



The whole wheat effect and refined with E-selectin polymorphism on breast cancer

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Abstract: Wheat is rich in sources of fiber, oligosaccharides, and resistant starch, simple carbohydrates which may have a protective role against carcinoma. Additionally, Whole wheat/bran as well includes contains phytochemicals such as flavonoids, lignans, folate, phytosterols, phenolic acids, and tocopherols. The above phytochemicals suitable forms antioxidant and cholesterol-reducing activities. Phytoestrogens are regarded as especially essential in the preventative measures of hormonally dependent malignancies such as breast cancer (BC). In this study lowered BC risk has been associated with whole grain/bran consumption with an odds ratio (OR=0.24 and 95 %CI=0.10-0.56). Wheat/bran appears to have a reliable protective impact against BC. While intake of white bread has been associated with a high risk of BC (OR=2.63 and CI 95 %=1.07-6.48). Also, the E-Selectin (SELE) Single nucleotide polymorphisms (SNPs) rs5353 A/G and rs932307 C/T were investigated using the sanger sequences approach. There was a positive association between genotypes (rs5353 GG+AG) and (rs932307 TT+CT) with rate consumption of wheat-white/bread and these genotypes were more frequent in patients had BC in comparison with a significant difference (P=0.03) (P=0.01), respectively. The genotypes (GG+AG) frequency of the rs5353 polymorphism and (TT+CT) genotypes of the rs932307 polymorphism in the present study had a high risk of cancer with (OR=3.05), (OR=4.17) respectively. While these genotypes showed no significant association with the rate of whole grain consumption in patients and control. Therefore, the type of wheat may associate with increased incidence of disease-related with type of Polymorphism because some present genotypes of SNPs showed high (OR) which may refer to their positive associated with disease, the white wheat consumption may active the risky association between SNPs and BC.

Key words: wheat, whole grain, fiber, breast cancer, SELE, SNPs

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1. Introduction

Wheat is a major food and energy source all over the world which is high in carbohydrates, proteins, vitamins, and minerals. Whole grains are linked to a lower risk of obesity, diabetes and cancer mortality.¹ A growing number of studies over the past few decades have suggested a correlation between whole grain consumption and a reduced risk of cancer.² This may be attributable to the abundance of phytochemicals,³ anti-oxidant, anticancer and anti-inflammatory.⁴ The association between whole grain consumption and BC was first proposed in the late eighties and early nineties.⁵

Consecutive research linked the postulated protective effect to lignans and Alkylresorcinols present in whole wheat^{6,7} and converted into enterolactone and enterodiol by mammalian microflora. The end product has phytoestrogenic characteristics and can affect estrogen hormone signaling and interaction.⁸ An in vitro study found that components of wheat flour inhibit the proliferation of breast cell lines (MDA-MB-231, and MCF-7, ER+, TNBC, triple-negative) via inducing apoptosis.⁹ whole grain also known as 1,3-dihydroxy-5-n-alkylbenzenes are an important category of polyphenol lipids present in whole grain/wheat, rye and barley.^{10,11} whole grains are supposed to inhibit cancer growth and may be helpful in slowing carcinoma progression.¹² In functional in vitro other studies, whole grains were found to inhibit the growth of MCF-7 BC cells. By five Alkylresorcinols isolated (Homalomena Wendland Schott /Arecae) with IC50 values ranging from 8.24 to 42.17M.¹³ Another product of wheat/bran with an anti-cancer effect is a flavone C, glycoside called Triticuside which was found that reduce the proliferation of BC cells (MCF-7 with MDA-MB-231) via mitochondria apoptosis and also the Akt/mTOR signaling pathway.

Robust linkages were frequently reported between (vascular cell-adhesion molecule 1, & intercellular-adhesion molecule 1) and Precedent research demonstrated that the levels of TNF-R2 and SELE were decreased in people following increased whole grain/bran intake.¹⁴

Aims: This study evaluates the impact of whole wheat/bran and refined wheat on the risk of breast cancer in women, in addition to evaluating the association between SNPs in the SELE gene and consumption of different types of wheat in order to better understand the roles of food as a risk factor in cancer progression.

