Journal of Cancer Research and Treatment, 2020, Vol. 8, No. 1, 1-6
Available online at http://pubs.sciepub.com/jcrt/8/1/1
Published by Science and Education Publishing
DOI:10.12691/jcrt-8-1-1

Rapid Screening of Blood Mitochondrial D310 and D315 Mutations in Breast Cancer Patients

Ferdowsi Akter[1], SM Hasan Israfil[2], Mohammad Shawkat Ali[3], A. S. Shamsur Rouf[4], Mohammad Sahajadul Alam[5], Mohammad Shafiqur Rahman[6], Gazi Nurun Nahar Sultana[7,*]

[1]Department of Pharmacy, East West University, Dhaka, Bangladesh
[2]Department of Pharmacy, State University of Bangladesh, Dhaka, Bangladesh
[3]Department of Clinical Pharmacy and Pharmacology, University of Dhaka, Dhaka, Bangladesh
[4]Department of Pharmaceutical Technology, University of Dhaka, Dhaka, Bangladesh
[5]Department of Surgical Oncology, National Institute of Cancer Research and Hospital, Dhaka, Bangladesh
[6]Institute of Statistical Research and Training, University of Dhaka, Dhaka, Bangladesh
[7]Centre for Advanced Research in Sciences, University of Dhaka, Dhaka, Bangladesh
*Corresponding author: nngazi@gmail.com

Received April 08, 2020; Revised May 10, 2020; Accepted May 17, 2020

Abstract **Aims:** 'Poly C' stretch extending from 303 to 315 nucleotide positions known as (D310) by GeneBank within the non-coding region HVR-II of mitochondrial DNA (mtDNA) has been identified as a mutational hotspot of primary cancer. We aimed to find this mutation in Bangladeshi breast cancer patients and control samples for screening the changes in poly C stretches. **Materials and Methods:** We have analyzed a total of 98 breast cancer blood samples and 98 healthy control blood samples and extracted DNA from blood samples for direct sequencing using ABI®3130 Genetic Analyzer. **Results:** We observed 70.41% C insertion at 310 regions (P<0.001, OR = 16.30 and 95% CI=7.83-33.93) in breast cancer patient whereas it is present only in 12.24% healthy individuals. No alterations were observed in 29.59% breast cancer samples. We also check the mutation pattern at D315 regions, but no significant observation was found (P=1.00). **Conclusions:** This is the first study from Bangladeshi breast cancer (BC) patients indicating a relatively high frequency of D310 mutations, which suggests that mtDNA instability at D310 may be a common characteristic of BC, study also supports the hypothesis that mtDNA D310 screening may represent additional blood based biomarker for breast cancer prognosis.

Keywords: *mtDNA, D310, Poly C stretch, breast cancer*

Cite This Article: Ferdowsi Akter, SM Hasan Israfil, Mohammad Shawkat Ali, A. S. Shamsur Rouf, Mohammad Sahajadul Alam, Mohammad Shafiqur Rahman, and Gazi Nurun Nahar Sultana, "Rapid Screening of Blood Mitochondrial D310 and D315 Mutations in Breast Cancer Patients." *Journal of Cancer Research and Treatment*, vol. 8, no. 1 (2020): 1-6. doi: 10.12691/jcrt-8-1-1.

1. Introduction

Breast cancer, a multifactorial disease, is a leading cause of morbidity and mortality in women worldwide [1]. It is a common cancer of women in the USA, Western Europe as well as in developing countries like Bangladesh [2]. Breast cancer is gradually becoming the most common cancer in Bangladeshi women, both from rural and urban areas [3,4].

