RESEARCH ARTICLE

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Involvement of microRNA-423 Gene Variability in Breast Cancer Progression in Saudi Arabia

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Abstract

Aim: microRNA-423 is an oncogenic factor which is frequently upregulated in cancer. However, associations with breast cancer risk remain inconsistent. Therefore, we investigated the prevalence of microRNA-423 rs6505162C>T gene variation with breast cancer susceptibility in Saudi women. **Methodology:** This study was conducted on 100 breast cancer patients and 124 matched healthy individuals. Genotyping of the microRNA-423 rs6505162C/T gene variation was performed by using the amplification refractory mutation system PCR method (ARMS-PCR). **Results:** A significant difference was observed in the genotype distribution between the breast cancer cases and controls (p=0.0001), the frequencies of the genotypes CC,CT and TT being 25%, 52% and 23% in patients and 65%,20% and 15% respectively, in controls. The microRNA-423 C>T variant was associated with an increased risk of breast cancer in codominant models for (OR = 6.73, 95 % CI, 3.50-12.97; RR 2.35(1.67-3.30, p=0.0001) the microRNA-423TT genotype and (OR = 4.14, 95 % CI, 1.93-8.87; p=0.0003) microRNA-423CT (OR= 6.73, 95% CI, 3.50-12.97; p=0.0001) and also with the dominant model (OR 5.6(3.14-1.01), p=0.0001) CT+TT vs CC) with a non-significant association for the recessive model (OR=1.75, 95%CI=0.08-3.44, P=0.139, TT vs CC+CT). The T allele significantly increased the risk of breast cancer (OR =2.63, 95 % CI, 1.77-3.91; p=0.001) compared to the C allele. Some 6.73 ,4.14 and 2.63 fold increased risk of developing breast cancer was associated with TT and CT genotypes and the T allele of microRNA-423 in the northwestern region of Saudi Arabia. **Conclusion:** Our findings indicate that the microRNA-423 TT genotype and the T allele are associated with an increased susceptibility, metastasis and advanced stage of breast cancer in Saudi Arabian patients. Further studies with larger sample sizes are necessary to confirm our findings.

Keywords: microRNA-423- SNP- Single-nucleotide polymorphism- ARMS-Amplification refractory mutation system

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Introduction

Breast cancer poses a major threat to public health worldwide, and its burden continues to increase. Breast cancer is the most common malignancy tumor among women, which accounts for 25% of all cancer cases in women all over the world, and it is the principal cause of female cancer-related death (Globocan, 2008). Saudi Arabia has the lowest rate of breast cancer incidence in the Arab world. The nationwide average of incidence in the Kingdom of Saudi Arabia is 22 patients for every 100,000 women. Breast cancer was the ninth leading cause of death for females in the Kingdom of Saudi Arabia (KSA) in 2010 (Mokdad et al., 2014). In 2011, the registry of King Faisal Specialist Hospital and Research Centre reported that the number of breast cancer cases has increased considerably, especially among the younger age group (Annual Report of the Tumor Registry, 2011) whereas in the United States alone, a total of more than 2.8 million women suffered from breast cancer in 2015, and the morbidity of breast cancer is still increasing fast in recent years, therefore the breast cancer has become a serious threat to the health and life of women worldwide (McGuire et al., 2015). The breast cancer is a multistep, multistage complicated disease involving multiple factors, among which genetic factors are considered to play an important role (Mehrgou et al., 2016).The identification of the susceptible genes of breast cancer is of great importance that can lead to better diagnosis, treatment and possible prevention of breast cancer. MicroRNAs are non-coding RNA molecules that can act as oncogenes or tumor suppressor genes (Shivdasani, 2006). There are more than 1000 miRNA genes in the human genome which regulate the translation or degradation of human messenger RNA by sequence complementarity (Li et al., 2017). MicroRNAs regulate approximately 30% of human genes and could increase or suppress the expression of multiple target genes, including cancer-associated genes, and thus are involved in many physiological and pathological processes such as cell proliferation, cell differentiation, cell apoptosis,

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carcinogenesis (Carthew, 2006; Xie and Sadovsky, 2016).

