Fibroblast growth factor receptor 2 (FGFR2) rs2981582T/C polymorphism and susceptibility to breast cancer in Saudi women

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1. Introduction

Breast cancer (BC) is the predominant type of cancer among women in the world. Globally, most incidence of breast cancer in 2018 has been reported in Belgium (Sharma, 2021). It is the second cause of mortality following lung cancer (Bray et al., 2018). However, diets are mostly likely to positively affect quality of life for BC patients (Pocciello et al., 2020). It has been documented that physical activity appears to reduce the risk of recurrence and mortality among BC patients (Cannito et al., 2021). Fibroblast growth factor receptor 2 (FGFR2) is a tyrosine kinases receptor that controls cell differentiation, proliferation, and apoptosis (Powers et al., 2000). FGFR2 plays a significant role in different cancers (Shoji et al., 2015). Breast cancer tumor and cell line revealed in FGFR2 overexpression (Penault-Llorca et al., 1995; Adnane et al., 1991).

The gene FGFR2 is located in 10q26 chromosome and comprises 20 exons (Kato, 2008). Several studies have confirmed that the FGFR2 gene has an important role in susceptibility to breast cancer. The association was confirmed with polymorphisms within FGFR2 that cause activation of FGFR2 signaling pathways up and/or downstream in breast cancer (Lei and Deng, 2017). Five single nucleotide polymorphisms (SNPs), rs2981579, rs11200014, rs1219648, rs2981582, and rs2420946, within FGFR2, was discovered by two genome association studies and showed association with BC (Easton et al., 2007; Gruhler et al., 2007). The polymorphisms remained within the linkage disequilibrium block in intron 2 (Liu et al., 2013). Genetic association of FGFR2 gene and different types of cancer was found in gastric cancer (Shoji et al., 2015), endometriosis (Zhao et al., 2008), pancreatic cancer (Nomura et al., 2008), squamous cell carcinoma in
the lung (Liao et al., 2014), and ovarian cancer (Meng et al., 2014). Several studies have been performed to confirm the role of the FGFR2 gene in susceptibility in different populations including Chinese (Liu et al., 2013), North India (Siddiqui et al., 2014), European Americans, and African Americans (Rebbeck et al., 2009). It was proven that FGFR2 gene intron 2 polymorphisms were associated with BC in several populations. One of the most important SNP in intron 2 which showed a strong association with the risk of breast cancer is rs2981582 (Wang et al., 2016). Several studies have found the association between FGFR2 gene polymorphisms rs2981582 and BC risk (Liang et al., 2015; Liu et al., 2013; Xia et al., 2015; Wang et al., 2016; Chen et al., 2016). However, no study investigated the role of rs2981582 gene polymorphism and susceptibility to BC in Saudi women.

This work was conducted to detect the association of rs2981582 variant and susceptibility to BC in Saudi women, to assess the association of this polymorphism with estrogen receptor (ER), progesterone receptor (PR), and human epidermal receptor 2 (HER2) status in Saudi women.

2. Materials and Methods

2.1. Ethical considerations

Ethical approval was obtained from Taibah University (TUCDREC/20170607) and King Fahad Hospital Ethical Committees.

2.2. Samples

Data of archive samples were retrieved from the King Fahad Hospital database in Madinah. Different BC subtypes were included. DNA was extracted from 137 cases (Tumor) and 98 healthy controls. The distribution of cases according to age is as follows: Premenopausal < 50 is 62 (45%), Postmenopausal ≥ 50 is 67 (49%) Samples were formalin-fixed paraffin-embedded breast tissue (FFPE). Samples were anonymous, and codes were used to identify samples. The FFPE samples were either mastectomy or biopsy from breast tissue.

2.3. Genotyping

The heating out method which was modified from phenol-chloroform protocol was used for DNA extraction. PCR was conducted in a 25-μL mix. The i-Taq PCR dried master mix from InTROn Biotechnology. To the mix, 1 μL of 10 mM forward primer and reverse primer as described previously (Liu et al., 2013) was added. 1 μL of 100 ng/μL DNA and the volume was completed to 25 μL with ddH2O. PCR carried out using forward primer: 5’CCCTTTGGAGACGTGAGCC3’ and reverse primer: 5’CACG-CACCAGTGGACTC TGC3’ PCR conditions consisted of 35 cycles following a hot start at 95 °C for 3 min. Each cycle included the following three steps: DNA denaturation (20 s at 95 °C), primer annealing (1 min at 56 °C), and primer extension (1 min at 72 °C). There was a final extension cycle for 5 min at 72 °C. Genotyping was carried out with PCR-RFLP using the HinfI enzyme and confirmed by sequencing of few samples with different genotypes.

2.4. Statistical analysis

The deviation from Hardy-Weinberg equilibrium for FGFR2 rs2981582 has been tested, then odds ratios (ORs) and 95% confidence intervals (CIs) were used to calculate the strength of genetic associations using AssociatORRR software at (https://www.genecalculators.net/associatorrr-cc.html). Statistically significant results were considered if the p-value was <0.05.

3. Results

137 BCE cases and 98 control records were retrieved from the Department of Histopathology at King Fahad Hospital. The result of Histopathological types of breast cancer showed that invasive/infiltrating ductal carcinoma was the most common among women 82.5% (n = 113). Grade II and III, showed the highest rate 43.1% (n = 56), and 42.3% (n = 55) respectively. The frequency of cases with negative ER, PR and HER2 were 37.8% (n = 45), 37.8% (n = 45) and HER2 were 67.8% (n = 38) respectively. Triple-negative hormone showed the lowest rate 13.9% (n = 19).