In recent years several studies identified mutations in the non-coding and coding regions of mitochondrial DNA (mtDNA) and have investigated their potential use as a somatic marker for early tumor detection [5]. Mitochondrial DNA (mtDNA) mutations were detected in many cancer e.g. 70% of colorectal [6], 46% head and neck, 64% bladder, 43% lung [7], 80% pancreatic [8], 60% ovarian [9], and 61% breast cancers [10]. Mitochondria are small cellular organelles which are responsible for majority of cellular energy production; they are the major site of reactive oxygen species (ROS) generation and play vital role in regulating apoptosis in mammalian cells [11]. Human mitochondrial DNA (mtDNA) is a 16,569 bp double stranded circular molecule, and each mitochondrion contains multiple copies of DNA. mtDNA is located in the inner membrane of mitochondria and is more susceptible to damage by free radical produced by oxidative phosphorylation (OXPOS) and exhibits a mutation rate >20 fold higher than that of the nuclear genome [12]. The reasons for higher mutation rate are also due to absence of introns and protective histone and non-histones proteins [13], lack of repair mechanisms, inefficient DNA proof reading and defective clearance of damage mitochondria [14]. Mitochondrial D-loop is a non-coding control region of mtDNA located between nucleotides 16010-15578bp which contains *cis*-regulatory elements required for replication, transcription, and maintenance of mtDNA. The D-loop of mtDNA has unstable homopolymeric C-stretch known as

D310 which is a mononucleotide repeat of Cs that varies from 12 to 18 Cs interrupted at nucleotide position 310 by T (303'CCCCCCCTCCCCC' 315). Mutations affecting these sites are considered significant in cancer pathology. The first stretch of Cs can vary from seven to nine in normal people. This region has been extensively studied in many human cancers, and Sanchez-Cespedes was the first to identify D310 as a mutational hot spot in cancer [15].

Since there is no study about the frequency of D310 and D315 region mutations inBangladeshi breast cancer patients, the aim of this study was to evaluate the frequency of mtDNA D310 and D315 mutations in blood samples of breast cancer patients in Bangladesh.

2. Material and Methods

2.1. Sample Collection

Total 98 blood samples were taken from the breast cancer patients during pathological test at National Institute of Cancer Research and Hospital (NICRH), Mohakhali, Dhaka. Time frame of sample collection was 2014 to 2017. A healthy group of age matched 98 female individuals from mainstream population was also included. Blood samples from healthy individuals were considered as control (Table 1). All two populations share the same ethnicity and nationality and reside in Bangladesh. Subjects with breast cancer were interviewed with patient's consent. From volunteers 3mL of blood samples were collected in EDTA coated tubes. The samples were transported to the laboratory at 4°C cool box and kept at -20°C until DNA extraction was done. This work was approved by the local ethical committee of Bangladesh Medical Research Council (BMRC) and University of Dhaka.

2.2. DNA Isolation

DNA was isolated by standard proteinase K treatment followed by phenol/ chloroform/ isoamyl alcohol extraction both from breast cancer patients and control individuals blood. Quantification of DNA was performed by Nano Drop-2000® Spectrophotometer and 0.8% agarose gel electrophoresis was done to observe the efficacy of extraction.

2.3. Amplification of D-loop by Polymerase Chain Reaction (PCR)

A 420 bp fragment of the non-coding D loop including D310 and D315 sequence was amplified by Polymerase chain reaction. For amplification 40 ng of DNA was used in a final 50µL master mix containing 10mM Tris-HCl (pH 8.8), 1.5 mM $MgCl_2$, 100mM dNTPs, 1U of TaqMan™ DNA Polymerase (Applied Biosystem USA) and 1µM of each primer. The forward primer and the reverse primer were used for PCR amplification using Bio-Rad PCR machine. PCR condition included an initial denaturation at 95°C for 5 min, followed by denaturation at 94°C for 30 s, 58°C for 30 sec, and 72°C for 2 min followed by 35 cycles and final extension step at 72°C for 7 min [16].

2.4. Gel Electrophoresis

Amplification of PCR product was confirmed by1.5% agarose gel electrophoresis in1×TE buffer using a 1kb pus ladder (used as a marker) for assessing the size of the amplified product.

2.5. Sequencing of the D310 & D315 Region

The ABI-prism Big Dye Terminator BDT v3.1 containing ampliTaq polymerase, dye terminators (fluorescent label), dideoxynucleotide triphosphate, magnesium chloride, was used for direct sequencing of PCR product for specific primers. The sequencing PCR was performed on the basis of following cycling conditions: initial denaturation at 95°C for 1 min followed by 94°C for 10s, 55°C for 30s, and 60°C for 4 min for 30 cycles. Cycle sequencing products were purified by ethanol precipitation and dissolved in Hi-Di formamide for sequencing with ABI®3130 Genetic Analyzer.

