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The polymorphism of Apelin gene rs2235306 and serum Apelin level in breast cancer women in Samarra- Iraqi population

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ABSTRACT

Breast cancer (BC) is the most common cancer leading to death in women and the fifth type of cancer leading to death worldwide. The study was done on 59 women, including 20 non-breast cancer women as a control group, their ages ranged from (30-74 years) and 39 women with breast cancer their ages ranged from (29-80 years). The results showed significant increase ($p \leq 0.05$) in the concentration of Apelin in women with BC compared with non-breast cancer women. This finding suggests that Apelin may promote carcinogenesis. The result of Apelin rs2235306 polymorphism using T-ARMS-PCR technique show no difference in the allele frequencies between BC patients and control group, the results also show that there is no association of the observed genotypes (TT&TC) with serum Apelin concentration in the studied Population. The result of the study suggest that the serum Apelin level can be independent of Apelin rs2235306 polymorphism which should be confirmed by further studies on larger populations.

The aim: The aim of this study was to determine Apelin level in serum and *apln* rs2235306 polymorphism in breast cancer (BC) patients in Samarra city-Iraq.

Introduction

Breast cancer is the most common cancer leading to death in women and the fifth type of cancer leading to death worldwide, despite the development of detection and treatment methods, there is an increase in the incidence and mortality of the disease in middle and Poor countries. In Iraq, breast cancer accounts for one third of cancer cases in women [1,2,3].

Apelin is endogenous peptide derived from 77 amino-acid pre-pro-peptide, was discovered as a high-affinity ligand to the G-protein coupled receptor APJ. the gene codes for Apelin called *apln* located on chromosome Xq25–26.1, that codes for 77 preproprotein of amino acids that undergo to proteolytic to form bioactive Apelin like Apelin-36, Apelin-17, apelin-13 [4].

Apelin belongs to the adipokines family, which are produced from fatty tissue which is not only a place to store fat, but consider as endocrine gland secrete many active substances such as TNF, IL-6, Leptin and others, which has many properties such as they act as inflammatory agents, regulate blood pressure,

glucose Homeostasis, fat metabolism, formation new blood vessels from existing vessels [5,6]

Apelin and its receptor APJ play role in Differentiated physiologic and pathological process and in both Angiogenesis, lymphangiogenesis and tumor cell proliferation [7,8]. Apelin was discovered after the discover of the gene coding for APJ which called *aplnr* located on chromosome 11, intronless in the coding region, amino acid sequence is 31% similar to the angiotensinII receptor type1, however Angiotensin not bind to APJ nor Apelin bind to angiotensin receptor [9,10].

Although the main function of Apelin is not clearly known but the widespread of Apelin- APJ in adult and fetal tissue show that Apelin is associated with many physiological and pathological processes in the body which starts from early stages of cardiovascular development in the embryos, this participation of the Apelin-APJ system strongly supported the idea that Apelin plays a major role in physiological process, infections and oxidative stress and it is involved in

many diseases including heart failure, obesity, diabetes and cancer [11,12,13].

Apelin was found to be abundantly excreted in milk and high level of Apelin-protein and pre-pro-Apelin mRNAs found in mammary gland, and in cultured human breast carcinoma cell line high level of Apelin mRNA was expresses [14].

Objectives and Methods

The study was done on 59 women, including 20 non-breast cancer women as a control group and 39 women with breast cancer.

Apelin levels

Intravenous blood was taken from all samples, infected and uninfected women, the serum was obtained by leaving the samples to coagulate at room temperature before centrifugation for 15min at 2000RPM, and the serum was stored at -20°C until use.

The level of Apelin was measured using ELISA technique based on quantitative sandwich principle according to manufacturer's instructions (cusabio biotech, USA).

