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THE ASSOCIATION BETWEEN CANCER OF THE BREAST AND THE ABO AND RHESUS D ANTIGEN PHENOTYPES IN LAGOS, NIGERIA: A CASE-CONTROL STUDY

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ABSTRACT

Objective: To determine whether inheritance of ABO and Rhesus D antigen phenotypes constitute a risk factor for development of cancer of the breast.

Design: A case-control study.

Setting: Patients with histologically diagnosed cancer of the breast referred to Radiotherapy department of the Lagos University Teaching Hospital over a six-month period were included in the study (cases). Healthy blood donors at the Donors Clinic of the hospital over the same period of time were also studied (Control A). Women attending family planning clinic were also studied and referred to as Control B.

Materials & Methods: Routine ABO and Rhesus D-phenotyping was carried out on washed red cell specimens of all cases and controls. The odds that cancer of the breast was due to presence of a given phenotype was determined (odds of cases). The odds that the given phenotype is present in the normal population without cancer of breast was also determined (odds of control). The Odds Ratio (OR) given by odds of cases/odds of control served as an estimate of the Relative Risk (RR) that the given phenotype is associated with the cancer of the breast.

Result: 107 cases, 2,243 controls A and 122 controls B were studied. Of the cases, only one was a male, the others were females. Their ages ranged between 24 and 85 years with a median of 45 years. The controls A were all males with ages ranging between 22 and 55 years. Controls B were females with ages ranging between 21 and 47 years.

A, B, O, AB, Rhesus D positive and Rhesus D negative phenotype distribution amongst the cases, controls A and B were 28, 22, 54, 3, 97 and 10; 589, 440, 1143, 71, 2142 and 101; 27, 25, 70, 0, 117 and 5 respectively.

Of all the phenotypes, only the inheritance of Rhesus D antigen gave a relative risk (RR) significantly below unity. RR = 0.45, standard error = 0.76 and $P<0.05$ when cases were compared with controls A. RR = 0.41, SE = 0.56 and $P>0.05$ when cases were compared with control B.

Conclusion: negative association was established between inheritance of Rhesus D antigen and the development of cancer of the breast. Rhesus D antigen phenotype may be protective against cancer of the breast.

KEYWORDS: ***ABO Blood group, Rhesus D blood group, relative risk, attributable risk, Absolute risk. Carcinoma of the Breast.***

INTRODUCTION

Breast cancer causes about 20% of cancer deaths among females and has been called the foremost cancer in the women[1,2]. The incidence has been on the increase over the past nine decades such that in the U.S.A., about 1 in 9 women will develop the cancer in their lifetime. As such, breast cancer has assumed a public health significance[3]. This cancer has received and is still receiving a great deal of appropriate publicity and intensive study with focus on its origin (aetiology), diagnostic methods and treatment[3].

The cause of this neoplasm is not definitively certain but available epidemiological data point to three sets of influences that may be important in breast cancer. These are

1. Genetic factors
2. Hormonal imbalances, and
3. Environmental factors.

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Of these sets of influences, genetic predisposition has been claimed to undoubtedly exist[4].

In mice, high cancer strains can be achieved by genetic inbreeding. In particular, mutations in the tumour suppression gene – P^{53} - located on chromosome 17p13.1 has been shown to predispose an individual to development of a variety of cancers such as cancers of the breast, colon and lungs[5,6]. Homozygous loss of the P^{53} gene has been said to occur in 30-50% of patients with breast cancer[6]. The presence of Li-Fraumeni Syndrome (Mutations of P^{53} gene) in large percentage of patients with breast cancer and the epidemiological findings of markedly increased risk of breast cancer in the first degree relatives of propositus implies that the search for genetic basis of this cancer must continue with vigour[3]. In fact, a breast cancer susceptability gene has been traced to a small locus on chromosome $17q^{21}$ in a large number of families in whom breast cancer develops at an early age[7].

The goal of the present communication is to determine whether it is possible to link or associate cancer of the breast with easily accessible genetically determined antigens on human red blood cells. These antigens have hitherto been found to be a useful marker for a number of other neoplasms such as[8] cancers of the salivary gland, the colon, the ovary and the stomach[9]. Recurrent thrombosis has been shown to be associated with inheritance of blood group A[8] presumably due to increased level of factor VIIIc amongst these group[10]. Blood group inheritance has also been linked with susceptability to malaria infection[11].

