Original Research Article

Detection of BRCA2 Receptor Gene polymorphism in Sudanese Patients of Prostate Cancer in Khartoum state

Abstract

Back ground: Prostate cancer (PCa) is a genetically complex disease with multiple predisposing factors affecting presentation, progression, and outcome. This was analytical case-control study conducted between January 2019 up to January 2021.

Objective: This study was aimed to investigate the effect of BRCA2 polymorphism in the etiology of prostate cancer among Sudanese patients.

Method: The study population was selected as one hundred patients with prostate cancer as case group and thirty healthy individuals as control. A total of 5 ml EDTA anti-coagulated venous blood samples were obtained from prostate cancer patients that admitted for routine follow-up of chemotherapy treatment, for hormones and remain of blood samples were kept at liquid nitrogen until used for DNA extraction and genes polymorphisms at institute of tropical diseases of Khartoum.

Results: One hundred and thirty persons were included, 100 patients were men with prostate cancer as case group while thirty were healthy subjects as control with mean age of case was 67.41years. The result showed that (87%) of patients with wild type *BRCA2* 6174delT alteration and (13%) with mutant type. The mean result of testosterone level, Insulin G. F level, and PSA level in associated to *BRCA2* 6174delT was insignificant difference between patients with mutant and wild genotype (P.value= 0.91, 0.061, 0.449, respectively).

Conclusion: This study was concluded the most frequent prostate cancer patients (87%) with wild type *BRCA2* 6174delT and there was no significant correlation in testosterone level, Insulin G. F and PSA level when correlated to *BRCA2* 6174delT with different genotype

KEY WARD: genetic polymorphism of BRCA2, Prostate cancer (PCa)-Sudan

INTRODUCTION

Prostate cancer (PCa) accounts for the second commonest cause of male cancer-related deaths in the United States (Jemal A et al, 2011) and the sixth worldwide, with more than 250 000 deaths a year (Ferlay J et al, 2010). Thus, it is essential to identify those patients with potentially lethal forms of PCa at their presentation. PCa is rarely diagnosed in men younger than 50 years, but its incidence rises rapidly thereafter. Excluding advanced age and African-American ancestries, the strongest risk factor for the disease is a family history of PCa (Carter BS et al, 1992; Eeles RA, 1999; Edwards SM, Eeles RA, 2004). PCa is one of the common cancers with a large genetic component, as up to 42% of the risk could be explained by inheritance from studies about twins (Lichtenstein P et al, 2000).

BRCA1 and BRCA2 are tumour suppressor genes and both are inherited in an autosomal dominant fashion with incomplete penetrance. Tumorigenesis in individuals with germline mutations in the BRCA genes requires somatic inactivation of the remaining wild-type allele. Both genes encode large proteins that function in multiple cellular pathways. BRCA1 is a key player in cellular control systems, having been linked to a range of cellular processes, such as DNA damage response and repair, transcriptional regulation and chromatin modeling (Gudmundsdottir K, Ashworth A, 2006; Boulton SJ, 2006). By contrast, BRCA2 function seems to be limited to DNA recombination and repair processes, being of particular importance in the regulation of RAD51 activity (Gudmundsdottir K, Ashworth A, 2006; Boulton SJ, 2006; Venkitaraman AR, 2002). BRCA1 or BRCA2 function loss is associated with a deficiency in repairing DNA double-strand breaks (DSBs) by the conservative mechanism of homologous recombination (HR). Therefore, cells have to repair these lesions through other non-conservative and potentially mutagenic mechanisms. This genomic instability may underlie the cancer predisposition caused by deleterious mutations in the BRCA genes, although the reason why these mutations are particularly associated with some specific types of cancer, such as breast, ovarian and PCa, remains unknown (Turner N et al, 2004). Francis et al. have proposed that BRCA2 may act as a tumor suppressor in epithelial prostate tissue and its functional loss predisposes to premalignant prostatic lesions. This study was aimed to investigate the role of BRCA2 in susceptibility in prostate cancer (Francis JC et al, 2010).

