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Frequency of BRCA1 Mutations in B&H Breast and Ovarian Cancer Patients

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Abstract

Incidence of breast cancer ranges from 27 per 100,000 in Middle Africa and Eastern Asia to 92 per 100,000 in Northern America. It is the fifth most common cause of death from cancer in women, with an estimated 522,000 deaths per year (6.4% of the total). Autosomal dominant inheritance of these cancers is characterized by transmission of cancer predisposition from generation to generation, with around 5-10% of all breast cancers being associated with inherited mutations in *BRCA1*, *BRCA2* and other genes. Breast and ovarian cancers are strongly associated with *BRCA1* and *BRCA2* mutations. In this study, we genotyped *BRCA1* gene for large genomic rearrangements in breast and ovarian cancer patients from Bosnia and Herzegovina, with aim to assess frequency of large *BRCA1* mutations (exon deletions/duplications) in this group. We collected 59 breast cancer samples, as well as other data concerning patients' histopathological parameters of tumor, like age at diagnosis, cancer type, TNM class, cancer grade, as well as estrogen, progesterone and Her2/neu expression. Following DNA extraction from breast cancer samples (tissue after biopsy), BRCA1 mutations were identified by Multiplex Ligase - Dependent Probe Amplification (MLPA) analysis. Biostatistical analyses were conducted using MedCalc v.9.2.0.0 software. In all statistical tests $p<0.05$ was considered significant. Mean age at diagnosis was 54±1.75 (range 17 – 80). *BRCA1* genomic rearrangements were found in 22% of breast and ovarian cancer patients. Statistically significant associations and correlations were found between *BRCA1* genomic rearrangements and cancer type, estrogen, progesterone and Her2/neu expression, but not cancer grade, size, invasiveness or patients' age.

Introduction

Breast cancer is the second most common cancer in the world, and leading cancer in women (World Cancer Research Fund International, 2017). In Bosnia and Herzegovina, according to the Institute for public health, Federation of Bosnia and Herzegovina breast cancer was leading cancer in women in 2012, with incidence of 42.6 per 100000, while ovarian cancer was fourth with incidence of 11.8 per 100000. The exact cause of breast cancer is still unknown, but link between the disease and certain risk factors, like age, gender, family and personal history of breast cancer, genetic factors are well supported (Feuer et al., 1993; Dupont et al., 1994; Helzlsouer et al.,1995; Johnson et al., 1995; Braden et al., 2014; Garcia-Closas et al., 2014; Sprague et al., 2015). Germline mutations in different genes have been identified as a major contributor to the risk of developing this disease (National Collaborating Centre for Cancer (UK), 2013). Linkage analyses have proven the existence of autosomal dominant predispositions to develop breast and ovarian cancer, which have led to the identification of highly penetrant genes as the causes of inherited cancer risk. The most common inherited mutations are those of *breast cancer susceptibility genes* 1 and 2- *BRCA1* and *BRCA2*. Since the introduction of *BRCA1* gene in 1994 (Albertsen et al., 1994; Futreal et al., 1994; Miki et al., 1994; O'Connell et al., 1994), and *BRCA2* in 1995 (Wooster et al., 1995), there have been numerous studies of prevalence and genetic background of breast and ovarian cancer (Newman et al., 1988; Castilla et al., 1994; Marcus et al., 1996; Neuhausen et al., 1996; Breast Cancer Consortium, 1997; Peto et al., 1999; Wilson et al., 1999; Frank et al., 2002; Foulkes et al., 2003; Seong et al., 2014; Aloraifi et al., 2015; Menes et al., 2015; Kuchenbaecker et al., 2017; Yamauchi & Takei, 2018). Women with inherited *BRCA1* or *BRCA2* mutations have up to 80% probability of developing breast cancer during their lifetime, and its development often starts at younger age than in women without inherited mutations in these genes (King et al., 2003; Kuchenbaecker et al., 2017). Women with these germline mutations also have an increased risk of developing ovarian cancer. The most frequent mutations in *BRCA1* and *BRCA2* genes involve deletions or insertions of a few bases or single–base substitutions, resulting in premature stop codons. Early studies for identification of these mutations used protein truncation test (PTT), single strand conformation polymorphism (SSCP), southern blotting and long–range PCR, but technical difficulties, in most cases, have limited screening for large genomic rearrangements. Multiplex ligase-dependent probe amplification (MLPA) for screening large genomic rearrangements of *BRCA1* gene gave a new perspective on diagnosis, prognosis, therapeutical approaches and prevention of breast cancer (Schouten et al., 2002; Brandão et al., 2012; McVeigh et al., 2017; Meisel et al., 2017). MLPA is simple, robust and cost-efficient method for basic screening for *BRCA1* mutations (Hogervorst et al., 2003; Cho et al., 2014; Maistro et al., 2016; Eid et al., 2017; Jakimovska et al., 2018). The frequency of *BRCA1* genomic rearrangements within high and medium risk families varies considerably among different populations. *BRCA1* mutations are by far the most common in Russia (79%), then in Israel (47%) and Italy (29%). In Europe (excluding Italy), the percentage of *BRCA1* mutations varies between 20–25%, depending on country (Szabo & King, 1997; Karami & Mehdipour, 2013). Also, this type of mutations in *BRCA* genes explain 4–10% of sporadic breast and ovarian cancers.

