

# Serum Levels of Alanine Aminopeptidase and Aggrecan in Women with Breast Cancer: A Comparative Study

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## Abstract

**Background:** The term “breast cancer” refers to a malignant tumor that has developed from cells in the breast and is a major public health problem in women throughout the world. It is the most common cancer among women both in developed and developing countries.<sup>(1)</sup> The aminopeptidases (AP) constitute a group of enzymes with closely related activities.<sup>(2)</sup> Aminopeptidases catalyze the cleavage of amino acids from the amino terminus of protein or peptide substrates. The enzyme exhibited low molecular weight of 58,000 by gel chromatograph.<sup>(3)</sup> Aggrecan is a proteoglycan, or a protein modified with large carbohydrates hormone; the human form of the protein is composed from 2316 amino acids long and can be expressed in multiple isoforms due to alternative splicing.<sup>(4)</sup>

**Materials and Method:** This study was designed as a cross-sectional, hospital based study. Forty-two women with breast cancer were included in this study, their ages were from 24 to 70 years, and they were from the center and the periphery of Salah Al-din Governorate. Forty-four apparently healthy women with negative family history of breast cancer were included in this study as a control group, they ages were of a comparable age. By using a sterile disposable syringe, 10 mls of venous blood sample was drawn in a plain tube from each women included in this study for the serum measurement of alanine aminopeptidase and Aggrecan by using ELIZA technique.

**Results:** The mean serum level of alanine aminopeptidase was elevated in breast cancer women comparing with the control group (71.73±1.5 versus 15.29±0.9 ng/ml). The result was highly significant at a P value of less than 0.01. While the mean serum level of aggrecan was found among women with breast cancer (41.81±11.5 ng/ml) compared to control group which was 7.27±3.6 ng/ml. The result was highly significant at a P value of less than 0.001. This study also found that there was a moderate positive correlation between S. AAP activity and S. aggrecan in breast cancer women (R<0.7).

**Conclusion:** There were an increase in serum levels of both the alanine aminopeptidase and aggrecan in women with breast cancer.

**Keywords:** Breast cancer, alanine aminopeptidase, and aggrecan.

## Introduction

Breast cancer is the most common malignancy in women, with incidence rates generally increasing

throughout the world and this increase being attributed to socio-economic and demographic changes.<sup>(5,6)</sup> It carries a high incidence and mortality from 27 types of major cancers and for all cancers combined since 2012 and are now available in the Series of the International Agency for Research on Cancer with 1.67 million patients in the world.<sup>(7)</sup> Breast cancer is the most dangerous disease that threatens women lives in Iraq, every year 1000-1200 new cases are recorded, 98% of which affect women, and 2% of which affect men. Breast cancer occupies 14% of the

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whole disease in different cancers and a rate of 1-6 of women are affected from every 100000 women.<sup>(8)</sup>

Alanine aminopeptidase (AAP) is one of exopeptidase, it is composed of 967 amino acids.<sup>(9)</sup> Alanine aminopeptidase its function is to release the N-terminal amino acid residues from polypeptides and protein, isolated in a highly purified state from human liver, kidney, and pancreas were investigated with regard to their different electrophoretic mobility. While the molecular weights are identical, different isoelectric points were found.<sup>(10)</sup> Alanine aminopeptidase may hydrolyze several biologically active peptides. Its activity was significantly higher in males than in females.<sup>(11)</sup> Alanine aminopeptidase spreads in various regions of mammalian body, because it is responsible for many physiological functions inside the human body, like kidney, pancreas, liver,<sup>(12)</sup> and intestine specially in duodenum.<sup>(13)</sup>