MATERIALS AND METHODS

This study was undertaken in Khartoum state during the period from January 2018 to January 2021. A total of One hundred prostate cancer patients were included in this study. Patients who had other form of malignancies were excluded. Venous blood (5 ml) samples were collected in EDTA tubes from all participants. Serum prostate specific antigen (PSA) levels were measured by competitive chemiluminescence immunoassay. The remainder of the blood samples were kept under liquid nitrogen (AT -20°C) until used for genotyping. DNA was extracted by chemical method. Genotyping was performed by PCR-RFLP method using *Eco147I* enzyme at 37°C. PCR and digestion products were visualized on 1.5% agarose electrophoresis. Data were analyzed by statistical package for social science (SPSS), version 16. Qualitative data were presented as mean and SD.

List 1: Genotyping of *BRCA2*6174delT Polymorphism:

BRCA2 6174delT	FCP	AATGATGAATGTAGCACGC	Allele specific
	FMP	GAATTTTTAGCACAGCAAGG	
BRCA2 6174delT	RCP	GTCTGAATGTTCGTTACT	Sequencing
	6174delT_F	AACGAAAATTATGGCAGGTTGTTAC	
	6174delT_R	CGAAAGGTGAACGACATGATTTAGG	

RESULTS

One hundred and thirty persons were included in case control study, one hundred were men with prostate cancer as case group while thirty were healthy subjects as control, the mean age of case was 67.41years ranging from 43 to 93 years old and the mean age of control was 63.43years, but this difference between the patients and controls group was statistically insignificant (P-value > 0.052). The proportion of patients in correlated to geographical and tribal distributions showed that highest among patients came from western Sudan (47.9%) followed by Northern (30.2%), Central (16.7%) and least from Eastern (5.2%) and the frequency of controls were most from Northern Sudan (43.3%) followed by western (33.3%), Central (20%) and less from Eastern (3.3%) with statistically insignificant (0.459).

Most of food consumption was wheat (63.3%) followed by millet (32.7%) and at last bean (4%) and all this not associated with prostate cancer with statistically insignificant (P-value > 0.187). When correlated to general appearance, the most of patients (67.0%) were well when compared with control group and 31% of patients appear as ill but just 2% of patients were severe ill with statistically significant among general appearance (P-value = 0.001). The frequency of patients in associated to previous family history with cancer reported that majority of patients (92%) with negative history and 8.0% of patients with positive history when compared with control groups with statistically insignificant (P-value = 0.110).

The distribution of patients in associated to medication showed that majority of patients were treated with hormone therapy (56%) followed by Zometa &casodex therapy (40%) and about (19%) of patients were treated with surgery and only 2% of patients were treated with radiotherapy.

Table 1: Age, tribe, traditional food, general appearance, family history with cancer, and Medications among patients with prostate cancer and controls group

Characteristics		**Patient N=100**	**Control N=30**	**P-value**
Age mean±SD		67.41±9.46	63.43±10.67	0.052
Tribe[a] N(%)	Northern	29(30.2)	13(43.3)	0.459
	Eastern	5(5.2)	1(3.3)	
	Western	46(47.9)	10(33.3)	
	Central	16(16.7)	6(20.0)	
Traditional food[b] N(%)	Wheat	62(63.3)	17(56.7)	0.187
	Millet	32(32.7)	9(30.0)	
	Beans	4(4)	4(13.3)	
General appearance N(%)	Sever ill	2(2.0)	0	0.001*
	Ill	31(31.0)	0	
	Well	67(67.0)	30(100)	
Family history with cancer N(%)	Yes	8(8.0)	0	0.110
	No	92(92.0)	30(100)	
Medications N(%)	Zometa &casodex	40(40.0)	-	
	Chemotherapy	26(26.0)	-	
	Surgery	19(19.0)	-	
	Hormone therapy	56(56.0)	-	
	Radiotherapy	2(2.0)	-	

The frequency of *BRCA2* 6174delT alteration among prostate cancer patients showed that wild type (87%) was more frequent than mutant type (13%).