2.6. Sequence Analysis

The purified sequencing PCR products were analyzed by electrophoresis in the ABI-Prism 3130 Genetic Analyzer (Applied Biosystem, USA). The sequence patterns were observed and edited by using Mac-based software (Auto Assembler V 3.0) and BioEdit Sequence Alignment Editor V 7.0.9.0 (http://www.mbio.ncsu.edu/bioedit/bioedit.html). The sequences were aligned by using bl2seq tool of NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and compared with the revised Cambridge Reference Sequence, rCRS (NCBI Reference Sequence: NC_012920.1) [17]. By using Mitomap (www.mitomap.org) polymorphisms of mtDNA were compared with the mitochondrial genome database of world population.

2.7. Statistical Analysis

Chi-squared test was used to compare the distributions of polymorphism in normal and tumor blood samples. It was used to compare the prevalence of mutant allele heteroplasmy between normal and cancer samples. Logistic regression analysis was also done. All statistical analyses were carried out with the STATA software (version-14). P value of <0.05 was considered statistically significant [18].

3. Results

3.1. High Frequency of Somatic D310 Mutations in Breast Cancer Samples

In this study, we screened for mitochondrial D-loop mutations in 420 bp of the hypervariable region II which includes the PCT (D310 and D315) repeat. This is a common mutational hot spot of the mitochondrial genome. Ninety eight breast cancer and equal number of healthy samples were taken. We compared the mutation frequency of D310 and D315 sequences between two study groups. In all positive samples, the reaction was repeated for confirmation of the mutational changes.

Table 1. D310 and D315 mutation frequency between two study groups along with Odds Ratio, *P-value* and 95% CI.

Mutation	Study group	Mutation presence	D310 (%)	D315 (%)	OR	*P-value*	95% CI
D310	Healthy	Present	12.24	9.18	16.30	<.001	7.83-33.93
		Absent	87.76	90.82			
D315	Breast cancer	Present	70.41	9.18	1.00	1.00	0.379 -2.636
		Absent	29.59	90.82			

A total of 98 breast cancer and equal number of healthy individuals comprises the study population of this research. Among them somatic mutation (defined as different mtDNA sequences between tumor and normal blood samples) for D310 sequence occurred in 70.41% of breast cancer (69 out of 98) and 12.24% of healthy control (12 out of 98) subjects (P-value= <0.001, with OR= 16.30 and 95% CI values of 7.83-33.93) (Table 1). For D315 sequence somatic mutation rate is 9.18% for breast cancer (9 out of 98) and 9.18% (9 out of 98) for healthy individuals (*P*=1.00 with OR of 1and 95% CI values of 0.379-2.636) (Table 1). Here significant mutational variation is absent in D315 sequence.

3.2. C_8TC_6 Mutation is More Common in Breast Cancer Samples

Polymorphisms in the D310 sequence in a normal human population consist of cytosine repeats variations, which range most commonly between seven and nine cytosine repeats [19]. In this study we observe polymorphic changes in D310 position and found high abundance of C_7TC_5 in healthy control sample (Figure 1) and C_8TC_6 abundance in breast cancer samples due to C insertion (Figure 2). Because of the high abundance of C_7TC_5 sequences in the healthy samples it is reasonable to consider C_7TC_5 as normal alleles. From these results, we hypothesized that any variation from these sequences is likely due to either somatic mutations or germ-line polymorphisms and in that conclusion; we found two C insertions in D310 sequence.

3.3. Frequency of Homoplasmy and Heteroplasmic mtDNA Mutation

Homoplasmic mutation means it affects all copies of mtDNA, whereas those which were present only in a proportion of mtDNA copies were defined as heteroplasmic. Thus, a coexistence of two or more peaks on the electropherogram indicated heteroplasmy [25] (Figure 3). Homoplasmic mutation dominates over heteroplasmic mutation in this study in both study groups (92.86% in breast cancer patients and 96.94% in healthy individuals). And the mutation frequency didn't vary significantly between two study groups (Table 2).

