Assessing the Prevalence and Impact of *KRAS*-rs712 Polymorphism in Breast Cancer Development Among Bangladeshi Women: A Case-Control Study

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.)

School of Pharmacy
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Spring 2026

Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing a degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. We have acknowledged all main sources of help.

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Approval

The thesis titled "Assessing the Prevalence and Impact of *KRAS*-rs712 Polymorphism in Breast Cancer Development Among Bangladeshi Women: A Case-Control Study" submitted by Abid Al Hasan (22146005), Fatima Akter Rifa (22146094), Sadia Tasnim (22146063), and Shahjin Choudhury (22146071) of Spring, 2026 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

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Ethics Statement

The investigation was conducted in accordance with all ethical principles to ensure that no one participating in it was harmed; their rights were not violated, and they were not neglected. The study was cleared by the Institutional Review Board (IRB No. BRACUIRB120220005) of BRAC University. The study plan was reviewed by the Institutional Review Board (IRB) which gave it the green light to ensure that it would abide by all the rules and morals. All participants who were interested in being part of the study were required to fill in a form indicating that they consented to participate in it. Individuals who participated in the study informed them about all becoming necessary about their objectives, procedures, the potential risks, and advantages of the study.

The research adhered to the provisions in the Declaration of Helsinki. All those who participated were informed that they were not facing much danger since it had been planned out. Undertaking this work on biological samples, all the attention was paid to the safety of the data to preserve privacy and identity. It was clarified that the information was confidential, and it would only be utilized in this study. They could only be viewed by people who were entitled to the data. This was to ensure that there were very strict guidelines regarding data security and ethics.

Abstract

Breast cancer affects Bangladeshi women especially poor women. The fact that diagnosis and treatment have improved means that even though people can no longer die due to lack of tests, some individuals still end up dying owing to the time-consuming nature of tests. Single nucleotide polymorphisms (SNPs) are a form of genetic mutation that increases the probability of an individual developing breast cancer by a considerable margin. To determine whether there was any relationship between the higher probability of getting breast cancer among Bangladeshi women and the *KRAS* gene, scientists examined the *KRAS* gene polymorphism (rs712). There were 124 healthy people and 112 people with breast cancer in the case-control study. These findings should be validated and how this difference influences the course of breast cancer should be established through more studies. The distribution of genotypes of rs712 was tested by Hardy-Weinberg equilibrium. TT genotype was linked with reduced risk of breast cancer with an Adjusted Odds Ratio of 0.065 (95% CI:0.009-0.504) and GT was associated with a potential risk of breast cancer with an Adjusted Odds Ratio of 1.870 (95% CI:1.071-3.263, p-value:0.028). However, following subsequent consideration of multiple testing and restricted covariate regulation, such associations are to be approached with caution. These results are to be supported by larger research studies with complex adjustment of confounders and categorization of multiple tests.

Keywords: Breast cancer, *KRAS*, SNP, Predictive biomarker, Cancer risk.

Dedication

- Abid Al Hasan (22146005)

This thesis is for parents who have been constant in my life; who might have made sacrifices and offered me excellent advice during my school life. I would also like to say thanks to my wife, Mariam Akter. She has always supported me and helped me when I was at the lowest point in my life. Lastly, Rajin, my only friend in university, Olif and Milan, has always been with me and assisted me throughout the past four years.

- Shahjin Choudhury (22146071)

I dedicate this thesis to my father who constantly supported me and removed all the thorns from my path to make this journey easier and my mother who always dreamt big for me. I want to thank my best friend Susoma living miles apart for being an inspiration to me. A part of this thesis is dedicated to my cousins, friends (especially Adiba, Maliha and Farhana) and teachers for their constant support and guidance. Lastly, I would like to thank an angel without whom I would never have been able to make it through.

- Fatima Akter Rifa (22146094)

I would like to dedicate this thesis to my parents for letting me have the privilege to do anything I ever wanted without any worry in the world and for always believing in me. I would also like to dedicate this to my husband Shahriar Polin for being my biggest supporter. I would like to thank my sister Suraiya Akter Rita for never letting me give up. I would like to thank some amazing people who have made my university life easier and cherish able. I would also like to thank sir Dr. Md. Aminul Haque and ma'am Dr. Sabrina Sharmin. Last but not least, I would like to thank me for not giving up and for always believing in Allah's plan.