Genomic DNA extraction and genotyping

DNA was extracted from blood samples stored in EDTA solution using the method described By [15], the extracted DNA was stored at -20°C until use. The SNP (single-nucleotide polymorphism) of *apln* rs2235206 was detected using the T-ARMS-PCR technique (tetra Amplification refractory mutation System polymerase chain reaction). The gene fragment to be studied was amplified by using the following specialized primers:

Forward inner primer:

(5'CCCCCTGCACACCATCTGCTT-3')

Outer Forward primer:

(5'AAGTG GTGCAGGGTATCTTGGGT-3')

Reverse outer primer:

(5'-AAGGA GCCAAGGAAGGAA CAGAGC-3')

Reverse inner primer:

(5'-GGGACA GGGATCTAGATGCAGGAAG-3') [16]

PCR reactions were performed in a total volume of 25µL containing: 12µL Master mix, Distilled water 7µL DNA 2µL, 1µL of each primer Go Taq G2 Green master mix Promega.

Thermal cycling conditions were as following: an initial denaturation step at 95°C for 5 min; followed by 35 cycles of denaturation at 95°C for 30s, annealing at 58°C for 30s and extension at 72°C for 60s and ending with a final extension at 72°C for 10min. Thermal cycling was performed in an Eppendorf Mastercycler machine (Eppendorf, Hamburg, Germany).

PCR products were electrophoresis on 2.0% agarose gel. The product sizes of *apln* rs2235306 polymorphism for two outer primers (control band) were 458bp, for C Allele the band size was 295 bp and 208bp for T allele.

Statistical analysis

Statistical analysis was Performed using SPSS (Statistical Package for the Social Sciences software), T-test was used to compare the mean of variables between patients and control group. data are presented as means \pm standard deviations (SD). P value (≤ 0.05) was considered statistically significant. We use Chi-square test for genotype frequencies in according with Hardy-Weinberg equilibrium.

Result and Discussion

The results showed a significant increase in the level of Apelin in patients with breast cancer compared to control group as show in the table (1).

Table 1: Comparison of Apelin level between patients and control groups.

variables	patients	control	p value
Apelin pg/ml	2307	867	
\pm SD	± 1001	± 252	0.000

The results of the present study agree with the findings of Salman *et al* [17]. which indicated an increase in the level of Apelin in breast cancer women at postmenopausal stage, also in histological study, high level of Apelin mRNA is expressed in cultured human breast carcinoma cell line [14].

Studies on patients with differentiated type of cancers, including lung, oral and rectal cancer, showed increase in the concentration of Apelin, however the relationship between Apelin high concentration and the presence of cancer tumors is not fully understood, but it's believed to belong to the role of Apelin in the formation of blood vessels since it encouraging the formation of new blood vessels and it is considered a vascular generator promotes formation of blood vessels. increased Apelin concentration induce by hypoxia thought to be one of the mechanisms involved in the formation of new blood vessels in tumors and the mechanism similar to VEGF (vascular endothelial growth factor) as well as some studies found that increase Apelin concentration linked with the return of tumors and progress stage of cancer and low Survival rate. It is also believed that Apelin works through the of ERK1/2 pathway to promote the growth and metastasis of tumors [18,19,20,21,22].

Since the tumors can not grow without new blood vessels, drug that inhibition angiogenesis factors like Apelin thought to be an excellent way to treat cancer, additionally it have fewer side effects than other drugs used in cancer treatment, bevacizumab antiangiogenesis drug works to prevent the formation of new blood vessels in solid tumors such as colon, rectum, lung, breast, glial and kidney cancer. The expression of the Apelin gene was found to be less in the case of Bevacizumab utilization [23]. However the results of our studies should be considered preliminary.

The results of the electrophoresis on the agarose gel showed the presence of three band, the 458 bp band, which represents the control band, which gives evidence of the accuracy of the technique used, band

of 295 bp for C allele and a band of 208bp for t allele as shown in figure1.

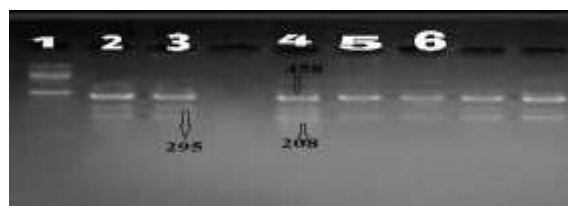


Fig. 1: pattern of Apelin rs2235306 polymorphism on agarose gel electrophoresis by use T-ARMS-PCR technique. Band size were 458 bp for two outer primer and the 295bp for C allele and a band of 208bp for two inner primer.