2. Experimental

A total of 100 Iraqi women who visited the breast clinic in the Oncology Teaching Hospital, Medical City-Iraq. This study was during the period of 28/01 to 11/08/2020 after giving informed written consent. This study was approved by the ethical committee of Medical City directorate. Group 1 included sixty women who had recently been diagnosed with breast cancer. Group 2 included forty age-matches, apparently healthy individuals served as a control. Any patients with other systemic diseases and those who were taking any hormone-modifying drug were excluded from the study. The dietary intake food items of all participants were assessed using a researcher-administered quantitative nutrition frequency questionnaire (FFQ). Whole-wheat bread includes (barn, corn flakes, and barley), while white bread includes (white flour, baguette and sweetened bread). Therefore, present patients and control are divided into subgroups according to wheat type consumption.

Eight to ten milliliters of blood were collected from the patient using EDTA tubes). Genomic DNA extraction using Norgen Biotek kit - Canada, The quantity and quality of extracted DNA were investigated based on the OD 260/280 ratio using Nano-drop (QubitTM4 Invitrogen – USA). A ratio between 1.7-1.9 was considered good. Extracted (DNA) samples were kept at -20 °C. The amplification of targeted genes was performed using the PCR method.

2.1. Polymerase chain reaction

Each 25 µL reaction was contained 3 µL of DNA sample, 7.5 µL of H₂O free, 7.5 µL of PCR master mix have MgCl₂, dNTP mix, from (Promega - USA) and 2 µL of the PCR primer mix (*Table 1*) used into

Table 1. Primer sequence for SELE gene of (exon 1 +intron +exon 2+intron) region

	No Sequences	Tm	Product size	by
E-Sele seq F	AGCAGTGATACCCACCTGAGA	57°C	970	Second Author
E-Sele seq R	TTTGTGACATGGCCCAGACTT			

SNPs genotypes. DNA was amplified using the PCR assay (Applied Biosystems-USA) conditions PCR were as follows: the sample was initially denaturation at 95 °C, 5 min; followed 45 cycles denaturation at 94 °C; 30s; and Annealing 57°C; 30s, followed Extension 72 °C; 40s; Final extension 72 °C, 10 min and hold at 8 °C. And has been screened genotyping of (rs5353 A/G and rs932307 C/T), by Sequencing Panel Sanger from a company (MacroGen-South Korean), which examined frequently the SELE gene in patients with BC and was used to detect variations.

2.2. Statistical-Analysis

The genotype of SELE and wheat consumption was statistically analyzed using the computer program SPSS, Inc (Chicago-USA) (Statistical Package for Social Sciences) version 25. Allele frequencies of SELE genes were calculated by the direct gene counting method, using Geneious Software Prime 11.1.5 (Auckland New-Zeland), and genotypes of SELE were presented as frequencies and percentages. Differences between groups were assessed by odds ratio (OR) and were also estimated to define the association between SELE genotypes and wheat

consumption in patient and control groups. The OR value can range from less than one (negative association) to more than one (positive association). This investigation was used to assess the potential connections between wheat consumption and genetic variations of the SELE gene and cancer risk in population.

3. Results and Discussion

3.1. Consumption of wheat and the Risk of BC

This study analyzed wheat consumption association with breast cancer. According to the results, comparing patients to control revealed that white bread consumer showed a significantly increased frequency as patients (43.3 %) vs (22.5 %) control (P=0.03). This submitted a positive association between white bread consumption and BC scored OR value of (2.63). The whole grain/bran consumer patients recorded a low percentage (33.3 %) vs high healthy consumer of whole grain (67.5 %) in the control and the difference was highly significant (P=0.001). The whole grain/bran consumption was a preventive fraction (PF) of such a negative

Table 2. Association between wheat type consumer and BC frequency in study groups