MATERIALS AND METHODS

SUBJECTS

107 consecutive patients with the histological diagnosis of cancer of the breast referred to Radiotherapy Department for radiation therapy were recruited into the study. 2,243 healthy blood donors seen at the Blood Donors Unit of the Lagos University Teaching Hospital (over the same period of time - July 1998 to 9th February, 1999) were used as controls (control A). Family Planning Clinic attendees with no breast cancer (122) were recruited as control B. All the subjects were bled into plain bottles by drawing about 5ml of blood from one of the antecubital veins. Informed consent was sought and verbally obtained from all subjects.

ABO BLOOD GROUPING

All reactions were carried out in test tubes. Red cells were separated from the clotted specimens, washed in saline and 5% suspension made in saline. They were then reacted with ABO blood grouping anti-sera, anti-A, anti-B and anti-A+B supplied by Novopath™ Monoclonal Antibody Diagnostic reagents. As in-built controls, subjects cells were also reacted with own serum (negative auto-control) and with AB serum (negative control). Subjects' sera were also reacted with standard, A, B and O cells (obtained from the Blood Bank of the Lagos University Teaching Hospital) to detect the presence of naturally occurring ABO antibodies (serum grouping).

RHESUS BLOOD GROUPING

All subjects were typed for only rhesus D-antigen using tube technique. Monoclonal anti-D serum was obtained from the same manufacturer as ABO antisera. Subjects that typed apparently Rh-D negative were confirmed to be truly negative by carrying out indirect Coomb's test.

STATISTICAL METHOD

Risk Measurement

Of the measures of risk available (Absolute Risk, Relative Risk [RR], Attributable Risk, Odds Ratio, Standardised Mortality Ratio and Proportionate Mortality Ratio), it is only the Odds Ratio that can effectively be determined in a case-control study of this type[12]. Although relative risk can never be determined from case-control study[13], its value can be estimated from odds ratio if the disease under study is rare[14].

Calculation of the Odds Ratio (OR)

This requires formulation of a 2 by 2 contingency table as below

	Cases with Ca Breast	Controls without Ca Breast	Total
Presence of a particular Red Cell Antigen Phenotype	a	b	a+b
Absence of the Phenotype	c	d	c+d
	a+c	b+d	a+b+c+d

The odds that ca. breast is due to presence of the phenotype = a/c = odds of cases

The odds that the phenotype under study is present in the normal population without ca. breast = b/d = odds of control

$$\text{The odds ratio (OR)} = \frac{\text{odds of cases}}{\text{Odds of controls}} = \frac{a}{c} / \frac{b}{d} = \frac{ad}{bc}$$

Relative Risk (RR) OR = ad/bc

Interpreting the value of RR[12]

If RR <1 The risk in the given phenotype is lower than in the control. Negative association. ??protective phenotype

If RR = 1 No association established

If RR >1 Risk of Ca. Breast in the given phenotype is more than the risk in the normal population. Positive association is established. ??causative phenotype.

The significance of RR above or below unity is determined by calculating chi-square using the formular[14]

$$\text{chi-square} = \frac{[ad - bc]^2 [a+b+c+d]}{[a+c][b+d][a+b][c+d]}$$

and the standard error (SE) of RR is given by[14]

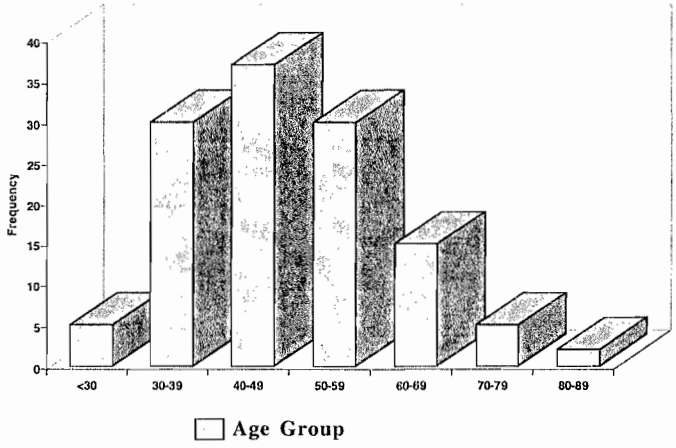
$$SE(RR) = OR\sqrt{1/a+1/b+1/c+1/d}$$

RESULT

Patients' Demography

A total of 107 reference subjects (cases), 2,243 controls A and 122 controls B were studied in a six-month period. The cancer predominantly affects women in their 4^{th} to 6^{th} decades - *figure 1*. Age range was 24 - 85 years, the median age being 45 years. Male:female ratio was 1:106. Controls A were predominantly males age ranged between 22 and 55 years while controls B were females age ranged between 21 and 47 years.