Materials and methods

Tumor biopsy specimens from breast and ovarian cancer patients have been consecutively collected at Sarajevo Clinical Center during the period of two years. After biopsy, tissue for molecular-genetic analysis was preserved in phosphate buffered saline (PBS) pH 7.4, and frozen at –20^{o}C prior to DNA isolation. The total of 59 breast and ovarian cancer specimens were collected together with corresponding patient data: family history, age, place of birth, cancer type, tumor-node-metastases (TNM) class, cancer grade, as well as estrogen, progesterone and Her2/neu expression status. Samples were coded and identity of patients remained anonymous throughout the study. For the purpose of method normalization and interpretation of results, genomic DNA of five breast and ovarian cancer asymptomatic and family history free females was used. DNA was isolated from tumor tissue samples using modified salting-out procedure

(Miller et al., 1988). DNA concentration was determined using UV spectrophotometer, and working dilutions of 20 ng/μl were made. Following DNA extraction, *BRCA1* genomic rearrangements were identified by MLPA analysis, using *BRCA1* P002 kit (MRC-Holland, Amsterdam, the Netherlands). This kit contains 34 probes, 9 control probes and 25 mutation specific probes which include exons 1-24 of *BRCA1* gene (with the exception of exon 4). Hybridization, ligation and PCR reaction was performed on Eppendorf Mastercycler gradient (Eppendorf, Hamburg, Germany) according to the manufacturer's instructions. An ABI PRISM 310 automated sequencer and ABI 3500 genetic analyser (Applied Biosystems, Foster City, CA) were used to separate the amplicons with Genescan-LIZ 500 (Applied Biosystems, Foster City, CA) as internal size standard. Individual electropherograms were normalized and compared to electropherograms of control (reference) samples using Coffalyser.NET sotware (MRC-Holland, Amsterdam, the Netherlands). This method does not detect point mutations like direct sequencing (with the exception of probes that are designed to detect point mutations), since probes are designed to detect the most common *BRCA1* exon deletions/duplications. Additional biostatistical analyses were conducted using MedCalc v.9.2.0.0 software. We tested the differences in variation using χ^2 test or Fisher's exact test, and rank correlation using Kendall's Tau correlation test. In all statistical tests $p<0.05$ was considered significant.

Results and Discussion

Out of 59 tumor bioptic specimens collected during the study, 95% were breast cancer tissue specimens, and 5% were ovarian cancer tissue specimens. Since the tumor specimens were collected at the first biopsy following the diagnosis, we calculated the age of patients at the time of diagnosis of cancer. Mean age of the patients was 54 ± 1.7, with youngest patient 17 years old, and oldest patient 80 years old. Most patients were 55 years old at the time of the first diagnosis.

The most common histological type was ductal breast carcinoma (47%), followed by lobular type of breast carcinoma (33%) and the rest were tubular, medullar and mixed cancer types. Estrogen and progesterone expressions ranged from negative expression (0) to highest expression (8), according to histopathological findings. Her2/neu expression ranged from 0 (negative expression) to 3+ (highest expression). 20.3% specimens were estrogen negative, while most of the specimens (32.2%) had highest expression of this marker. This was not the case with progesterone and Her2/neu expression, since most of the specimens were negative for these markers (30.5% and 55.9%, respectively).

Genomic rearrangements on *BRCA1* gene were identified with Coffalyser.net output (Figure 1) as charts. Unchanged exons were marked in black color dots, deletions were marked in red dots, and duplications in blue color. In our tested population, *BRCA1* genomic rearrangements were found in 22% of cases. All of the mutations were found in breast cancer specimens, and none was found in ovarian cancer specimens. In 38.5% of *BRCA1* mutation positive cases we identified only one rearrangement per sample. In rest of the samples we identified from two to six genomic rearrangements (depending on a sample). The most common mutations were found in exons 5, 15 and 16 of *BRCA1* gene. In addition, we found combinations of rearrangements, meaning that two identical rearrangements were found in more than one sample. The most common combination of rearrangements (duplication of exon 15 and 16) was found in 23% of breast cancer specimens.