Characteristic	Variables	Patients (n=60)	Control (n=40)	Test Result		
		Freq. %	Freq. %	OR.	(95%CI)	P -value
White-bread	Consumers	26 (43.3%)	9 (22.5%)	2.63	1.07 - 6.48	0.03*
	Non-Consumers	34 (56.7%)	31 (77.5%)	0.38	0.15- 0.93	
Mixed consumption	Consumers	14 (23.3%)	4 (10.0%)	2.73	0.83- 9.03	0.09
	Non-Consumers	46 (76.7%)	36 (90.0%)	0.36	0.11- 1.20	
Whole grain/bran	Consumers	20 (33.3%)	27 (67.5%)	0.24	0.10 - 0.56	0.001***
	Non-Consumers	40 (66.7%)	13 (32.5%)	4.15	1.77- 9.74	

Significant P ≤ 0.05

association was OR (0.24), between whole grain consumption, and BC.

The whole view for Table 2 shows that consumer subgroups for white and mixed grain recorded a highly significant Odds' ratio comparing to non-consumers within group (2.63 and 2.73) respectively, consequently this result conclude that consumer refined wheat may have positive associated with BC, and consumption refined wheat could consider as etiological fraction related with increase susceptibility to BC.

At the same time, patients non consumer whole grain showed high significance (OR=4.15) with very high CI which could be explained as a result of non-consumption whole grain and that may associate with increase susceptible of individual for BC.

3.2. Association of the White-bread with SELE gene SNPs Polymorphism in BC patients

Regarding the comparison among SELE SNPs genotypes, white bread consumption with BC, genetic polymorphism of SELE gene was investigated in two positions rs5353 and rs932307 (Fig. 1 and Fig. 2).

Both genotypes GG and AG showed non-significant increasing frequency in patients comparing to control with consumption of whole grain (10 %) and mixed (5.0 %). While TT and CT showed a significant increase in patients' frequency with whole grain consumption (18.3 %) without any frequent in patients and control with mixed consumption (0.0 %). TT and CT genotype was significantly in patients compared to control (31.7 % and 10 %) respectively. Such positive association scored OR value of (3.05) in rs5353 polymorphism, and scored OR value of (4.17) in rs932307 polymorphism with White-bread consumption in BC.

As general view in both positions of SNPs, the genotypes G/G + A/G and T/T + C/T in all sub-study groups according to type of wheat consumption showed a high score of OR, which OR, range from 1.37-4.9 for GG + AG and OR rang were 4.17-4.26 for TT + CT, All these leads to concluded that kind of genotype recorded a positive associated with BC as an etiological factor. In which females who carry these genotypes may be more susceptible to disease even when consumer whole grain wheat.