Over 50% of microRNAs genes are located in cancer-associated genomic regions or fragile sites, and have been found to be involved in carcinogenesis as tumor suppressors or oncogenes (Calin et al., 2004). In recent years, the genetic variants in miRNAs have been identified as oncogenes of many different types of cancer and found to play an important role in initiation and development of malignancies (Chang et al., 2016).It is reasonable to say that single-nucleotide polymorphisms (SNPs) in miRNAs genes might alter miRNAs expression, maturation and can alter the effects of microRNAs on their target genes, possibly leading to abnormal biological metabolism and modified cancer susceptibility (Nicoloso et al., 2010). The microRNA-423-3p was found to promote cell proliferation, migration, and invasion in cell lines and animal models. It was reported that microRNA-423-5p (miR423-5p) is an oncogenic factor that is frequently upregulated in carcinoma (Kong et al., 2017).

Studies have reported the association between the SNP microRNA-423 rs6505162 C>A and cancer risk in several cancers with contradictory outcomes. It was reported that miRNA-423 rs6505162 C>A polymorphism was associated with the overall survival and the recurrence-free survival of colorectal carcinoma. However, no studies have reported the association between miRNA-423 rs6505162 C>A polymorphism and susceptibility of Breast carcinoma. The rs6505162 SNP is located in the pre-miR-423 and maps to 17q11.2, with a nucleotide alteration from C to A. The aberrant expression of both mature forms of the miR-423 (named as miR-423-3p and miR-423-5p) has been observed in numerous disorders including cancer like breast cancer, mesothelioma, head and neck cancer (Kasashima et al., 2004). Recently it was reported that both mature forms of miR-423 were highly expressed in infiltrating ductal carcinomas of women who later went on to develop metastasis (Lerman et al., 2011). Besides the association between the SNP rs6505162 in pre-miR-423 and cancer risk has been frequently evaluated in diverse populations but not in Saudi Arabia. Similarly it was published recently that pre-micro-423 rs6505162 increases risk of familial Breast Cancer in families with a strong history of Breast cancer in a South American population (Morales et al., 2016). In one of the study it is indicated that miR-423- rs6505162 is associated with reduced breast cancer risk (Smith et al., 2012) whereas in another study it is indicated that miR-423- rs6505162 is associated with both overall survival and the recurrence-free survival in colorectal cancer (Xing et al., 2012). It was reported that the miR-423 rs6505162 affects the mature micro expression thereby plays a potentially oncogenic role in breast tumorigenesis (Zhao et al., 2015). Several studies suggests that the miR-423- rs6505162 is most likely to contributes to susceptibility to breast cancer, especially in Asians but no data available in Saudi Arabia . Therefore, we investigated the association of microRNA-423 rs6505162C>T gene variations with breast cancer progression and susceptibility in our population.

Materials and Methods

The study was approved by the Research Ethical Committee of the University of Tabuk, Saudi Arabia. Written informed consent was obtained from all participants involved in the study.

Inclusion criteria :The study was case control study and included 100 clinically confirmed Breast cancer patients and 100 sex-matched healthy women with no history of any types of cancer and not related to the patients.The exclusion criteria included women with any other previous history of cancer. After obtaining written informed consent, and assessing the clinicopathological findings an approximately 4ml peripheral blood sample was collected from each and every patient as well as from the sex matched healthy controls in EDTA by venipuncture in EDTA vials .

DNA extraction

The genomic DNA was extracted by using DNeasy Blood Kit (cat 69506) from Qiagen (Germany) as per the manufactures instructions and then the DNA was dissolved in nuclease-free water and stored at 4°C until use. The quality of the extracted DNA was checked by running the sample in 1% agarose gel.

The quantity of the extracted DNA is determined by absorbance at 260 nm and 280nm using a spectrophotometer or NanoDrop™ (Thermo Scientific, USA).

Genotyping for miR-423 rs6505162C/T)

Allelic-specific tetra-primer amplification was performed on the genomic DNA using a tetra-primer ARMS PCR approach. The miR-423 rs6505162 C/T genotyping was detected by using amplification-refractory mutation system PCR .The ARMS primers were designed by using Primer3 software as depicted in Table 1.The ARMS-PCR was performed in a reaction volume of 25uL containing template DNA (50ng), FO -0.30uL , RO -0.30uL , FI-0.20uL , RI -0.20uL of 25pmol of each primers and 10uL from GoTaq® Green Master Mix (cat no M7122) (Promega, USA). The final volume of 25 uL was adjusted by adding nuclease free ddH2O .Finally 2ul of DNA was added from each patient.