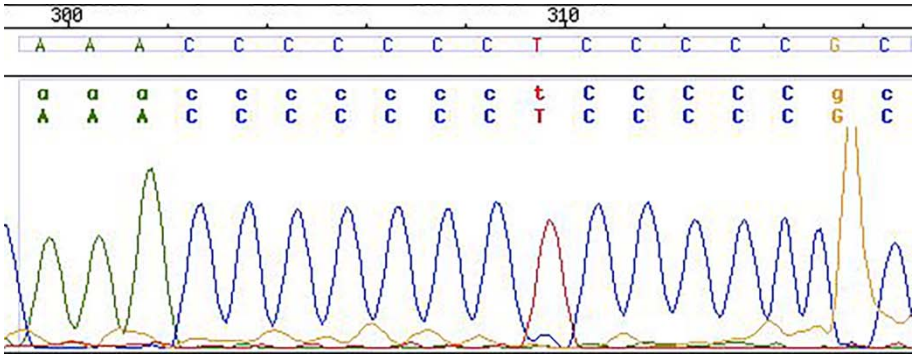

Figure 1. Healthy control having mitochondrial DNA C_7TC_5 sequence stretch

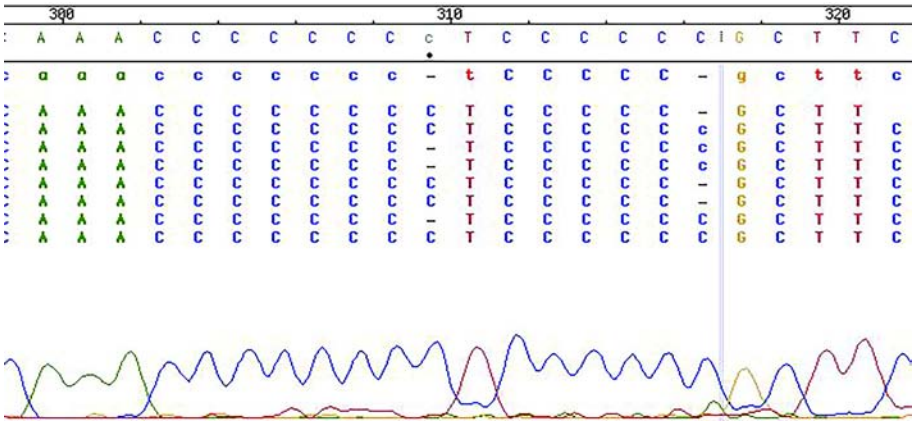

Figure 2. Breast cancer patients having mitochondrial DNA C_8TC_6 sequence stretch

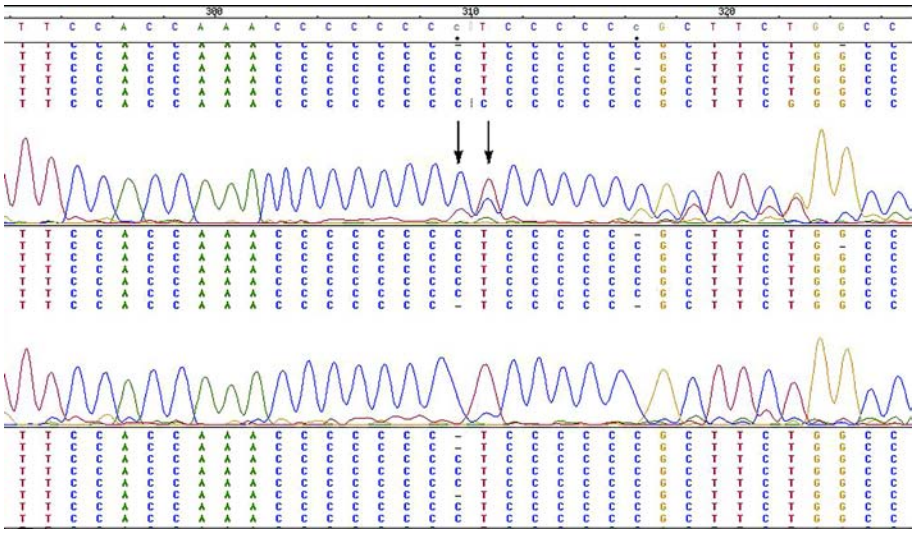

Figure 3. Heteroplasmy in breast cancer patient's mtDNA sequence

Table 2. Prevalence and frequency of heteroplasmy in the two groups of study subjects

Study Subjects	Homoplasmy	Heteroplasmy
Healthy	96.94 % (95)	3.06% (3)
Breast cancer	92.86% (91)	7.14 (7)