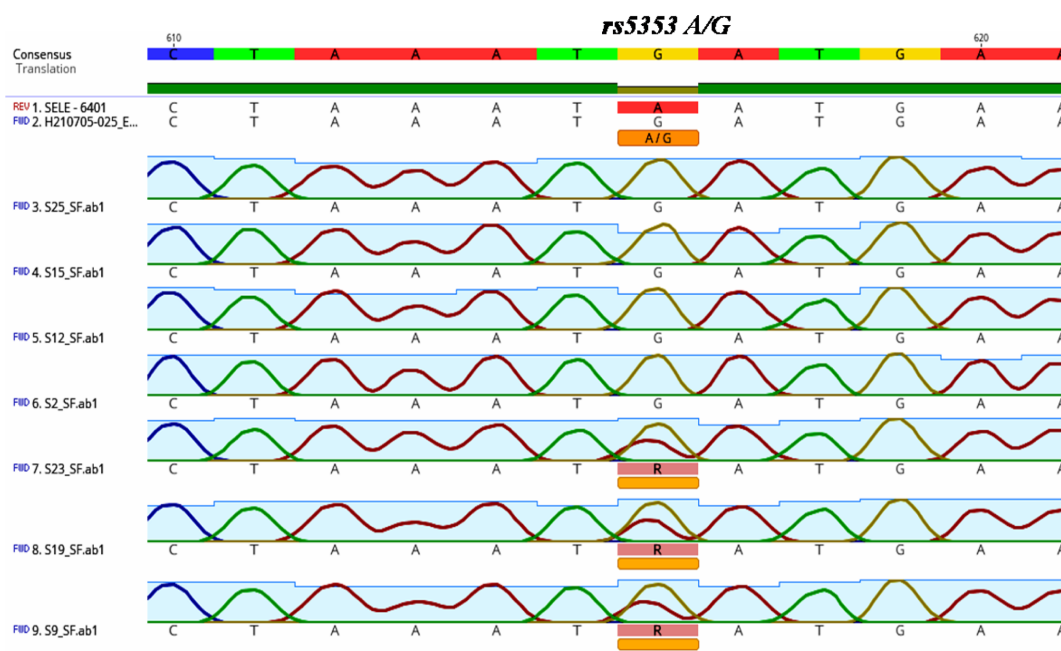


Fig 1. The sequence of SELE gene when alignment with reference sequencing of NCBI location: showed substitute nucleotide from AA to GG and AG in breast cancer patients and healthy control.

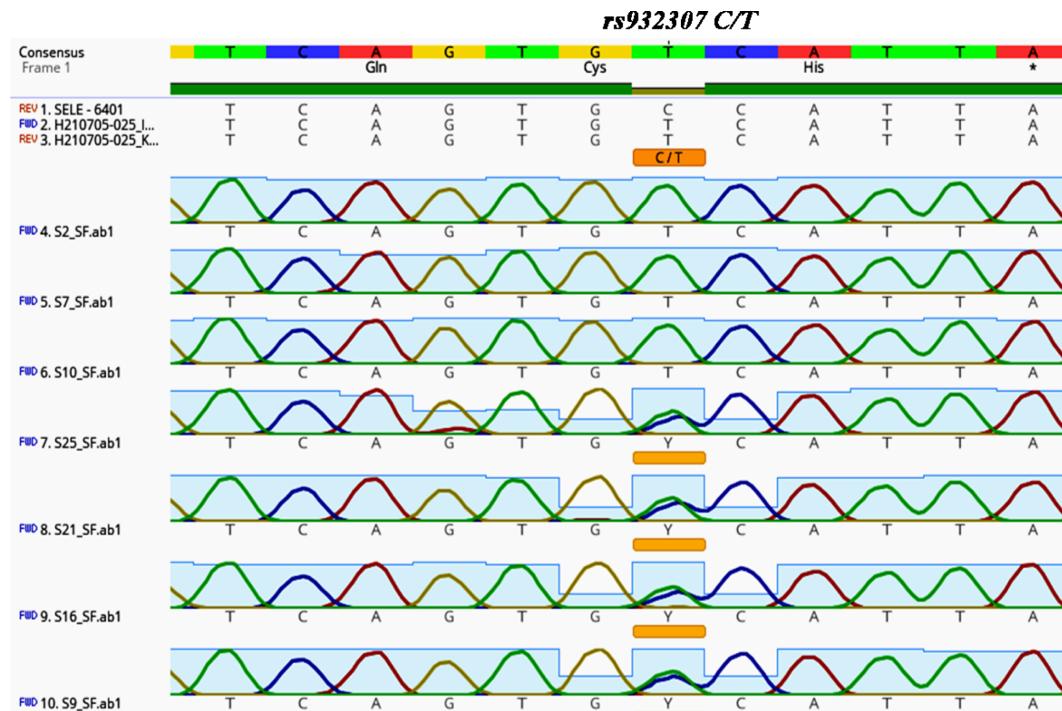


Fig 2. The sequence of SELE gene when alignment with reference sequencing of NCBI location: showed substitute nucleotide from CC to TT and CT in breast cancer patients and healthy controls.