The prevalence of genomic rearrangements in *BRCA1* gene was correlated and associated with collected patient data (Table 1). Our results concur with previously obtained data in numerous studies on histopathological signature of *BRCA1* mutation carrier cancers. Regarding association between cancer type and BRCA1 genomic rearrangements, χ^2 test did not show statistically significant association ($p=0.214$). We did not find association between cancer histological type and mutations types. Nevertheless, as it was shown in this study, there is a considerable number of patients with *BRCA1* genomic rearrangements. These data were comparable with published data, so we can conclude that there is no significant difference between Bosnia and Herzegovina and rest of the Europe (Szabo & King, 1997; Karami & Mehdipour, 2013), or USA (Frank et al., 2002).

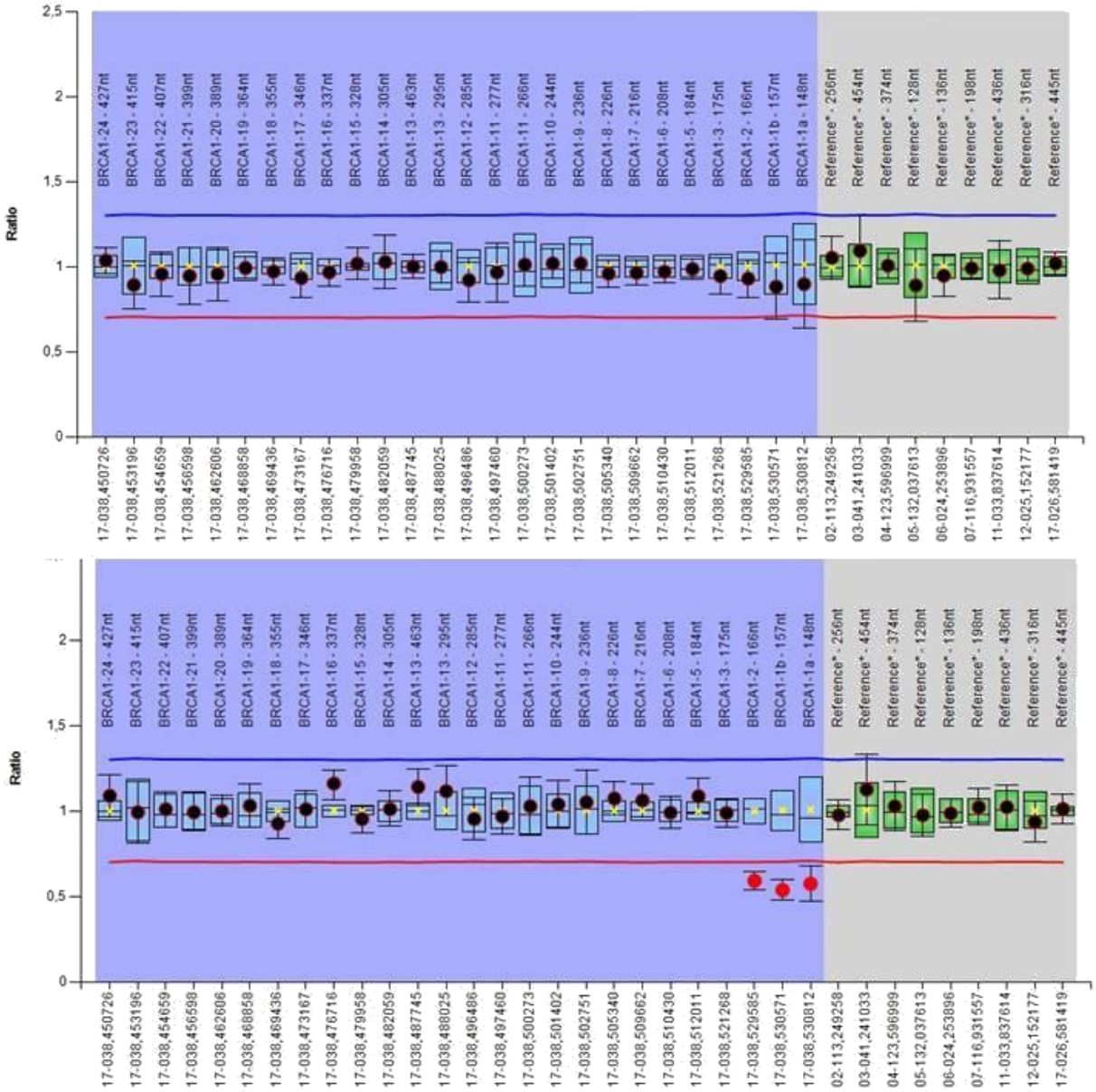


Figure 1. Coffalyser output of control sample (upper chart) and sample with heterozygous deletions of exons one and two (lower chart