Table 3. Demographic and clinicopathological characteristics of breast cancer patients and healthy controls

Parameter	Case (%) (n = 98 breast cancer patients)	Control (%) (n = 98 healthy control)
Age (mean ± SD)	28.28±10.40	26.26± 12.39
Language group	Indo-European	Indo-European
Rural	87	62
Urban	11	36
Family history		
Yes	7 (7.14)	Data unavailable
No	73 (74.49)	
Data unavailable	18 (18.37)	
Hormonal status:		
Estrogen receptor (Positive)	21(48.84)	No test performed
Estrogen receptor (Negative)	22 (51.16)	
Hormonal status:		
Progesterone receptor (Positive)	19 (44.19)	No test performed
Progesterone receptor (Negative)	24 (55.81)	
Hormonal status:		
Her2 receptor (Positive)	31(29.55)	No test performed
HER2 receptor (Negative)	13 (70.45)	
History of contraceptive pills	88 (89.8%)	41 (41.8%)
The lump was detected by the patient herself	79 (80.6%)	Not applicable
Women who sought medical advice within the first month.	19 (19.4%)	Not Applicable
Breast cancer stage		
T0	12 (10.64)	Healthy individuals
T1	19 (20.21)	
T2	26 (26.60)	
T3	24 (25.53)	
T4	16 (17.02)	
Blood Group		
A+	15 (53.57)	13 (46.43)
B+	26 (44.83)	32 (55.17)
B-	0 (0.00)	2 (100)
AB+	11 (40.74)	16 (59.26)
AB-	0 (0.00)	1 (100)
O+	31 (51.67)	29 (48.33)
O-	0 (0.00)	1 (100)

3.4. Correlation of D310 & D315 Mutation with Demographic and Clinicopathological Characteristics

Demographic and clinicopathological characteristics of breast cancer patients and healthy controls are shown in Table 3.

4. Discussion

Mitochondrial dysfunction and its association with cancer development have been studied over the last several years [20]. In tumorigenesis; altered proliferation, oxidative metabolism, phosphorylation, apoptosis are seen which are hypothesized to be occurred due to altered mitochondrial function [21]. This study is about effect of mtDNA mutation on breast cancer. In this study we have investigated the frequency of mutation in a regulatory region of mtDNA called as D-loop. Within the loop D310 region poly-C repeats is a mutational hot spot. In breast cancer 72% of all D-loop mutation occurs in the D310 region [22].

Analysis of D310 and D315 sequences in our patients suggest high mutation frequency in case of D310. The frequency of D310 mutation was found in 70.41% of breast cancer patients. Whereas this mutation is present only 12.24% in healthy individuals. In case of D315 mutation we found equal number of mutations in both study groups (9.18%) (Table 1). Increase/decrease in the cytosine residue numbers in the poly-C region may affect the rate of DNA replication by impairing the binding of polymerase and other trans-acting factors [23]. Reduced mtDNA copy number has been found in 72% of breast cancers with mtDNA D-loop mutations [24]. The decrease in mtDNA content in breast cancer may consequently increase mitochondrial genomic instability, causing alterations in energy metabolism and promoting tumor development [25]. Thus it can be said that D310 mutation might have role to promote the early development of breast cancer by depleting mtDNA content in cells, thus altering energy metabolism and accelerating genetic instability [26]. Further studies are

needed to confirm the role of D310 & D315 mutation in carcinogenesis.

This study shows "C" insertion in 310 regions, whereas $C_7TC_5$is most abundant in healthy control in contrast to breast cancer patients where C_8TC_6 is most abundant. This is an interesting finding in this study; in most of the studies they found multiple insertion or deletions [25,27] in same ethnic people but here we found a fixed insertion in breast cancer patients and a fixed C_7TC_5 sequence for healthy people.

This study also found 96.94% homoplasmy in breast cancer patients and 92.86% in healthy individuals. Ratio of homoplasmy to heteroplasmy is very high here. Some groups have also provided evidence that most mtDNA mutations are homoplasmic [28,29,30]. It is unclear how certain mutations go towards homoplasmic state during breast cancer development. During the time of multiple generations mtDNA may acquire various mutations, some of which may not be advantageous for cell survival and will be eliminated. Mutations in the mtDNA that complement the nuclear genome function may dominate and lead to homoplasmy [21].