Table 3. Analysis of the relation between Wheat / SELE polymorphisms with BC

Variables	rs5353 A/G		OR. CI 95 %	P	rs932307 C/T		OR. CI 95 %	P
	Patients (n=60)	Control (n=40)			Patients (n=60)	Control (n=40)		
White-bread	G/G+A/G	21 (35.0 %)	6 (15.0 %)	0.03	T/T+C/T	19 (31.7 %)	4 (10.0 %)	0.01
	A/A	39 (65.0 %)	34 (85.0 %)		C/C	41 (68.3 %)	36 (90.0 %)	
Mixed consumption	G/G+A/G	3 (05.0 %)	0 (00.0 %)	0.3	T/T+C/T	0 (00.0 %)	0 (00.0 %)	0.8
	A/A	57 (95.0 %)	40 (100 %)		C/C	60 (100 %)	40 (100 %)	
Whole grain/bran	G/G+A/G	6 (10.0 %)	3 (07.5 %)	0.7	T/T+C/T	11 (18.3 %)	2 (05.0 %)	0.07
	A/A	54 (90.0 %)	37 (92.5 %)		C/C	49 (81.7 %)	38 (95.0 %)	

Significant $P \leq 0.05$

The SNP rs5353 wild genotype AA showed a negative association with disease, so AA may have a protective effect, OR=less than one in all subgroups, that means all females with wild genotype AA maybe not be susceptible to BC.

But the genotypes (AA) in position rs5353 and (CC) in position rs932307 frequency was higher (90.0 % and 92.5 %) respectively, in the Patients who had BC and control with Whole grain consumption (Table 3). The genotype CC for rs932307 SNP

showed OR ratio of 0.24 in subgroup with white bread and 0.23 with whole grain consumers but OR equal to 1.49 for individuals who consume mixed wheat which means this genotype has a protective fraction but losing its protective effect with mixed consumption with turning into etiological fraction, OR became more than one (OR=1.49). This result could explain an etiological or environmental factor in present study related with type of wheat could change the effect of CC genotype from protective

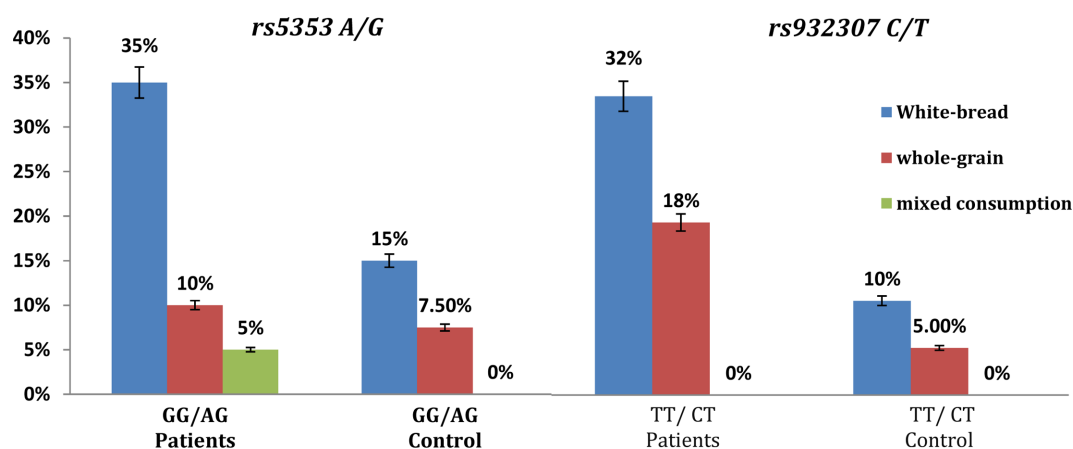


Fig 3. Effects wheat consumption on mutant genotypes of SNPs, of the SELE gene in breast cancer patients.

into etiological effect.

The present study pointed out that white bread consumption recorded the highest frequency in BC patients from control, was associated with OR value of 2.63; the observation suggests it's may susceptibility role in BC of Iraqi women patients. Because white bread maybe had a healthy effect on the Iraqi population, in contrast with whole grain which showed very high frequency in control compared to patients' with probability whole grain consumption had a preventive fraction against disease. Whole grains and regarding products in the diet can be a practical approach to BC protection and administration. Whole grain food stuffs are an appealing good option for widespread, lengthy-term use in a variety of populations due to their inherent safety. Also, consumption of whole grains/wheat has been related to a lower risk of BC in previous studies.¹⁵ A recent Greece study indicated that consuming whole wheat over 7 times per week was connected to a decreased risk of BC in women aged 44 to 68.¹⁶ Another study on Iranian population suggested that eating foods high in resistant starch, like whole grain bread, could minimize the risk of BC in women range aged 25 to 65.¹⁷ Moreover according to a 2017 cohort research, eating whole grains decreases the BC risk by 47 percent, but no clear link among whole grain consumption of food and other adiposity associated carcinoma.²