- Sadia Tasnim (22146063)

To start with, I would like to thank Allah for giving me this life and the opportunity to reach this milestone. I am grateful to my parents who have given me unconditional love, have always supported me, and encouraged me to bring out the best in me. I am also thankful to my family, friends, and special mention to my maternal uncle Saiful Islam who has always inspired me to become a better individual through the wisdom and benevolence he has. Last but not the least, I am thankful to my superman because of his support and strength in all my ups and downs.

Acknowledgement

We are deeply grateful to Almighty Allah for providing us with the strength, patience, and opportunity to conclude this research assignment well. First and foremost, we want to thank Dr. Md. Aminul Haque, Associate Professor at BRAC University's School of Pharmacy, for allowing us to undertake this research with his aid. His constant support, intelligent advice, encouragement, and profound understanding have been extremely beneficial to us during the complete thesis effort. We are also extremely thankful to the faculties at BRAC University's School of Pharmacy for always pushing us, helping us with our academics, and being dedicated to their work. All these things helped us graduate from university. We also want to thank Rufaida BioMeds from the bottom of our hearts for allowing us to utilize their labs, which made it easy and fast for us to perform our research. Lastly, we want to thank everyone who has assisted us with this research in any way.

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List of Acronyms

BC	Breast Cancer
SNP	Single Nucleotide Polymorphism
KRAS	Kirsten Rat Sarcoma Viral Oncogene Homolog
PCR	Polymerase Chain Reaction
PCR-RFLP	Polymerase Chain Reaction–Restriction Fragment Length Polymorphism
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
miRNA	Micro Ribonucleic Acid
MAPK	Mitogen-Activated Protein Kinase
PI3K	Phosphatidilinositol 3-Kinase
HWE	Hardy–Weinberg Equilibrium
aOR	Adjusted Odds Ratio
CI	Confidence Interval
BMI	Body Mass Index
ER	Estrogen Receptor
PR	Progesterone Receptor
HER2	Human Epidermal Growth Factor Receptor 2
EDTA	Ethylenediaminetetraacetic Acid
IRB	Institutional Review Board

Chapter 1

Introduction

The prevalence of breast cancer is one of the most widespread types of cancer in women globally. The main effect is the uncontrolled development of aberrant breast cells. It may infiltrate surrounding organs and tissues and most often begins with the epithelial cells of the ducts or lobules. There are various varieties of breast cancer which include triple-negative, hormone receptor-positive, and HER2-positive. Every one of these kinds responds and reacts differently to treatment and rehabilitation at the clinic. The worldwide cancer prevalence is greater in females compared to males, with breast cancer taking the number 1 position among all types of cancer at over 25 percent (Mohthash et al., 2020). Women are the most susceptible to this form of cancer. It demonstrates the extent to which it has impacted the lives of people who are ill and dying around the world because approximately 1.67 million new cases are identified every year (Mohthash et al., 2020). By the year 2040, the statistics on breast cancer have been projected to reach over three million new cases and 1 million deaths (Arnold et al., 2022). Although there has been better detection and early treatment, breast cancer remains a health issue in especially developing countries such as Bangladesh with a weak healthcare system.

The number of breast cancer cases has tremendously increased in the world. Developed nations have greater frequencies of the disease because they have better screening methods. Nevertheless, the developing nations such as Bangladesh have increased rates of death although it has emerged as a major criminal hidden health issue due to its presence. The most common type of cancer in Bangladeshi women is breast cancer, with 22.5 incidences per 100,000 (Shamsi, 2021). In 2018, there were 12,764 new cases and 6,846 deaths, a high fatality rate. Most of the female cancer deaths in Bangladesh are the result of breast cancer (Shamsi, 2021).