In this study demographic data suggests that, 87% patients are from rural area compared to 11% urban population [31]. This finding opposes other studies where mostly urban dwellers having western food habit, environmental pollution and advanced life style. The reasons for this in rural women might be due to lack of modern screening techniques or proper awareness and educations which are yet to be unrevealed. Another important finding is the use of contraceptive both in urban women 88% and rural women 89% consistent with other studies [32]

The analysis of clinicopathological characteristics showed no significant association between age, BC stages, and the presence of mitochondrial DNA mutations at the D310 region. This is consistent with other studies where no significant association between mtDNA instability, mutations, and clinicopathological parameters including sex, age, tumor size, stages, and durations of clinical course was identified [25,33].

Acknowledgements

Authors are thankful to the Centre for Advanced Research in Sciences (CARS), University of Dhaka, for financial support. We are also thankful to blood donors of NICRH for their cooperation. We are grateful to Proyash Roy, Assistance Professor, Department of Genetic Engineering and Biotechnology, University of Dhaka for his partial involvement in this project. Special thanks to Dr. Mizanur Rahman, former Professor of surgical oncology, National Institute of Cancer Research Hospital, Dhaka, Bangladesh.

References

[1] Tazhibi, M., Feizi. A. Awareness Levels about Breast Cancer Risk Factors, Early Warning Signs, and Screening and Therapeutic Approaches among Iranian Adult Women: A large Population Based Study Using Latent Class Analysis. Biomed Res Int. 306352:1-9. Sep. 2014.

[2] WHO (2019) Breast cancer: prevention and control. [Online] Available: www.who.int/cancer/detection/breastcancer/en/, [Accessed Aug 28, 2019].

[3] Sultana, G.N.N., Rahman. A., Hossain. C.F., et al. Mitochondrial DNA Mutations- A Candidate Biomarkers for Breast Cancer Detection in Bangladesh. Chin J Cancer, 31 (9): 449-454. Sep.2012.

[4] Hussain, S.A., Sullivan, R. Cancer Control in Bangladesh. Jpn J Clin Oncol, 43 (12): 1159-1169. Oct.2013.

[5] Kirches E. MtDNA As a Cancer Marker: A Finally Closed Chapter? Curr Genomics 18 (3): 255-267. Jun. 2017.

[6] Greim H, Albertini RJ. The Cellular Response to the Genotoxic Insult: The Question of Threshold for Genotoxic Carcinogens. Royal Society of Chemistry, UK, Cambridge, 2012, 1-20.

[7] Fliss, M.S., Usadel, H., Caballero, O.L., et al. Facile detection of mitochondrial DNA mutations in tumors and bodily fluids. Science. 287 (5460): 2017-2019. Mar.2000.

[8] Jones, J.B., Song, J.J., Hempen, P.M., et al. Detection of mitochondrial DNA mutations in pancreatic cancer offers a "mass"-ive advantage over detection of nuclear DNA mutations. Cancer Res 61: 1299-1304. 2001.

[9] Liu, V.W., Shi, H.H., Cheung, A.N., et al. High incidence of somatic mitochondrial DNA mutations in human ovarian carcinomas. Cancer Res, 61(16): 5998-6001. Aug.2001.

[10] Parrella, P., Xiao, Y., Fliss, M., et al. Detection of mitochondrial DNA mutations in primary breast cancer and fine-needle aspirates. Cancer Res, 61(20): 7623-7626. Oct. 2001.

[11] Wang, C., Youle, R.J. The Role of Mitochondria in Apoptosis. Annu Rev Genet, 43: 95-118. 2009.

[12] Santos, R.X., Correia. S.C., Zhu, X., et al. Mitochondrial DNA Oxidative Damage and Repair in. Aging and Alzheimer's Disease. Antioxid Redox Signal, 18 (18): 2444-2457. Jun.2013.

[13] Kim, H-R., Won, S.J., Fabian, C, et al. Mitochondrial DNA Aberrations and Pathophysiological Implications in Hematopoietic Diseases. Ann Lab Med, 35 (1): 1-14. Dec. 2014.