Since our sample included approximately the same number of patients with invasive and non-invasive breast cancer, we conducted correlation and association analyses between this histopathological parameter and *BRCA1* genomic rearrangements. Biostatistical analysis between rearrangements and invasive carcinoma or cancer grade did not show statistically significant correlation or association. Identification of histopathological parameters that characterize *BRCA1* mutation carriers is not simple because of their similarity to cancer types in sporadic cancers. Still, it is possible to find some reference data about histopathological parameters, which characterize *BRCA1* mutation carrier cancer types, which are mostly medullar, tubular and lobular cancer types (Breast Cancer Linkage Consortium, 1997). Carcinoma in *BRCA1* mutation carriers are highly proliferative, of high grade, and tubular differentiation (Eisinger et al., 1996; Marcus et al., 1996; Breast Cancer Linkage Consortium, 1997; Thompson & Easton, 2004). The fact we were unable to detect correlation between *BRCA1* genomic rearrangements and cancer grade, may be explained by small sample size, and the fact that cancer grade evolves with disease progression. It should be noted that cancer grade is extremely high in our sample (84% of

individuals of G2 or G3 cancer grade), so we can hypothesize that most of the cancer cases in this respective sample are not diagnosed at early stages of the disease.

Table 1. Correlations and associations of genomic rearrangements in BRCA1 gene with additional collected patient data. Statistically significant values accentuated in bold

	BRCA1 genomic rearrangements	
Histopathological parameter	**Kendall's Tau**	**χ^2 test**
Cancer type	P=0.1121	P=0.0214
TNM Class	P=0.3223	P=0.8833
Invasivity	P=0.8644	P=0.8353
Cancer grade	P=0.6356	P=0.3183
Estrogen expression	P=0.0272	P=0.0304
Progesterone expression	P=0.0223	P=0.0439
Her2/neu expression	P=0.9778	P=0.0438
Age	P=0.3495	P=0.7824

In our further genotype to phenotype relationship analysis, we did not find any association between TNM class of cancer and *BRCA1* genomic rearrangements.

The best described biological parameters in breast cancer are estrogen, progesterone and Her2/neu immunohistochemical expression. Negative correlation was shown by analysis of rearrangements and estrogen expression (p=0.0272). Negative correlation was also evident between genomic rearrangements of exon 24 and estrogen expression (p=0.0248); on the other hand, we did not observe correlation between other single mutations and estrogen expression. Just as described previously (Hu, 2008), we confirmed negative correlation between mutations and progesterone expression (p=0.0223), as well as association between these two parameters (p=0.0439). Association between genomic rearrangements and Her2/neu expression was also detected in this study (p=0.0438). Immunohistochemical markers are one of the markers for characterization of *BRCA1* mutation carriers' carcinoma, because these carcinomas are mostly estrogen, progesterone and Her2/neu negative (Nielsen et al., 2004; Palacios et al., 2005).

In addition, we analyzed relations between genomic rearrangements and age at diagnosis of carcinoma, but we did not find any correlation or association between these two parameters.

Since gene expression profiles in *BRCA1* mutation carriers and in sporadic cancers significantly differ from each other (Hedenfalk et al., 2001), innovations in gene expression technology also created the potential to determine breast and/or ovarian cancer type on molecular level. Downregulated expression of biological markers in BRCA1 carriers can be explained by the fact that mutations in different genes induce the formation of breast tumors by separate pathways.

Conclusions

It is estimated that breast cancer is one of the most common cancers in Bosnia and Herzegovina, although the exact number of breast cancer patients at the state level is unknown (there is no national patient database). The frequency of *BRCA1* mutations in breast and ovarian cancer patients was also unknown. *BRCA1* genomic rearrangements were found in 22% of breast cancer samples and 0% in ovarian cancer samples. We found statistically significant association between cancer type, estrogen and progesterone expression and Her2/neu expression but not with TNM class, cancer grade, invasivity or age of patients. n case that is possible to diagnose *BRCA1* carrier carcinoma without the genetic testing but based on histopatological phenotypes only, it would be a crucial move towards eligible patient selection for predictive and presymptomatic genetic testing.

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