FGFR2 rs2981582 allele and genotype frequencies were in Hardy-Weinberg equilibrium (p-value > 0.05). Table 1 shows the allele and genotype distributions of rs2981582 in cases and controls. Recessive allele (C) showed significantly higher frequency in cases than in control (P = 9.69 x 10^-5) with Odds Ratio = 2.3, %95 CI = 1.5 ~ 3.4. Genotype distribution was different in cases and controls (p = 0.019). CC and T/C genotype were predominant in cases.

 Frequencies of the rs2981582 alleles and genotypes in BC cases, association with ER, PR, and HER2 status were demonstrated in Table 2. When rs2981582 genotype and allele rates in BC cases were compared between ER, PR, HER2 positive and ER, PR, HER2 negative. The findings revealed no significant differences (p-value > 0.05). However, when the hormonal receptor status is compared with the controls. There was a significant deviation in allele and genotype distribution in ER+, PR+, PR- and HER2- tumors. However, highly significant differences in alleles distribution were found among ER+ (p = 6.8 x 10^-4), PR+ (p = 4.6 x 10^-4), and HER2- (p = 5.9 x 10^-4).

4. Discussion

The current research was carried out to detect an association between rs2981582T/C polymorphism within the FGFR2 gene and susceptibility to BC in Saudi women and to assess the association of this polymorphism with ER, PR, and HER2 status in Saudi women. FGFR2 gene was investigated widely for its crucial effect on BC tissue growth. There was no single study on the FGFR2 gene and susceptibility to BC in Saudi Arabia. SNPs within the intron 2 of FGFR2 have been linked to a 5–10% elevated chance of BC (Liang et al., 2015; Wang and Ding, 2017). The most important SNPs within intron 2 of FGFR2 gene are: rs2981582, rs1219648, rs2981579, rs2912778 and rs2420946 (Liu et al., 2013; Wang et al., 2016). FGFR2 rs2981582T/C polymorphism associated with BC in different populations. Due to a lack of research on the FGFR2 gene in Saudi Arabia, FGFR2 rs2981582T/C has been chosen. The results obtained from this study showed a genetic association between rs2981582T/C and susceptibility to BC in Saudi women. The role of rs2981582T/C polymorphism in FGFR2 gene expression regulation was not fully explained. Intron 2 variants act as enhancer and induce upregulation of FGFR2 expression in breast cancer tissues, which may lead to the formation of a tumor. Additionally, it was shown that there is numerous known binding site.
of transcription-factor within FGFR2 near rs2981582T/C (Huijts et al., 2011; Meyer et al., 2008). The two genome studies conducted in European women identified 10q26 (FGFR2) locus (Easton et al., 2007) and the rs2981582T/C have been replicated in Chinese populations (Li et al., 2016; Cen et al., 2013; Fu et al., 2012). African and European American (Rebbeck et al., 2009), along with Hispanic and non-Hispanic (Slattery et al., 2011). This result showed that CC or TC was significantly predominant in cases than controls. Consistently with our results T allele of FGFR2 rs2981582T/C was associated with a low likelihood of BC and has also revealed and TT protects Han Chinese (Chen et al., 2016). Many studies have shown a positive association of FGFR2 rs2981582 T allele with a high risk of BC. A significant association between BC risk and T/C genotype of FGFR2 rs2981582T/C was found among Pakistani women. However, homozygote TT genotype was not associated (Mazhar et al., 2016). The definitive association between rs2981582T/C and BC is still inconclusive, this may be due to various factors such as regional and ethnic differences. Interracial differences may cause variations in the prevalence and types of BC. Therefore, a variant discovered in one population may not have a similar impact on other populations. Several validated studies have shown inconsistent results in terms of ethnic and pathological characteristics. Therefore, verification by the intrinsic subtypes (ER+, PR- and HER2+) is crucial. The current study demonstrated that luminal A (48.8%), which is ER and PR positive, and HER2 negative was the most frequent subtype among cases. Luminal A shows a good prognosis, where the chances of treatment are better, and the response is good. A previous study in Saudi Arabia was estimated luminal A to be 58.5%, (Alnegheimish et al., 2016). No allelic or genotype association between FGFR2 rs2981582T/C and susceptibility to breast cancer were found when cases stratified by hormonal status, ER+ compared to ER-, PR+ to PR-, HER2+ to HER2- in cases, however, allele and genotype frequencies in ER+, PR- and HER2- tumors were significantly different between cases and controls. Many studies identified a strong association of FGFR2 gene rs2981582 polymorphism in ER+ rather than ER- patients. In a previous study, a strong relationship was found between FGFR2 and positive estrogen hormone (ER+). The results suggested reduction of FGFR2 expression was found in breast cancer ER+ patients (Campbell et al., 2016). Therefore, looking at different tumor subtypes and their relation to BC is an important etiologic issue. FGFR2 polymorphisms showed significant association for (ER+) than (ER-) (Liang et al., 2015; Siddiqui et al., 2014; Wang and Ding, 2017; Shan et al., 2012; Cen et al., 2013; Fu et al., 2012). FGFR2 SNPs and BC subtypes and hormone exposure were studied in a woman of European and an African-American origin. The results confirmed FGFR2 had a role in BC predisposition and the impact was mainly on ER+ and PR+ tumors (Rebbeck et al., 2009). FGFR2 polymorphisms were showed an association with ER-/PR- and ER+/PR+ in Hispanic and non-Hispanic respectively (Slattery et al., 2011). Strong evidence was provided for an association between the FGFR2 gene and HER2-negative disease (Cox et al., 2016).
Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Rawya Ibrahim Rabeh Alia Alraddadi, Rawya Ibrahim Rabeh Alia Alraddadi, Razan Jamaan Nafaa Alamri, Weam Talal Yehya Shehbi et al. Saudi Journal of Biological Sciences 28 (2021) 6112–6115

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