Thermocycling conditions

The amplification conditions used were at 94 °C for 12 minutes followed by 35 cycles of 94oC for 35sec, 62 °C for 40 sec, 72 °C for 40 sec followed by the final extension at 72 °C for 10 minutes. The amplification products were separated by electrophoresis through 2% agarose gel stained with 0.5µg/mL ethidium bromide and visualized on a UV transilluminator. Primers FO and RO flank the exon of the miR-423-rs6505162 C/T gene, resulting a band of 336bp to act as a control for DNA quality and quantity. Primers Fwt and RO amplify a wild-type allele (C allele), generating a band of 160bp, and primers FO and Rmt generate a band of 228bp from the mutant allele (T allele) as depicted in Figure 1. The best temperature was determined to be 62 °C in the temperature range of 55 °C to 62 °C tested with a gradient PCR thermocycler. The number of cycles was increased from 30 to 42

cycles, significantly enhancing the yields of all three PCR products. Together, these changes resulted in a more robust amplification of the mutant allele and a less competing reaction from the control, as shown by the relative intensities of the corresponding bands on agarose gel electrophoresis.

Statistical analysis

Group differences were compared using Student's two-sample t-test or one-way analysis of variance (ANOVA) for continuous variables and Chi-squared for categorical variables. Deviations from Hardy-Weinberg disequilibrium (HWD) was calculated by Chi-square (χ^2) goodness-of-fit test . The differences in the miR-423 gene allele and genotype frequencies between groups were evaluated using Chi-square test. The associations between miR-423 (rs6505162 C>T) genotypes and risk of breast cancer were estimated by computing the odds ratios (ORs), risk ratios (RRs) and risk differences (RDs) with 95 % confidence intervals (CIs). Allele frequencies among cases as well as controls were evaluated by using the Chi–square Hardy-Weinberg equilibrium test. A p-value < 0.05 was considered significant. All statistical analyses were performed using Graph Pad Prism 6.0 or SPSS 16.0.

Results

The Hardy-Weinberg Equilibrium Analysis

The genotype distributions and allele frequencies of the SNPs located in the miR-423 gene showed that no deviation was not detected in HWE (all p > 0.05) (χ^2 = 0.44 P=0.612) in the patient group similarly the genotype distributions and allele frequencies of the SNPs rs6505162 of miR-423 showed that no deviation was not detected in HWE (all p= 0.05) (χ^2 = 0.52 p=0.712) in the control. Thus, we chose 10% samples from normal control group randomly to the review genotyping results, showing that the accuracy rate was more than 99%.

Study population

This population-based case–control study was done on 100 histologically confirmed Breast cancer patients and 124 Sex matched healthy women with no history of any types of cancer and not related to the patients. This research was approved by the Research ethics committee, University of Tabuk and written informed consent was obtained from all the subjects before enrollment.

Clinicopathological Characteristics of Breast Cancer Patients

All demographic features of the subjects are depicted in Table 2. Of 100 consecutive breast cancer patients, 28 (28%) patients were below or equal to 40 years age and 72 (72%) were above 40 years of age. Of breast cancer cases 38 (38%) were in early (I and II) stage and 62 (62%) cases were in advanced stages (III and IV). Histological grading of the patients tumor showed that 16 (16%), 33 (33%) and 51 (51%) were in grade I, II and III respectively. Metastasis status of patients showed that 86 (86%) patients had distant metastasis and 35(35%) do not show distant metastasis. Based on the receptor status, out of 100 Breast cancer cases 51(51%) were positive for Her2/neu, 65 (65%) were carrying estrogen receptor and 61(61%) were +ve for progesterone receptor.