Table 2: Distribution of *BRCA2* 6174delT polymorphisms among prostate cancer patients

Genotype		**Patient N(%)**
BRCA2 6174delT	Wild	87(87.0)
	Mutant	13(13.0)

The mean result of testosterone level in associated to *BRCA2* 6174delT was insignificant difference between patients with mutant genotype (2.62 ng/ml) and without mutant gene (2.47 ng/ml) with P.value= 0.91. The mean of Insulin G. F level in correlated to *BRCA2* 6174delT was insignificant increased in patients with mutant genotype (27.88 ng/ml) compared to those with wild type (14.98 ng/ml) with P.value = 0.061. The mean of PSA level in correlated to *BRCA2* 6174delT genotype was insignificant more increased in patients with wild type (19.64 ng/ml) than those with mutant genotype (12.46 ng/ml) with P.value = 0.449

Table 3: Correlation between hormonal parameters and *BRCA2* 6174delT gene among prostate cancer

Genotype		Testosterone ng/ml mean±SD	Insulin G. F ng/ml mean±SD	PSA ng/ml mean±SD
BRCA2 6174delT	Wild	2.47±4.71	14.98±22.39	19.64±32.18
	Mutant	2.62±2.39	27.88±25.82	12.46±28.51
	P-value	0.911	0.061	0.449

The mean age of patients in correlated to *BRCA2* 6174delT genotype was 66.52 years for patients with wild genotype and 73.38 years for patients with mutant genotype with statistically significant different between two patient with P.value=0.014.

The frequency of *BRCA2* 6174delT genotype according to tribal distribution showed the most frequent patients with wild from Western (54.2%) followed by Northern (26.5%), central (14.5%), and Eastern (4.8%) and most frequent patients with mutant genotype were from Northern (53.8%) followed by Central (30.8%), and (7.7%) from Eastern and Western with statistically significant (P.value= 0.020)

The distribution of patients with *BRCA2* 6174delT when correlated to general appearance showed that most of patients with wild type (63.2%) were well, (34.5%) were ill and (2.3%) of patients were severe ill, and also observed the most of patients with mutant type were well (92.3%) and less patients were ill (7.7%) with statistically insignificant (P-value = 0.114).

The distribution of *BRCA2* 6174delT genotype in associated to family history with prostate cancer reported that about (94.3%) of patients with wild type without history of disease and (5.7%) with family history when compared to mutant type, we found about (76.9%) without history and (23.1%) with history of disease with statistically significant (P-value = 0.032).

Table 4: Age, tribe, general appearance and family history in associated with *BRCA2* 6174delT gene among prostate cancer patients

Risk factor		*BRCA2* 6174delT		P-value
		Wild	Mutant	
Age mean±SD		66.52±9.59	73.38±5.85	0.014*
Tribe N(%)	Northern	22(26.5)	7(53.8)	0.020*
	Eastern	4(4.8)	1(7.7)	
	Western	45(54.2)	1(7.7)	
	Central	12(14.5)	4(30.8)	
General appearance N(%)	Sever ill	2(2.3)	0	0.114
	Ill	30(34.5)	1(7.7)	
	Well	55(63.2)	12(92.3)	
Family history with cancer N(%)	Yes	5(5.7)	3(23.1)	0.032*
	No	82(94.3)	10(76.9)	

DISCUSSION

Prostate cancer is the most common cancer in Sudanese men. Despite the substantial public health impact of prostate cancer little is known about its a etiology. The accepted risk factors for the development of prostate cancer are advanced age, familial predisposition and potentially ethnicity. One hundred and thirty individuals were included in the present study, one hundred were patients with prostate cancer as case group while thirty were healthy subjects as control.