[14] Sanchez-Cespedes, M., Parrella, P., Nomoto, S., et al. Identification of a mononucleotide repeat as a major target for mitochondrial DNA alterations in human tumors. Cancer Res. 61(19): 7015-7019. Oct.2001.

[15] Sultana, G.N.N., Rahman, A., Karim, M., et al. Breast Cancer Associated mitochondrial NADH-dehydrogenase subunit-3 (ND3) polymorphisms *G10398A and T10400C) in Bangladeshi women. J. Med. Genet. Genomics, 3 (8): 131-135. 2011.

[16] Tanaka, M., TakeyasuT., Fuku N, et al. Mitochondrial genome single nucleotide polymorphisms and their phenotypes in the Japanese. Ann N Y Acad Sci. 1011: 7-20. Apr.2004.

[17] Krystek, Jr. SR., Metzler William J., Novotny J. Hydrophobicity Profiles for Protein Sequence Analysis. CurrProtoc Protein Sci 2.2: 1-13. 1995.

[18] Sheskin, DJ. Handbook of parametric and nonparametric statistical procedures. Chapman & Hall/CRC, USA, 2004, New York, 221-239.

[19] Ha, P.K., Tong, B.C., Westra, W.H., et al. Mitochondrial C-tract alteration in premalignant lesions of the head and neck: a marker for progression and clonal proliferation. Clin Cancer Res. 8 (7): 2260-5. Jul.2002.

[20] Modica-Napolitano, J.S., Singh, K. Mitochondria as targets for detection and treatment of cancer. Expert Rev Mol Med 4(9): 1-19. Apr.2002.

[21] Josephine, S. Modica-Napolitano, Mariola Kulawiec, et al. Mitochondria and Human Cancer.Current Molecular Medicine, 7(1): 121-131. Mar.2007.

[22] Tseng, L-M., Yin, P-H., Chi, C-W., et al. Mitochondrial DNA mutations and mitochondrial DNA depletion in breast cancer. Genes Chromosomes Cancer, 45(7): 629-638. Jul.2005.

[23] Fliss. M.S., Usadel, H., Caballero, O.L., et al. Facile detection of mitochondrial DNA mutations in tumors and bodily fluids. Science 287(5460): 2017-2019. Mar.2000.

[24] Hertweck, K.L., Dasgupta, S. The Landscape of mtDNA Modifications in Cancer: A Tale of Two Cities. Front Oncol 7: 262. Nov. 2017.

[25] Avagliano, A., Ruocco, M.R., Aliotta, F., et al. Mitochondrial Flexibility of Breast Cancers: A Growth Advantage and a Therapeutic Opportunity. Cells 8(5): 401. Apr.2019.

[26] Xu C, Tran-Thanh D, Ma C, et al. (2012) Mitochondrial D310 mutations in the early development of breast cancer. Br J Cancer 106(9):1506. Apr.2012.

[27] Alhomidi, M.A., Vedicherla, B., Movva, S. Mitochondrial D310 instability in Asian Indian breast cancer patients. Tumour Biol 34(4): 2427-2432. Aug. 2013.

[28] Polyak, K., Li, Y., Zhu, H., et al. Somatic mutations of the mitochondrial genome in human colorectal tumours. Nat Genet 20(3):291-293. Nov. 1998.

[29] Darvishi, K., Sharma, S., Bhat, A.K., et al. Mitochondrial DNA G10398A polymorphism imparts maternal Haplogroup N a risk for breast and esophageal cancer. Cancer Lett 249(2): 249-255. Nov.2007.

[30] Richard, S.M., Bailliet, G., Páez, G.L., et al. Nuclear and mitochondrial genome instability in human breast cancer. Cancer Res 60(15): 4231-7. Aug. 2000.

[31] Fei, X., Wu, J., Kong, Z., et al. Urban-Rural disparity of breast cancer and socioeconomic risk factors in China. PLoS One 10 (2): e0117572. Feb. 2015.

[32] Kumle, M., Weiderpass, E., Braaten, T., et al. Use of Oral Contraceptives and Breast Cancer Risk. Cancer Epidemiol Biomarkers Prev 11(11): 1375-1381. Nov.2002.

[33] Covarrubias, D., Bai, R-K., Wong, L-JC., et al. Mitochondrial DNA variant interactions modify breast cancer risk. J Hum Genet 53(10): 924-928. Aug. 2008.