Genotype distribution of miR-423 rs6505162 C>T gene variation in Case-control

The genotype distribution of miR-423 rs6505162C>T

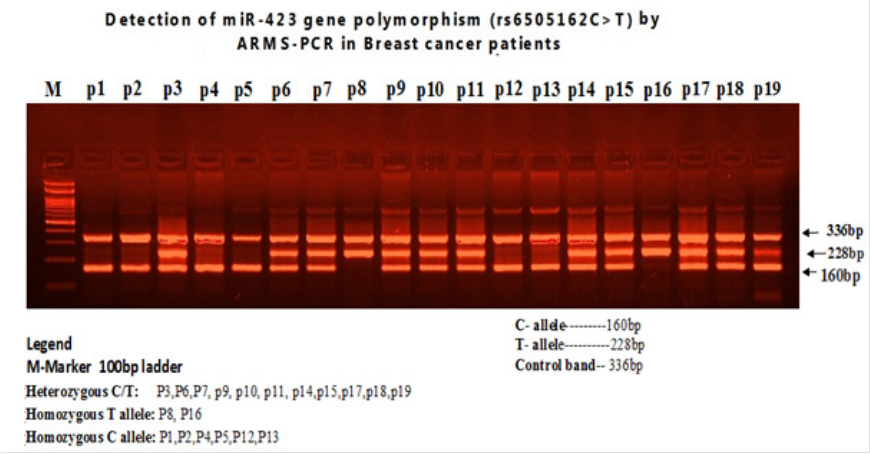

Figure 1. Amplification of miR-423 rs6505162 C>T Genotyping by Amplification-Refractory Mutation System PCR

Table 1. Primer Sequence of miR-423 Genotyping (rs6505162C/T)

Direction	Sequence	Product size
miR-423 FO:	5′-TTTTCCCGGATGGAAGCCCGAAGTTTGA-3′	336 bp
miR-423 RO:	5′-TTTTGCGGCAACGTATACCCCAATTTCC-3′	
miR-423FI (T allele):	5′-TGAGGCCCCTCAGTCTTGCTTCCCAA-3′	228bp
miR-423 RI (C allele):	5′-CAAGCGGGGAGAAACTCAAGCGCGAGG-3′	160bp

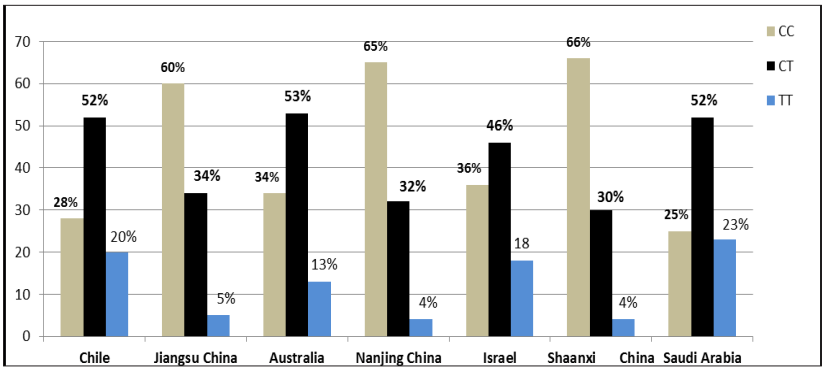

Figure 2. Frequency of miR-423- rs6505162C/T Polymorphism in Breast Cancer Patients of Different Populations

Table 2. Clinicopathological Characteristics of Breast Cancer Patients

Parameters	N=	%
Breast cancer cases	100	100%
High risk Breast cancer	26	26%
Healthy Controls	124	124
Age Group		
Age<40	28	28%
Age >40	72	72%
Stage Status		
Early (I & II)	38	38%
Advanced (III & IV)	62	62%
Grading Status		
Grade I	16	16%
Grade II	33	33%
Grade III	51	51%
Estrogen receptor status		
Positive	65	65%
Negative	35	35%
Progesterone Receptor status		
Positive	61	61%
Negative	39	39%
Her2/neu status		
Positive	51	51%
Negative	49	49%
Distant Metastasis status		
Positive	86	86%
Negative	14	14%

gene variation in cases and controls is summarized in Table 3. We observed a statistically significant difference in the frequencies of miR-423CC, CT and TT genotypes among patients and healthy controls (p=0.0001).This study reported significantly higher percentage of CT (52%) and TT (23%) genotypes in patients compared to controls GT (20%) and TT (15%) genotypes while lower percentage of CC (25%) genotype were reported in patients compared to control CC (65%) genotypes as depicted in Table 3.