Line number(1) Represent DNA ladder(100-1500bp), Line numbers (2&3&4) Represent the bands of (TC) genotype, Line numbers (5&6) Represent the bands of (TT) genotype

The result show the presence of two genotype: Heterozygous (TC) represented by the bands (458,295,208)bp and Mutant homozygous (TT) represented by the bands (458 ,208)bp.

The genotype frequencies of apelin rs2235306 polymorphism were done according to Hardy–Weinberg equilibrium in the patient group was ($\chi^2=25.7$), and in control subjects was (7.2). As shown in the table (2)

Table 2: Comparison of genotype between patient and control group.

genotype	Patient group NO (%)	Control group NO (%)
TC	35	15
TT	4	5

Apelin rs2235306 polymorphism did not appear to be associated with the breast cancer in the studied population.

We also investigated the relationship of Apelin rs2235306 genotypes with serum Apelin

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concentration in the studied population, the result show no significant differences in Apelin concentration according to differenced in genotype.

Genetic factors play an important role in the development and occurrence of the BC, Studies have shown that the traditional factors of infection such as age, hormonal factors and reproductive factors in many cases are not present, especially in women who develop BC at a young age. so it's became necessary to measure polymorphism of genes that believed it's have a role in the development of the disease.

The polymorphism of Apelin gene have studies in patients with heart disease, hypertension and diabetes. a study of five types of *apln* polymorphism in patients with coronary artery disease (CAD), show that allele frequency and genetic pattern of polymorphism were not significantly correlated with incidence of disease, In addition, a study involving CAD with hypertension patients and another study included patients with metabolic syndrome, the tow showed no difference in the allele frequency and genotype between the patients and control group. [24,25,26]. the polymorphism of Apelin found to be associated with the risk of atherosclerosis[27]

polymorphism of Apelin rs3115757 was found to be correlates with increased BMI and waist circumference in women in Chinese society and does not affect in men. The high regulation of Apelin gene in liver cells enhances formation of new blood vessels and enhances the susceptibility of invasion and the spread of cancer cells, Genetic and functional studies have shown that Apelin gene may affect the expression of Apelin.[28].

our results suggest that the plasma Apelin level can be independent of Apelin rs2235306, which should be confirmed by further studies on other populations.

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التعدد الشكلي لجين الأبلين rs2235306 ومستوى الأبلين في مصل الدم لدى النساء المصابات بسرطان الثدي في مدينة سامراء- العراق

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الملخص

يعد سرطان الثدي السرطان الأكثر شيوعاً المؤدي للوفاة في النساء والخامس بين انواع السرطانات التي تؤدي إلى الوفاة في جميع أنحاء العالم. أجريت الدراسة على 59 امرأة، تضمنت 20 امرأة غير مصابة بسرطان الثدي كمجموعة سيطرة، وتراوح أعمارهن من (30-74 سنة) كما وشملت 39 امرأة مصابة بسرطان الثدي تراوحت أعمارهن بين (29-80 سنة). أظهرت النتائج وجود ارتفاع معنوي ($p \leq 0.05$) في تركيز الأبلين لدى النساء المصابات بسرطان الثدي مقارنة بالنساء غير المصابات، وتشير هذه النتيجة إلى أن الأبلين ربما يشجع التسرطن. أظهرت نتائج تعدد الأشكال لجين الأبلين للموقع rs2235306 باستخدام تقنية T-ARMS-PCR عدم وجود أي فروقات في تكرار الأليلات بين المصابات وغير المصابات، وأظهرت النتائج عدم وجود فروق معنوية في تركيز الأبلين في المصل حسب الطرز الوراثة المشاهدة (TC&TT) وكان متوسط تركيزه أعلى لدى الأشخاص الحاملين للطراز الوراثة TT بالمقارنة مع الأشخاص الحاملين للطراز الوراثة TC. وتشير النتائج إلى أن مستوى أبلين المصل يمكن أن يكون مستقلاً عن تعدد أشكال في جين الأبلين للموقع rs2235306 والذي يجب تأكيده من خلال المزيد من الدراسات على أعداد أكبر من السكان.