The two present SNPs showed a significant variation

between BC patients and control with different wheat type's consumption (Fig. 3). The GG and AG genotypes recorded the highest frequency in BC patients compared to control with white bread consumption, an observation may focus on the role of rs5353 polymorphism in disease because the genotypes GG / AG were recorded a low frequency in control groups, this genotype accounted for about 15 % of control. Also, (TT/CT) genotypes frequency in position rs932307 was increased in patients compared to control with white bread consumption. An observation may highlight on the role of rs932307 polymorphism in disease. CC genotype showed the highest frequency in control group 90 % in Table 3. Found high frequency at position rs5353 and rs932307 in patients with white bread consumption, this may explain the progression of disease in these cases; the white wheat consumption may active the risky association between SNPs polymorphisms and incidence of BC. The result in Fig. 3, dis agree with results in a previous study about the Asian population which emphasizes at the mutant genotype of SELE contributing to disease development.¹⁸ However present study results about polymorphism goes with previous Iraqi study suggested that heterozygous genotype of SELE gene SNPs could be risk for diabetic Mellitus patients D.M.T2.¹⁹ Another Iraqi studies emphasize that heterozygous genotypes from different SNPs of SELE gene could be a risky genotype for D.M.T2 disease in Iraqi

population^{20,21} so it could be a risky genotype in SELE gene for BC.

The distribution of genotype with wheat consumption showed (Fig. 3) inverted distribution. The type of wheat consumption make the mutant genotype for both SNPs as the highly ratio.

Precedent research demonstrated that a concentration of tumor necrosis factor and SELE was lower in persons following heavy whole wheat and bran/rye eating. Qi with his colleagues found that females who ate a lot of whole wheat and bran had lower TNF-R2 levels. That was a research based on observation, and whole grains intake recorded was largely wheat.²² Also Zamaratskaia and his team in (2020) found the Levels of (TNF-R2 & SELE), and endostatin was lesser following consumption of the whole grain/bran compared with cellulose-added processed wheat products.¹⁴ In general, there is a scarcity of data on the association among (grain/bran) diet and endothelial dysfunction indicators, and unequal results had been mentioned. For instance, Qi et al. observed no link among whole grain/bran, and nutritional fiber diet and SELE.²² but De Mello and his team, showed reduced SELE levels in population with impaired glucose metabolism following ingestion fish oils and whole/grain reproducers, the last one also discovered a link among fiber consumption with SELE.²³ Carbohydrate quality in the diet It is beneficial to human health because it contains whole grains, dietary fiber, and sugars. Consumption of whole grains and cereal fiber can lower the risk of diseases such as cardiovascular disease and cancer. Through the effect of whole wheat on biomarkers of inflammation such SELE.²⁴ Also, some cytokines genotypes were found to have a significant relationship with wheat types consumption this polymorphism affects the regulatory role of the Interleukin-18 gene in responses, contributing to the development of disease.²⁵ and dietary patterns high in refined starches may activate the innate immune system and increase levels of SELE.²⁶

4. Conclusions

Whole wheat and bran consumption was positively

associated with lowered BC risk, whereas total refined wheat may related with the risk of breast cancer in females, OR=2.63. As a result, consuming more whole wheat in daily diet is a feasible technique for preventing breast cancer because whole grains are high in bioactive phytochemicals that have been shown to be useful. At the same time, the type of wheat was associated with an increased incidence of disease-related SNPs of SELE gene. White wheat consumption may activate the risky association between SNPs polymorphisms and the BC. While, Consumption of whole wheat/bran products significantly change rs5353 and rs932307 SNPs frequency in females with breast cancer, compared with refined wheat products with added cellulose. Future research into the health effects of phytochemicals included in whole grains should focus on the prevention of diseases.

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