In the present study we found the mean age of case was 67.41years ranging from 43 to 93 years old and the mean age of control was 63.43years with statistically insignificant difference between case and controls group (P-value > 0.052). That similar to case-control study done at

the University of Vienna from October 1998 to January 2001 by Andrea Gsur, *et al*, 2002, 380 individuals were included in this study, 190 patients with prostate cancer as cases and 190 individuals as control, the mean age was 65.9 years for cases and 66.5 years for controls with statistically insignificant difference between case and controls group (P-value =0.507). Another study done by Mohammed El Imam M. Ahmed, *et* al, 2009 study was carried out in Gezira Hospital-Sudan. A total of 194 elderly male patients, their mean age was 65 years (range 45-90). Also the mean age of incidence of prostate cancer agree with previous study conducted in Soba University Hospital, Khartoum during 2008–2010 by Elaimam and E. Abdel Raof Sharfi, 2013, the mean age of case was 65 years ranging from 40 to 90 years old. Also correspond to case-control study covers the 268 prostate cancer patients from May 2006 to December 2009 conducted in Sudan done by Hamad and AbuIdris, 2011, More than 73.9 % of cases occur in men over 66 years, with the largest number being diagnosed in the age period between 66 - 76 years. Also similar to another study conducted by Haala M. Gabra, *et al*, 2014, conducted in Fedail Hospital, Khartoum-Sudan during the period of December 2010 to March 2012, the mean age for them was 68.8.0±8.3years. The study, also, included 100 healthy males as the control group with matched mean age 66.7.0±8.1 years. In another previous study conducted by Ingle SP *et al*, 2013, 102 cases of prostate enlargements were selected. Most of the cases were in the age group of 60-79 years (89.2%). Few cases were in the 40-49 years age group (9.8%).

In present study, the proportion of patients in correlated to geographical and tribal distributions showed that highest among patients came from western Sudan (47.9%) followed by Northern (30.2%), Central (16.7%) and least from Eastern (5.2%) and the frequency of controls were most from Northern Sudan (43.3%) followed by western (33.3%), Central (20%) and less from Eastern (3.3%) with statistically insignificant (0.459). This corresponding to case-control study conducted in Sudan and done by Hamad and AbuIdris, 2011, 268 prostate cancer patients were included, the most of cases came from Central and Northern regions tribes and its distribution is similar to its normal census distribution. The most of patients about 104 patients came from Central ((38.8%) followed by 97 patients from Northern (36.1%), 57 patients (21.2%) from western Sudan and 10 patients (3.7%) from Eastern.

In our study, the frequency of patients in associated to previous family history with cancer reported that majority of patients (92%) with negative history and 8.0% of patients with positive history when compared with control groups with statistically insignificant (P-value = 0.110). That agrees with previous study conducted in Sudan by Hamad and AbuIdris, 2011, 268 prostate

cancer patients were included in this study, 18 (6.7%) of the patients had positive family history. The family history findings agree with Steinberg et al, 1990, who reported that approximately 15% of men with a diagnosis of prostate cancer were found to have a first-degree male relative (e.g., brother, father) with prostate cancer, compared with approximately 8% of the U.S. population. Approximately 9% of all prostate cancers may result from heritable susceptibility genes.

In our study, we found the frequency of *BRCA2* 6174delT alteration among prostate cancer patients showed that wild type (87%) was more frequent than mutant type (13%). That similar to study of A Mitra*et al*, 2008, there was a total of 20 cases and 20 controls, the frequency of mutant type of *BRCA2* 6174delT alteration among prostate cancer patients was about 7% of patients.

In our study we found the mean of PSA level in correlated to *BRCA2* 6174delT genotype was insignificant more increased in patients with wild type (19.64 ng/ml) than those with mutant genotype (12.46 ng/ml) with P.value = 0.449. That similar to study of A Mitra*et al*, 2008, there was a total of 20 cases and 20 controls, The mean of PSA level in correlated to *BRCA2* 6174delT genotype was insignificant increased in patients with wild type (32.8 ng/ml) than those with mutant genotype (24.3 ng/ml) with P.value = 0.583.

CONCLUSION

This study was concluded the most frequent prostate cancer patients (87%) with wild type *BRCA2* 6174delT and there was no significant correlation in testosterone level, Insulin G. F and PSA level when correlated to *BRCA2* 6174delT with different genotype.

ETHICAL APPROVAL AND CONSENT

The study was approved by the research committee at the faculty of Medicine, El imam Elmahdi university. Ethical approval was achieved from the university, Informed consents were taken from each subject before enrollment in the study.

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