Allelic distribution of miR-423 rs6505162 C>T gene variation in Cases-controls

The frequency of T allele (fT) was found to be higher among breast cancer patients (0.49) whereas, the lower frequency of T allele (fT) was observed among healthy controls (0.25). However frequency of C allele (fC) was found to be lower among breast cancer patients (0.51) whereas, the higher frequency of C allele (fC) was observed among healthy controls (0.75) as depicted in Table 3.

Correlation between miR-423 (rs6505162 C>T) gene variation and age

As depicted in Table 4, statistical analysis of the correlation between miR-423 (rs6505162C/T) gene variation in breast cancer patients revealed non-significant associations with age status (p=0.97) .The distribution of CT genotype increased slightly among younger patients (<40 years of age) (54% vs 51%) whereas the frequency of TT genotype increase among older cases (>40 years of age) (24% Vs 21%) .

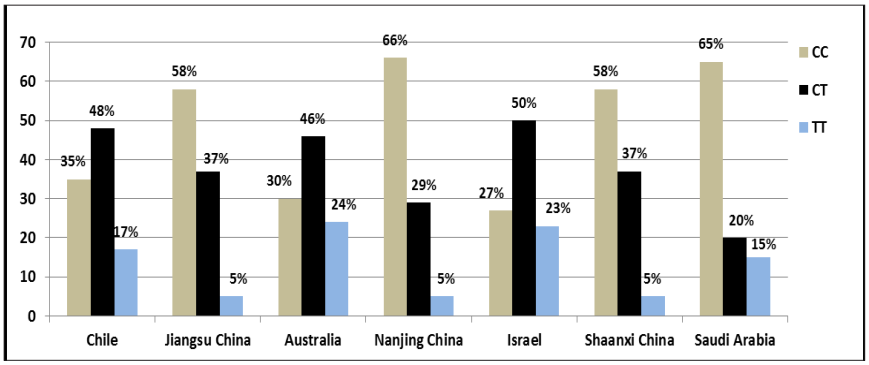

Figure 3. Frequency of miR-423- rs6505162 C/T Polymorphism in Gender Matched Healthy Controls

Table 3. Association Of Mir-423 Rs6505162 C>T Gene Variation in Breast Cancer Cases and Controls

Subjects	N=	CC	CT	TT	Df	X2	P value
Cases	100	25(25%)	52(52%)	23(23%)	2	37.42	0.0001
Controls	124	81(65%)	25(20%)	18(15%)			
Frequencies of T and C allele in cases					C=0.51		
					T=0.49		
Frequencies of T and C allele in controls					C=0.75		
					T=0.25		

Table 4 . Correlation between miR-423 rs6505162C/T Gene Variation and Clinicopathological Characteristics of Breast Cancer Patients

Clinical Parameter	N	CC	CT	TT	X2	P-value
	100	25 (25%)	52 (52%)	23 (23%)		0.81
Age Group						
Age<40	28	07 (25%)	15 (54%)	06 (21%)	0.06	0.97
Age >40	72	18 (25%)	37 (51%)	17 (24%)		
Stage status						
Early (I & II)	38	08 (21%)	20 (53%)	10 (26%)	13.5	0.0015
Advanced (III & IV)	62	17 (27%)	32 (52%)	13 (21%)		
Grading status						
Grade I	16	05 (31%)	06 (37%)	05 (32%)	1.52	0.82
Grade II	33	06 (18%)	18 (55%)	09 (27%)		
Grade I	16	05 (31%)	06 (37%)	05 (32%)	1.87	0.39
Grade III	51	14 (27%)	28 (55%)	09 (18%)		
Estrogen receptor status						
Positive	65	20 (31%)	35 (54%)	10 (15%)	7.28	0.02
Negative	35	5 (14%)	17 (49%)	13 (37%)		
Progesterone Receptor status						
Positive	61	15 (25%)	34 (56%)	12 (20%)	1.18	0.55
Negative	39	10 (26%)	18 (46%)	11 (28%)		
Her2/neu status						
Positive	51	14 (27%)	25 (49%)	12 (24%)	0.44	0.8
Negative	49	11 (22%)	27 (55%)	11 (23%)		
Distant Metastasis status						
Positive	86	20 (23%)	49 (57%)	17 (20%)	9.25	0.009
Negative	14	05 (36%)	3 (21%)	06 (43%)		

Correlation between miR-423 (rs6505162 C>T) gene variation and histological grade status

As depicted in Table 4, statistical analysis of the correlation between miR-423 (rs6505162C/T) gene variation in breast cancer patients with histological grade status revealed no significant associations in different grades (p=0.82).