The condition is usually not diagnosed early enough. Research indicates that a quite large proportion of the population are diagnosed with Stage III or IV cancer (Shamsi, 2021). Perhaps, the number of cases could grow due to many different factors, some of them include lack of knowledge, lack of belief in medical procedures, lack of access to good screening tools, and tendency to postpone medical services. Women are comparatively less likely to pursue prompt diagnosis and get proper treatment because of a few reasons such as cultural and economic factors among others. There are chances that breast cancer treatment in Bangladesh would be very stressful to patients and their families due to its high cost. This is because there is a shortage of finance available. Numerous individuals have been revealed as ready to pay for treatments which could conceivably lengthen their time on earth by ranking the outlay on health higher than any other expenditure on their agenda. On the other hand, it is still challenging to locate reasonably priced medical treatment. Intriguingly, the rate of breast cancer occurring in Bangladesh is earlier compared with the western countries. Research indicates that the highest rate of incidence can be 1520 years younger than in developed nations (Shamsi, 2021). Nevertheless, the urban and middle-class patients comprise the majority. But diagnosis and treatment in rural set-ups are more difficult. Considering this discrepancy, it is paramount to emphasize the importance of being diagnosed at an early stage and being able to utilize healthcare. Breast cancer is also far more common in other South Asian countries, for instance India. The rates of death are still higher even when it turns out that in poor countries the rates of new cases are now comparable to the rates in rich countries. This increasing trend demonstrates the relevance of developing research and health plans tailored to different regions since approximately one out of eight women are being diagnosed with this breast carcinoma thus making it one of the most common cancers affecting women across the globe.

Genetic mutation and polymorphism have a major influence on breast cancer. Genetic mutations refer to the alterations in the DNA which prevent cells from performing or

continuing their normal functions. Cancer is usually caused by these mutations. Changes in genetics, especially SNP, play a key role in the risk of modification of cancer. These modifications can affect the expression of genes, as well as protein activities and cellular communication. Polymorphisms can also influence the oncogenes and tumor suppressor gene expression in breast carcinoma, and this may interfere with the mechanisms of cell division, cell death and cell repair. Polygenic inheritance theory is proven by the accumulative impact of numerous polymorphisms in cancer development (Antoniou & Easton, 2003).

The focus of the current study is to identify additional genetic markers affecting the risk of breast cancer. Some of these genes mediate signaling pathways, hormonal actions and immune reactions. Knowledge of such inherent aspects is crucial in easily identifying and helping people early. The *KRAS* gene is a significant oncogene which has great effects on cell signaling pathways of controlling growth, differentiation and survival. It acts as a molecular switch in pathways like mitogen-activated protein kinase (MAPK) pathways and the phosphatidylinositol 3- kinase (PI3K) pathways (Wee et al., 2009). These pathways play an important role in cell survival and growth. *KRAS* gene mutations or polymorphisms can constitute a continuous activation of these pathways. This can cause an increase in the multiplication of the cell and the creation of cancer. The *KRAS* gene plays a role in regulating the growth, division, and development of cells through the assistance of the MAPK and PI3K pathways.

Alterations or changes in this gene can cause cancer and abnormal cells. This SNP of the *KRAS* gene is highly important in the format of rs712. This site is in the 3' 1untranslated region. MicroRNAs are the main way that this area affects genes. The miRNA binding of the polymorphism on rs712 is altered. This applies to microRNAs that prevent the growth of tumors like the let-7 that prevents the production of *KRAS*. *KRAS* passing through the rs712

mutation might cause the *KRAS* to be increasingly active, based on its ability to be bound by miRNA (Du et al., 2017). In case of over-expression, it will increase the number of signals that will result in increased uncontrolled growth of cancerous cells, arrest, and creation of tumors. Certain genotypes of rs712 are predisposing. Rs712 can cause cancer by changing *KRAS* post-transcriptional regulation, although it has not been examined as deeply as rs61764370. It contributes to breast cancer ambiguously and differently across groups. *KRAS* rs712 polymorphism can predispose breast cancer by means of changing gene functionality. Further studies are needed to determine its clinical value as a biomarker to diagnose and personalize treatment especially in Bangladesh. Although the number of breast cancer patients in Bangladesh is on the rise, not much genetic research is being conducted in the country. Most of the published research studies have largely consisted of clinical and statistical problems. Genetic material polymorphisms, such as *KRAS,* have not received much attention. Bangladesh requires population-based research because it has its own genetic and environmental peculiarities. The identification of genetic risk factors may result in improved early detection, improved disease prevention strategies, and more individuals to guarantee when implemented.

