to controls in the Durocher et al. study . Ovarian and breast cancer cases were from collected series of high-risk families, some of which likely were African-American, whereas control subjects were from geographic areas (Utah, Quebec) where few African-Americans reside. In view of the high frequency of the L allele in the African-American population, a slightly higher fraction of African-Americans among cases relative to controls would skew the distribution of allele frequencies between the groups. This is result and theory agree with our result because sudan is part and country in Africa.

Conclusion

In the present study, we investigated the BRCA1 mutations, specifically the single nucleotide polymorphisms (SNPs) rs799917, in Sudanese ovarian cancer patients. Our findings revealed no significant association between these polymorphisms and ovarian cancer.

Although it was detected, it was found to be rare. There was no significant correlation between this polymorphism and the cancer grade or stage, as classified by the International Federation of Gynecology and Obstetrics (FIGO) system.

The BRCA1 rs799917 polymorphism showed a potential association with an increased risk of ovarian cancer. However, similar to rs1799950, there was no significant relationship between the rs799917 polymorphism and the cancer grade or stage according to the FIGO classification.

These results highlight the complexity of genetic factors in ovarian cancer. Despite identifying the presence of these BRCA1 mutations, their impact on the disease's progression and prognosis remains unclear. This underscores the necessity for further research involving larger and more diverse populations to better understand the role of these and other genetic variants in ovarian cancer.

Limitation of the study

The study primarily focused on specific variants within the BRCA1 gene ,(the rs799917 variant).

Weaker Effect of Variants: It's possible that the variants examined in the study have a weaker effect on ovarian cancer risk in Sudanese women compared to other populations or compared to other genetic and environmental factors. Genetic variants may exert varying degrees of influence on disease risk depending on the population studied and the presence of other modifying factors.

1. **Interaction with Other Factors**: Genetic variants associated with ovarian cancer risk may interact with other genetic or environmental factors in complex ways. These interactions could modify the overall risk conferred by the variants examined in the study. Without considering these potential interactions, the study may not fully capture the nuanced relationships between genetic variants and ovarian cancer risk.

2. **Small Sample Size**: The study's small sample size could also contribute to the lack of association observed. With a limited number of participants, the study may lack the statistical power needed to detect significant associations between genetic variants and ovarian cancer risk. Larger sample sizes are generally required to increase the likelihood of identifying meaningful associations, especially when examining relatively rare genetic variants.

Considering these alternative explanations is important for interpreting the study findings in context and for guiding future research efforts aimed at understanding the genetic factors influencing ovarian cancer risk in Sudanese women.

Recommendation

Future research directions should prioritize conducting studies with larger and more representative populations, including both patients with ovarian cancer and control groups.

Overall, there is a critical need for continued research in this area to better understand the association between genetic variants and ovarian cancer risk, determine the frequency of genetic mutations, and inform effective prevention and screening strategies.

Comprehensive Analysis: Gene sequencing allows for a comprehensive analysis of the entire genome, not just specific known variants like BRCA mutations. By sequencing the entire genome, researchers can identify novel genetic variants that may be associated with ovarian cancer risk. This approach broadens the scope of investigation beyond known mutations and provides a more complete picture of genetic factors contributing to the disease.

Identification of Rare Variants: Gene sequencing can detect rare genetic variants that may not be captured by traditional genotyping methods. These rare variants could potentially have significant implications for ovarian cancer risk.

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