Correlation between miR-423 (rs6505162 C>T) gene variation and receptor status

As depicted in Table 4, statistical analysis of the correlation between miR-423 (rs6505162 C>T) gene variation in breast cancer patients with respect to receptor status including progesterone, estrogen, her2/neu receptors revealed non-significant associations. The higher distribution of heterozygosity (CT) of miR-423 was seen in ER- positive breast cancer patients than in ER- negative patients (54% versus 48%).The higher distribution of heterozygosity (CT) of miR-423 was seen in PR- positive breast cancer patients than in PR- negative patients (56% versus 46%).The higher distribution of heterozygosity (CT) of miR-423 was seen in PR- negative patients than PR- positive breast cancer patients (49% versus 55%).

Correlation between miR-423 (rs6505162 C>T) gene variation and metastasis status

As depicted in table 4, statistical analysis of the correlation between miR-423 (rs6505162 C>T) gene variation in breast cancer patients with metastasis status of breast cancer patients revealed a strong significant association (P<0.009).The higher distribution of heterozygosity (CT) of miR-423 was reported in breast cancer patients with distant metastasis status than negative patients (57% versus 21%).It can be indicated that CT

Table 5. Association of miR-423 rs6505162 C>T Gene Variation with Breast Cancer

Genotypes	Female Healthy controls (N=124)	%	Breast cancer patients (N=100)	%	OR (95% CI)	Risk Ratio(RR)	P-value
Codominant							
miR-423 -CC	81	65%	25	25%	1 (ref.)	1(ref.)	
miR-423 -CT	25	20%	52	52%	6.73 (3.50-12.97)	2.35 (1.67-3.30)	p=0.0001
miR-423 -TT	18	14.50%	23	23%	4.14 (1.93-8.87)	1.74 (1.21-2.49)	p=0.0003
Dominant							
miR-423 -CC	81	65.32%	25	37%	1 (ref.)	1 (ref.)	
miR-423 (CT+ TT)	43	34.70%	75	75%	5.6 (3.14-1.01)	2.09 (1.61-2.72)	p=0.0001
Recessive							
miR-423 (CC+ CT)	106	85.50%	77	77%	1 (ref.)	1 (ref.)	
miR-423 (TT)	18	14.50%	23	23%	1.75 (0.88-3.44)	1.31 (0.91-1.90)	p=0.139
Allele							
miR-423 -C	187	75%	114	53.77%	1(ref.)	1 (ref.)	
miR-423-T	61	25%	98	46.22%	2.63 (1.77-3.91)	1.61 (1.30-2.01)	p=0.0001

Table 6. Frequency of miR-423-rs6505162C/T in Breast Cancer Patients of Different Countries

First author	Year	Country	Ethnicity		N=	CC	CT	TT	N=	CC	CT	TT
Morales (28)	2016	Chile	Caucasian	BC	440	125 (28%)	229 (52%)	86 (20%)	807	284 (35%)	385 (48%)	138 (17%)
Zhang (41)	2015	Jiangsu China	Asian	BC	382	231 (60%)	131 (34%)	20 (5%)	189	110 (58%)	69 (37%)	10 (5%)
Smith (27)	2012	Australia	Caucasian	BC	179	60 (34%)	95 (53%)	24 (13%)	174	52 (30%)	80 (46%)	42 (24%)
He B (42)	2015	Nanjing China	Asian	BC	450	292 (65%)	142 (32%)	16 (4%)	450	299 (66%)	129 (29%)	22 (5%)
Kontorovich (26)	2010	Israel	Caucasian	BC	190	68 (36%)	88 (46%)	34 (18%)	206	55 (27%)	102 (50%)	49 (23%)
Ma (43)	2013	Shaanxi China	Asian	BC	192	127 (66%)	57 (30%)	8 (4%)	189	110 (58%)	69 (37%)	10 (5%)
Our study	2017	Saudi Arabia	Middle east	BC	100	25 (25%)	52 (52%)	23 (23%)	124	81 (65%)	25 (20%)	18 (15%)

genotype is associate with the disease progression and distant metastasis status.