This paper evaluates how genetic differences, especially on *KRAS* gene, affect the predisposition to breast cancer in Bangladesh. Breast cancer is prevalent in Bangladesh. It is relevant to study the genetics of breast cancer and acquire an understanding of why this population group is more prone to develop the disease and how it propagates. Bangladesh is associated with a high rate of death due to late diagnosis, ineffective screening procedure and lack of awareness. Genetic differences, especially single nucleotide polymorphism (SNPs) affect the risk of cancer as they can change the expression levels of genes and the communication between cells. Researchers have studied the cancer that involves using the *KRAS* gene that determines the growth and survival of cells. Alterations in the genes influencing the way cells operate can lead to the development and spread of the tumors.

Researching the molecular information of *KRAS* associated SNPs associated with breast cancer may make us understand more about the disease. This connection is of great importance to find it. Initially, it may enhance early treatment and screening measures that would assist the physicians to locate individuals who fall at risk before their condition deteriorates. Second, it may facilitate tracking down groups of individuals that are at high risk so that they can be continuously monitored and kept safe. Third, by examining some DNA patterns, genetic research could be beneficial in developing drugs that are more effective and have fewer or very less adverse effects. This type of research also prepares customized medicine. Individualized medicine alters the process of detecting, protecting and treating the genes in a person. Bangladesh does not possess enough money or resources yet, introducing genetic research into the sphere of healthcare could support people there. This analysis could contribute to reducing the number of patients with breast cancer, increasing the cases of survivors, and promoting more efficient and fair healthcare policies by discovering the DNA risk factors and endorsing early intervention.

Chapter 2

Methodology

2.1 Materials

TaKaRa taq DNA Polymerase Master Mix was procured from TAKARA BIO INC (Japan). Restriction enzyme from TAKARA BIO INC (Japan). DNA ladder from TAKARA BIO INC (Japan). SeaKem LE Agarose from Lonza (USA). Tris-borate-EDTA (TBE) buffer from Solarbio Life Sciences (China). DNA extraction kit from Favorgen (USA). Midori Green Nippon Genetics (Europe). Primers from Macrogen (Korea). Restriction digestion enzymes (New England Biolabs, USA).

2.2 Study design and participants

To establish the link between *KRAS* rs712 polymorphism and breast cancer, a hospital-based case control study was conducted in Bangladeshi population. For the following study, 112 confirmed breast cancer patients were recruited from the National Institute of Cancer Research and Hospital (NICRH) and 124 healthy patients were taken from the same region. The patient group included confirmed breast cancer female patients who were at least 18 years old and did not have any previous recording of any other cancer. Also, patients who received chemotherapy or radiotherapy before blood sample collection or with severe chronic system diseases were excluded. Structured questionnaires were used to collect demographic and clinical data including age, body mass index (BMI), number of children, occupation, education, socio-economic status, residential status and menopausal status.

2.3 DNA extraction and genotyping

Peripheral blood samples collected from the volunteers were stored in 15 ml tubes where EDTA was used as an anticoagulant. Then genomic DNA was extracted from these samples

using the commercial DNA extraction kit Favorgen, USA by the spin extraction column method. To isolate the genomic DNA, the blood samples were subjected to lysis and then the lysate was sent to the binding column. The DNA was then washed with multiple buffers after which nuclease free water was used to wash out DNA. This DNA was stored at -20°C and its purity was confirmed using 1% agarose gel.