Figure 1: **Age Distribution of 107 patients wtih Breast Cancer in Lagos, Nigeria.**



Histological Types of Breast Cancers

All the cases had histological report of cancer of the breast. Ductal cancer is the predominant histological type, 98 (91.6%) of 107 cases had this diagnosis. Of the 98 ductal cancers, 93 (95%) were invasive (infiltrating) and 5 (5%) were intraductal. Other less common histological types reported are shown in *Table 1*.

Table 1: **Histological Types of Malignant Breast Disease - 107 cases at the Lagos University Teaching Hospital**

	Disease	Number	%
	CARCINOMA		
1.	Intraductal Carcinoma	5	4.6
2.	Invasive Ductal Carcinoma (Nos)	93	86.0
3.	Invasive Lobular Carcinoma	3	2.8
4.	Invasive Papillary Adenocarcinoma	5	4.6
	SARCOMA		
5.	Malignant Cystosarcoma Phylloides	1	0.9

Prevalence of Phenotypes

Of the 107 cases, 28 (26.2%) had blood group A phenotype. The prevalence of this phenotype in the control is almost the same, 589 (26.3%) of the 2,243 controls A having the same phenotype. Although the prevalence of the phenotype in control B was lower (22%), the difference was not significant, P>0.05. Prevalence of other ABO phenotypes also vary between cases and controls - *Table 2*. There appears however to be significant excess of subjects (9.3%) without inheritance of rhesus D antigen in the patient group compared with the controls (4.5%) and 4.1% for blood donors and Family Planning Clinic Attendees respectively, P<0.05.

Phenotype-Cancer risk Association

The calculation of the risk that inheritance of a given phenotype may be associated with breast cancer is laid out in *Table 3*. None of the phenotypes of the ABO blood group system has a relative risk significantly different from unity when cases were compared with controls A and controls B. Inheritance of rhesus D antigen however appears to be protectively associated with this cancer, relative risk (RR) being significantly lower than unity - RR = 0.45; SE = 0.16 and P<0.05 when cases were compared with controls A. RR is also well below unity (0.41) when cases were compared with controls B with SE = 0.23 although P>0.05.

Table 2. **Distribution of ABO and Rhesus D Phenotypes in Patients with Breast Cancer, Blood Donors and Family Planning Clinic Attendees (FPCA)**

	Phenotypes	Number	No. Positive for the Phenotype	Prevalence
A	Cases	107	28	26.2
	Blood Donors	2243	589	26.3
	FPCA	122	27	22.1
B	Cases	107	22	20.1
	Blood Donors	2243	440	19.6
	FPCA	122	25	20.5
O	Cases	107	54	50.5
	Blood Donors	2243	43	50.9
	FPCA	122	70	57.4
AB	Cases	107	3	2.8
	Blood Donors	2243	71	3.1
	FPCA	122	0	0.0
Rh.D Negative	Cases	107	10	9.3
	Blood Donors	2243	101	4.5
	FPCA	122	5	4.1
Rh.D Positive	Cases	107	97	90.7
	Blood Donors	2243	2142	95.5
	FPCA	122	117	95.9

Table 3: **Calculation of the Relative Risks (Odds Ratio) of Breast Cancer in association with some Red Cell Antigen Phenotypes**