Correlation between miR-423 (rs6505162 C>T) gene variation and Stage of the disease

Early stage group included Ductal Carcinoma in Situ (DICS) patients in stage I and II of breast cancer. On the other hand, advanced stage group included breast cancer patients in stage III and IV. As depicted in Table 4, statistical analysis of the correlation between miR-423 (rs6505162C/T) gene variation in breast cancer patients revealed highly significant associations with stage status (p=0.0015).

Risk of Breast Cancer with miR-423 (rs6505162C/T) gene polymorphism in BC patients

A multivariate analysis based on logistic regression like odds ratio, risk ratio and risk difference with 95% confidence intervals were calculated for each group to estimate the association between the miR-423 (rs6505162C/T) variant and risk of Breast cancer in Saudi patients as depicted in table 5. Our findings showed that the miR-423 (rs6505162C/T) variant was associated with an increased risk of BC in codominant (OR=6.73, 95%CI=(3.50-12.97) p=0.0001, CT vs CC; and OR=4.14, 95%CI=(1.93-8.87), p=0.0003, TT vs CC), Dominant (OR=5.6, 95% CI= (3.14-1.01), p=0.0003, CT+TT vs CC), and Recessive (OR=1.75, 95%CI= (0.88-3.44), P=0.139, TT vs CC+CT) inheritance models tested. While, the T allele significantly increased the risk of BC (OR= 2.630; 95% CI= (1.77-3.91); p=0.0001 compared to C allele.

Discussion

Our study indicates that miR-423 rs6505162 might be associated with a increased risk of Breast cancer, however, this finding need to be evaluated further in larger samples, especially subgroup analyses. In addition, cancer-specific functional studies are especially needed to reveal the underlying mechanisms between miR-423 and the etiology of Breast cancer. The miR-423 - rs6505162 polymorphism was revealed to be associated with an overall increased risk of Breast cancer in our population. The higher prevalence of miR-423 rs6505162 CT (52%) and TT (23%) genotypes were reported in our Breast cancer patients than the healthy controls CT (20%) and TT (15%) respectively whereas the higher prevalence of GG genotype (65%) was identified in the healthy individuals than the breast cancer patients (25%) respectively. A growing number of studies also have been conducted to assess this polymorphism's association with the risk of Breast cancer, but the results were conflicting rather than conclusive. We analyzed the frequencies of miR-423- rs6505162C/T in different populations to further

eliminate heterogeneity as in Figure 2 and 3.

The prevalence of miR-423-rs6505162 TT genotype in our study group was higher than that reported in Jiangsu china (5%), Nanjing china (4%), Shaanxi china (4%), whereas the similar frequency was reported in Chile (20%), Israel (18%) and Australia (13%) as depicted in Figure 2.

Similarly the frequencies of miR-423- rs6505162C/T in gender matched healthy controls of different countries were compared with our study group as depicted in Figure 3. The prevalence of miR-423-rs6505162 TT genotype in the healthy controls was higher than that reported in Jiangsu china (5%), Nanjing china (4%), Shaanxi china (5%), Australia (13%) and somehow same prevalence was reported in Israel (18%) ,Chile (20%) as depicted in Figure 3.

It was reported that miR-423- rs6505162 affect the expression or processing of miR-423, therefore, studies evaluating the effect of this SNP in miRNA functionality are required. However, studies of the rs6505162 polymorphism on cancer risk have yielded inconsistent results (Ye et al., 2008). One of the study done in 2012, Smith et al., (2015) reported that the CC genotype of the rs6505162 SNP reduces the risk of breast cancer development, however, another study undertaken in 2009 suggested that the C genotype of rs6505162 offered an increased risk of developing both ovarian and breast cancer in Breast Cancer associated 2 (BRCA2) mutation carriers (Zhang et al., 2017) whereas in another study the miR-423 rs6505162C/A has been associated with an increased risk of primary ovarian insufficiency (Smith et al., 2012). Similarly it was reported that miR-423 rs6505162C>A might also be associated with a significantly increased risk of esophageal squamous cell carcinoma in patients who smoke (Yin et al., 2016).