After the completion of DNA extraction, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay was carried out using a thermal cycler (miniPCR, USA) and Emerald AMP GT PCR Master Mix was used to identify SNP at different polymorphic sites. The products of PCR were confirmed by 1% agarose gel electrophoresis. After PCR was completed, restriction digestion enzyme *MspI* was used at 37°C for 1 hour to digest the products. These digested fragments were separated on 3% agarose gel electrophoresis stained with Midori Green and then visualized under UV illumination. 20% of the samples were subjected to Sanger sequencing through a commercial service provider, the results of which was 100% concordant with PCR-RFLP thus confirming the validity of the genotyping results.

Gene, SNP	Primers' sequence F-Forward, R-Reverse	Thermal condition	Fragments, BP
KRAS rs712	F 5'-AAGGCATACTAGTACAAGTGGTAA-3' R 5'- TGTGTTCCCTCAATGTTTCAGT-3'	5 min of denaturation at 98°C, then 40 cycles of 98°C for 40s, 62°C for 40s, and 72°C for 50s. The final cycle had a 5 min extension at 72°C	426

Table 1: Primer sequences, thermal conditions and amplified products' size

2.4 Quantitative analysis

The data was statistically analyzed using R software (version 4.4.1; R Foundation for Statistical Computing, Vienna, Austria). Hardy-Weinberg principle was used to test Hardy-Weinberg equilibrium. Chi-squared tests were used to assess the differences in the distribution of single nucleotide polymorphism between healthy individuals and individuals with breast cancer. Age and BMI were used as covariables in multivariate logistic regression models to calculate Adjusted Odds Ratios with 95% confidence intervals.

2.5 Ethical considerations

The following study was conducted in Rufaida BioMeds as a final year thesis project by students at the School of Pharmacy, Brac University under the approval of Institute Review Board (IRB), Brac University (IRB No. BRACUIRB120220005). Prior to the beginning of this study, all the participants gave written informed consent. They had the freedom to withdraw at any point without any consequences.

Chapter 3

Result

The genomic DNA of 124 healthy volunteers and 112 breast cancer patients was collected. The research data was used to identify any association between breast cancer with *KRAS* rs712 by utilizing different genetic models. The data were tested for Hardy-Weinberg Equilibrium (HWE) using chi-square test. P value was calculated to be greater than 0.05 demonstrating that the genotype distribution followed Hardy-Weinberg equilibrium. Four types of models were used: additive model, dominant model, recessive model and over dominant model. All the models used GG phenotype as the reference group.

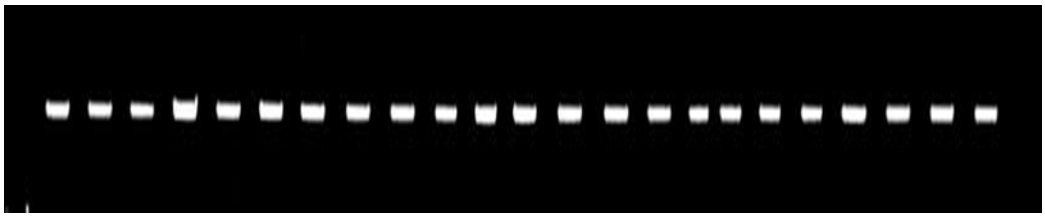

Figure 1: Presentative 1% gel image of the extracted DNA by spin column extraction method

DNA ladder after electrophoresis of 2% agarose gel is shown below. The results from gel electrophoresis showed two types of bands: G type at 154 and 272 bp and T type at 426 bp.

Figure 2: 426 bp DNA ladder after electrophoresis of 2% agarose gel

1 2 3 4

1=100 BP Ladder; 2=TT Genotype; 3=GG Genotype; 4=GT Genotype

Figure 3: Representative 3% gel image of digested samples by enzyme *MspI* at 37°C for 1 hour

Based on the bands at figure 2, *KRAS* rs712 polymorphism was identified at:

GG common homozygote giving band at 154 bp and 272 bp

GT heterozygote giving band at 154 bp, 272 bp and 426 bp

TT rare homozygote giving band at 426 bp

For these three alleles, adjusted odds ratios (aOR), 95% confidence intervals (CI), and p values were calculated using the multivariate logistic regression in R adjusted for age and BMI.