Phenotype		Cases with Carcinoma of the breast	Controls without Carcinoma of the breast ie Blood donors and FCPA	Total	OR ~RR	Standard Error	P- Value
Blood Group A	Present	28	589 (27)	617 (55)	1 (1.25)	0.22 (0.37)	(>0.05)
	Absent	79	1654 (95)	1733 (174)			
	Total	107	2243 (122)	2350 (229)			
Blood Group B	Present	22	440 (25)	462 (47)	1.06 (1.0)		
	Absent	85	1803 (97)	1888 (182)			
	Total	107	2243 (122)	2350 (229)			
Blood Group O	Present	54	1143 (70)	1197 (124)	0.98 (0.75)	(0.20)	(>0.05)
	Absent	53	1100 (52)	1153 (105)			
	Total	107	2243 (122)	2350 (229)			
Blood Group AB	Present	3	71 (0)	74 (3)	0.88 (0)	(0.52) (0.58)	>0.05 (>0.05)
	Absent	104	2127 (122)	2276 (226)			
	Total	107	2243 (122)	2350 (229)			
Rhesus D Antigen	Positive	97	2142 (117)	2239 (214)	0.46 (0.41)	0.16 (0.23)	<0.05 (>0.05)
	Negative	10	101 (5)	111 (15)			
	Total	107	2243 (122)	2350 (229)			

( ) Corresponding values for Family Planning Clinic attendees.

DISCUSSION

In the present study, breast cancer is found to be uncommon below the age of 25 years. This finding agrees with other previously reported epidemiological features of this cancer[3]. The age incidence appears to increase up to the perimenopausal years (40 - 50 years). Beyond this age group, frequency was noted to decline. Keane et.al[15] had earlier described two patterns of age related distribution of breast cancer - *"In countries where there is a low frequency of the cancer, the incidence increases progressively, plateauing at the perimenopausal years and then decreases. In countries where breast cancer is more common, the incidence increases progressively with age, except during the postmenopausal years where there may be a plateauing or decline in disease incidence."* There are currently no data to show whether Nigeria fits a low or high incidence country but the age distribution finding in the present study may fit Keane's low incidence pattern.

As is the experience elsewhere[16], ductal carcinoma is the most common histological type in Nigerians and by the time the diagnosis is made, most have become invasive, about 97% of all cases in the present series being invasive.

The prevalence of ABO phenotypes of both the cases (predominantly females) and control (predominantly males, i.e. blood donors) groups were practically the same. This buttresses the fact that inheritance of ABO alleles are not sex-linked and the choice of predominantly male sex as control group does not introduce sex bias in the prevalence of different phenotypes. The use of predominantly female controls (Family Planning Clinic Attendees) also showed that sex bias for ABO phenotype does not exist. The phenotypes prevalence reported - 23%, 22%, 50% and 3% for A, B, O and AB phenotypes respectively agrees perfectly with the reported prevalence for this environment (Worlledge et.al, 1974)[17].

The finding that inheritance of D antigen phenotype may be protective against development of breast cancer was unexpected as most reported phenotypes that have been associated with cancer risk had been ABO phenotypes[8,9]. The D antigen is a non-glycosylated hydrophobic protein which traverses the red cell membrane 13 times with only short segments extending outside the membrane[18,19]. The physiological function of this protein is uncertain. Its deficiency as occurs in Rhesus null phenotype is however associated with haemolytic anaemia with stomatocytosis[20]. It is also not certain whether D antigen is expressed on cells other than the red cells. Its absence has been documented on the leucocytes[21], platelets[22], saliva[23] and amniotic fluid[24].

Its protective effect on the development of cancer of the breast statistically documented in this study is difficult to explain. The finding may however partly explain the epidemiological observation that in the Far East, incidences of and death rates from breast cancer are approximately one-tenth of those in the West[15]. Whereas the prevalence of D antigens in the western population is 85%, 100% of Chinese population are Rhesus D-antigen positive[25]. Gamut of evidences abound in literature to show that development of breast cancer is associated with an underlying genetic pathology. These evidences include:

a. The description of mutations in the tumour suppressor gene p^{53} in the Li-Fraumeni Syndrome of hereditary breast cancer[26].

b. The description of prevalent mutations in the BRCA-1 (Breast Cancer and Ovarian Cancer-1) gene amongst patients with breast cancer[27].

c. The identification of BRCA-2 which has been linked with development of familial female and male breast cancers but not ovarian cancers[28], and

d. Using the cytogenetic methodology of loss of heterozygosity and the technique of comparative genomic hybridisation, many chromosomal abnormalities have been described in association with the cancer of the breast[29]. These include chromosome 1, the chromosome that bears Rhesus antigen genes.

Despite all these specific mutations, which have been identified with high frequency and proven relevance in breast cancer, there are certainly many more important genetic changes remaining to be fully elucidated. We submit that lack of inheritance of D-antigen or some mutations of its gene may be a contributory factor in aetiogenesis of breast cancer.

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