Many studies have shown that miR-423 rs6505162C/A or mutations in miRNA coding regions may alter miRNA expression and/or maturation, that may affect the occurrence of diseases (Jazdzewski et al., 2008). Therefore these previous studies imply that miR-423 rs6505162C/A has the potential to become a biomarker for the diagnosis of many clinical diseases including breast cancer. It was demonstrated that the SNP rs6505162 in pre-miR-423 affects mature microRNA expression, and miR-423 plays a potentially oncogenic role in breast tumorigenesis (Zhao et al., 2015). A few polymorphisms are located in the mature microRNA sequence which could directly affect the binding of microRNAs to hundreds of target mRNAs. This study has shown that miR-423 rs6505162C/A is associated with an increased risk of Breast cancer susceptibility in our study subjects. Several studies have evaluated the association between the miR-423 rs6505162C/A and cancer risk in diverse populations and in different types cancers, with contradictory outcomes. Nevertheless, there have been scarce association studies on this SNP and BC risk. It was indicated that rs6505162 was associated with a significantly increased risk of ovarian cancer b (Kontorovich et al., 2010) on the contrary; it was shown that it conferred a reduced risk of Breast cancer susceptibility (Smith et al., 2012).

Recently two studies concluded that the A allele of the rs6505162 increased the risk of breast cancer (Morales et al., 2016; Li et al., 2018) whereas the same allele presented a decreased risk of developing lung cancer and bladder cancer in two other studies (Yang et al., 2008; Kim et al., 2011). It has been reported that different expression patterns of miR-423 have been reported in various types of cancers, such as over-expression in head and neck cancer (Hui et al., 2010) laryngeal carcinoma (Guan et al., 2014) female genital system neoplasms (breast, cervical and endometrial) (Boren et al., 2008) and most of the digestive system neoplasms (gastric, pancreatic, hepatocellular) (Ali et al., 2012) and under-expression in mesothelioma (Chen et al., 2014).Some studies indicated that miR-423 acts as tumor suppressor in oral cancer (Roy et al., 2016) oncogene in hepatocellular carcinoma (Lin et al., 2011). Tumor-specific functional studies are especially needed to clarify biological effects of the tissue heterogeneity on the expression and function of miR-423 and to experimentally validate its potential targets, so as to illustrate the underlying mechanism and interpret the data appropriately.

One of the meta-analysis published but reported no associations between the rs6505162 SNP and breast cancer risk in any genetic model (Pollard et al., 2018). However, this meta-analysis included only two association studies involving miR-423 rs6505162 that was an important limitation to interpret the results. In our study, however our study indicated miR-423 rs6505162C>A is significantly associated with disease progression and susceptibility to Breast cancer. Our results are in accordance with the recent results obtained by Zhao et al., (2015) who demonstrated that the rs6505162C/A in pre-miR-423 affects mature miRNA expression and that miR-423 plays a potentially oncogenic role in breast cancer tumorigenesis. Common functional polymorphisms in the promoter region of microRNAs, based on multiple lines of evidence, might participate in transcriptional regulation and other biological processes, which interact to increase the risk of developing breast cancer. Our study supports that the miR-423-rs6505162 A alleles might be potential risk factor for breast cancer.

However, this finding needs to be evaluated further in larger samples, especially for subgroup analyses. The microRNA 423 expression in blood need to be determined and correlated with its polymorphism miR-423 rs6505162 whose occurrence may lead to decreased expression. In addition, cancer-specific functional characterizations are simultaneously needed to reveal the underlying mechanisms between miR-423 rs6505162 and the etiology of Breast cancer.

In conclusion, the findings indicated that microRNA-423 TT genotype and T allele are associated with an increased susceptibility, metastasis and advanced stage of Breast cancer patients of Saudi Arabian population. It can be used as a predisposing genetic marker for BC .Furthers studies with larger sample sizes are necessary to confirm our findings.

Disclosure

This manuscript is not under consideration by any other publication and has not been published elsewhere. Authors have declared that no competing interests exist.

Consent

All authors hereby declare that all experiments have been examined and approved by the Research ethics committee, University of Tabuk, and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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