Allele	Case (n=112)	HWE		Controls (n=124)	HWE		Genetic models	aOR	95% CI	p-value
		X^2	P		X^2	P				
GG Common homozygote	n=68 (60.714%	0.038	0.844	n=78 (62.903 %)	0.10 6	0.74 4	Additive model 1 (GT vs GG)	1.591	0.904-2.799	0.107
							Additive model 2 (TT vs GG)	0.076	0.010-0.594	0.014
GT Heterozygote	n=43 (38.393%)			n=31 (25%)			Dominant model (GT+TT vs GG)	1.970	0.648-1.856	0.730
TT Rare homozygote	n=1 (0.893%)			n=15 (12.097 %)			Recessive model (TT vs GT+GG)	0.065	0.009-0.504	0.009
							Over dominant model (GT vs GG+TT)	1.870	1.071-3.263	0.028

Note: Genotype frequencies were tested for Hardy-Weinberg equilibrium (HWE) using the Chi-square test (X^2).

Adjusted Odds Ratios (aOR), 95% Confidence Intervals (CI), and p-values were calculated using multivariate logistic regression in R. All models used the TT genotype (common homozygote) as the reference group.

$p > 0.05$ indicates that genotype distribution follows Hardy-Weinberg equilibrium.

Abbreviations: HWE – Hardy-Weinberg equilibrium, aOR – adjusted Odds Ratio, CI – Confidence Interval, X^2 – Chi-square value

Table 2: Association between breast cancer with *KRAS* rs712 polymorphism

3.1 Association of rs712 polymorphism with BC

In table 2, the allelic and genotypic frequencies of the *KRAS* rs712 polymorphism were analyzed for both breast cancer patients and control groups. The genetic models used were additive model 1 where GT was compared with GG, additive model 2 where TT was compared

with GG, dominant model where GT+TT was compared with GG, recessive model where TT was compared with GT+GG and over dominant model where GT was compared with GG+TT. The GG genotypes were slightly higher in the control group (62.903%) compared to the patient group (60.714%). The rare homozygote TT was also higher in the control group with a percentage of 12.097% compared to the patient group, which had a percentage of 0.893%. The rare heterozygote GT was 38.393% in the patient group, which was greater than the control group with 25%. The results of additive model 1 and dominant model were statistically insignificant as the p-value was greater than 0.05 and CI includes 1. However, the additive model 2, recessive model and the over dominant model yielded statistically significant results. In additive model 2 (TT vs GG), the p value was 0.076, suggesting that the results were significant statistically. The aOR was 0.076 (95% CI:0.010-0.594) which suggested that there was a reduced risk with genotype TT compared to GG. The recessive model had aOR of 0.065 (95% CI:0.009-0.504) suggesting that TT genotype was linked with reduced risk of breast cancer and had a protective effect over GT and GG genotypes. The over dominant model (aOR:1.870, 95% CI:1.071-3.263, p-value:0.028) suggested that GT was associated with a potential risk of breast cancer. However, in additive model 1 and dominant model GT did not show statistically significant association with breast cancer.

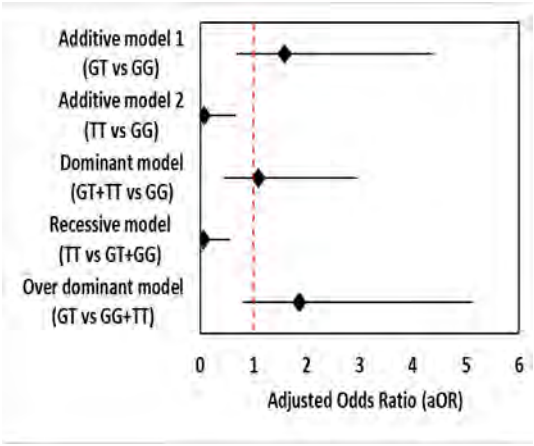

Figure 4: Forest plot illustrating the connection between breast cancer and the *KRAS* rs712 polymorphism

3.2 Socio-demographic characteristics

The demographic features of breast cancer patients and healthy volunteers are represented in table 2. The age of the patients was between 34 and 59 while the age of the healthy volunteers was between 34 and 60 with no significant difference between their age as indicated by a p value of 0.58. There was no significant difference between their BMI, occupation, level of education, socio economic status, residential status, and menopausal status as well. The volunteers were taken from three different occupations, housewife, working woman and student. Most of the women with breast cancer were working women with a percentage of 68% while only 5.4% were students. 53.57% of the patients were illiterate while 52.68% belonged to lower-class socio-economic status. 76% of patients with breast cancer were rural residents and the majority of them with a percentage of 63% were post-menopausal. From the table we can see that the p value was less than 0.05 for all the categories which indicated that there was no significant difference between the patient group and control group thus increasing reliability of the experiment.

Variable	Cases(n=112)	Controls(n=124)	P value
Age(years)	46.9±12.5	47.8±13.1	0.58
BMI (kg/m2)	25.7±4.9	25.2±5.1	0.32
Number of children	2.13±0.91	2.09±0.87	0.45
Occupation			0.46
Housewife	38 (33.9%)	44 (33.5%)	
Working women	68 (60.7%)	73 (58.9%)	
Student	6 (5.4%)	7 (5.6%)	
Level of education			0.71
Illiterate	60 (53.57%)	60 (48.38%)	
<10 years	40 (35.71%)	48 (38.71%)	
>10 years	12 (10.71%)	16 (12.9%)	
Socio-economic status			0.29
Lower class	59 (52.68%)	74 (59.68%)	
Middle class	45 (40.19%)	38 (30.66%)	
Higher class	8 (7.14%)	12 (9.68%)	
Residential status			1.0
Rural	76 (67.86%)	85 (68.55%)	
Urban	36 (32.14%)	39 (31.45%)	
Menopausal status			0.881
Premenopausal	49 (43.75%)	52 (41.94%)	
Postmenopausal	63 (56.25%)	72 (58.06%)	

Table 3: Demographic characteristics of breast cancer cases and healthy control

3.3 Histopathological reports of breast cancer patients

Histopathological data was collected from BC patients, which is summarized in table 4. The number of patients with variables such as breast lump, nipple discharge, retraction of nipple, deviation of nipple, weight loss and ulceration of overlying skin or nipple areola was recorded. Along with that, the clinical stage of the cancer and percentage of hormone receptors involved was also collected. 100% of the patients had breast lump, 21% had nipple discharge, 16% had retraction of nipple, 10% had deviation of nipple, 45% suffered from weight loss. Their histopathological examination identified invasive ductal carcinoma as the predominant type. In terms of clinical stage majority of the patients (46.43%) were identified as grade I. According to their hormone receptor profiling, 65.19% was estrogen receptor positive, 71.43% was progesterone receptor positive and 28.57% was HER-2 receptor positive. The size of the tumor was mostly between 2.1-5 cm while lymph node metastasis was present in 26.79% of the patients.

Variable	**Cases (n=112)**
Breast lump	112(100%)
Nipple discharge	21(18.75%)
Retraction of nipple	16(14.29%)
Deviation of nipple	10(8.92%)
Weight loss	45(40.19%)
Peau d'orange	44(39.29%)
Ulceration of overlying skin or/and nipple areola	11(9.82%)
Histology	
Ductal ca in situ	16(14.29%)

Invasive Lobular	12(10.71%)
Invasive ductal	84(75%)
Clinical stages	
Grade I	52(46.43%)
Grade II	42(37.5%)
Grade III	18(16.07%)
Hormone receptor	
ER+	73(65.19%)
PR+	80(71.43%)
Her-2+	32(28.57%)
Tumor size	
<2cm	6(5.36%)
2.1-5.0cm	62(55.36%)
>5.0cm	44(39.29%)
Lymph node	
Positive	30(26.79%)
Negative	82(73.21%)

Table 4: Histopathological data of breast cancer patients

Chapter 4

Discussion

The case control study involved investigating the relationship between *KRAS* (rs712) polymorphism and predisposition to BC among Bangladeshi women. Based on the logistic regression analysis results to estimate the adjusted odds ratios, confidence intervals, and p-values, the results suggest that the rs712 variant might be related to the risk of BC in this population. These findings are, however, to be read with caution as there were limitations in the study.

Breast cancer is a widespread cancerous disease which plagues scores of women in the world and is typically triggered by some genetic alterations that govern cell growth and multiplication (Semmler et al., 2019). *KRAS* is a common oncogene, which produces a protein that is a part of a cell division-controlling system (Prior et al., 2012). Variations in this gene particularly in the 3′-untranslated region (3CL-UTR), can have effects on the expression of a gene (Mayr & Bartel, 2009). It is also worthy to mark that the polymorphism in the position of the rs712 falls within a binding site of the tumor suppressive microRNA let-7 and any mutation in the site can interfere with binding of miRNA, resulting to change of activity of *KRAS* and contributing to carcinogenesis (Chin et al., 2008; Johnson et al., 2005).

In the current study the polymorphism of rs712 was found to be linked with the risk of BC. The findings are in line with previous research done in Southeast Iran which reported that the rs712 variant was as well strongly correlated with the risk of BC and TT genotype seemed to protect similarly in a recessive model (Safaralizadeh et al., 2012). Likewise, investigations of Iraqi women have shown a correlation of the TT genotype of the rs712 genotype and susceptibility of benign tumours of the breast (Al-Janabi et al., 2014). These results fulfil the hypothesis that rs712 could take part in the development of breast tumour.

However, all studies are not consistent. One case in point is a study conducted on the population of Chinese people (Han), which revealed that there is no significant correlation between the risk of BC and rs712. Instead, the TT genotype and T allele could make a patient of BC less prone to the metastasis of lymph nodes (Zhang et al., 2011). These discrepancies could be indicative of the intricacy of genetic-disease interactions that could be different based on ethnicity, genetic variation, environmental exposures, and sample sizes.

Overall, this study suggests that polymorphism of *KRAS* at rs712 is a potentially useful genetic determinant that puts Bangladeshi women at risk of developing breast cancer. However, further studies are needed employing bigger multi-centred samples, and functional studies to validate these findings and elaborate the biological processes.

Chapter 5

Conclusion

The study was done considering the aim of either confirming or refuting the fact that there is correlation between the rs712 and the probability of contracting cancer in breast among Bangladeshi women. There was a total of 112 breast cancer patients and 124 healthy control volunteers who took part in the study. Due to their involvement, we managed to get valuable information concerning the genetic disposition of the disease in the same community.

The findings of this study suggests that *KRAS* polymorphisms may affect breast cancer susceptibility in Bangladeshi women. The rs712 TT genotype might be particularly associated with reduced risk of breast cancer while the rs712 GT genotype might increase the risk of developing breast cancer. However, the GT genotype did not show a significant association with breast cancer when compared to GG in additive model and dominant model.

The outcomes of our study will present the confirmation that genetics can be applied in future with the scope of establishing the probability of getting cancer. Consequently, this would render it possible to carry out larger research that may involve multiple institutions to investigate the same ideas at a greater level and cast some light on outcomes. It was due to this fact that the identification of *KRAS* rs712 as a high-risk marker would facilitate easier constructions of early screening tools and specific intervention strategies that would target breast cancer detection and medicine. To be able to obtain a better understanding of the functional implications that this polymorphism has in the signaling pathways and the role it takes in the development of malignancies, it is necessary to perform additional research activities. Moreover, the interconnection of the genetic data with the information concerning the environment and the style of life will be used to facilitate the formulation of the individualized methods of the inhibition and therapy of breast cancer in Bangladesh.

Although it is clear, due to our data, that existing genetic predisposition is possibly linked to the *KRAS* rs712 polymorphism, further validation and study of genetic risk factors in breast cancer is nonetheless required. It is believed that with some time the evolution of genetic study in Bangladesh would ultimately lead to development of better diagnostic tools to manage early diagnosis, enhance specialized methods of therapeutics, as well as decrease the weight of the disease in the region at some stage in the future.

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