Aggrecan (ACAN) is a proteoglycan that present in all cellular and tissue compartments. It is one of the major structural component of cartilage therefore it is known as cartilage-specific proteoglycan core protein (CSPCP) or chondroitin sulfate proteoglycan 1 (may have >100 chondroitin sulphate chain) which are key to its function in the maintenance of a hydrated, compression-resisting matrix.<sup>(14,15)</sup> Aggrecan has a molecular mass more than 2,500 kDa.<sup>(16)</sup> The core protein (~300 kDa),<sup>(17)</sup> and has around 100 GAG chains attached to it.<sup>(18)</sup> It consists of two globular structural domains (G1 and G2) at the N-terminal end and one globular domain (G3) at the C-terminal end, separated by a large extended domain chondroitin sulfate (CS) heavily modified with GAGs. The two main modifier moieties are themselves arranged into distinct regions (N-G1-G2-CS-G3-C), a chondroitin sulfate and a keratin sulfate region. The three globular domains, G1, G2, and G3 are Involved in aggregation, hyaluronan binding, cell adhesion, and chondrocyteapoptosis. Along with type-II collagen, aggrecan forms a major structural component of cartilage, particularly articular cartilage. It's play an important role in function of joints.<sup>(18)</sup>

### Patients, Materials, and Method

This study was designed as a cross-sectional, hospital based study. The protocol of this study was approved by the scientific committee of Tikrit university college of medicine, and the agreement to collect the samples from patients was approved by the Salah Al-

din Health Directorate. The study was carried out at the Gynecology Outpatient and Oncology Unit in Samarra General Hospital-Samarra City-Iraq, from the 14 of November 2018 to the end of June 2019. A verbal consent was taken from each women included in this study whether considered as a case or control. The diagnosis of breast cancer was according to discussion of both the gynecologist and the histopathologist. The total number of women with breast cancer were 42, and their ages were form 24 to 70 years, and they were from the center and the periphery of Salah Al-din Governorate. Forty-four apparently healthy women with negative family history of breast cancer were included in this study as a control group, their ages were comparable to that of the women with breast cancer and they were also from the center and the periphery of Salah Al-din Governorate.

By using a sterile disposable syringe, 10 mls of venous blood sample was drawn in a centrifuge from each women included in this study after morning and allowed to clot at room temperature, then the tubes were centrifuged at 3000 rpm and the supernatant serum was used to estimate the levels of alanine aminopeptidase and aggrecan by ELIZA method.

**Statistical Analysis:** All the date collected in this study were analyzed by using the student t-test, the mean, standard deviation, and P-value was also considered. The significance was considered at a P value of less than 0.05.

### Results

This study showed that the peak age of women with breast cancer was between 44–53 years and its percentage was 35.71%, while the least age group was above 63 years and its percentage which was found to be 9.52%, see table (1).

**Table (1): Distribution of breast cancer women according to age.**

Age groups (years)	No.	Percentage
24-33	5	11.91
34-43	8	19.05
44-53	15	35.71
54- 63	10	23.81
>63	4	9.52
<b>Total</b>	<b>42</b>	<b>100</b>

**Table(2): Residence distribution of breast cancer women.**

Residence	No.	Percentage
Rural	11	26.19
Urban	31	73.81
Total	42	100

This study also showed that maximum rate of breast cancer was 73.81% where from the urban area, while 26.19% of breast cancer women was from rural area, see table 2.

This study demonstrated that the BMI was significantly higher at a P value of 0.001 in breast cancer women compared to control group (34.55±0.21 versus 29.91±0.44 Kg/cm<sup>2</sup>), as shown in table (4.3)

**Table (3): Comparison between women with breast cancer and the control group regarding body mass index (BMI).**

BMI (Kg/cm <sup>2</sup> )	Breast cancer women	Control group	T. test	P – value
No.	42	44	13.9	0.001 highly significant
Mean±SD	34.55±0.21	29.91±0.44		

P. value <0.01: highly significant.

As shown in table 4, the mean serum level of alanine aminopeptidase was elevated in women with breast cancer comparing with the control group (71.73±1.5

versus 15.29±0.9 ng/ml). The result was highly significant (P <0.01).

**Table (4): Serum alanineaminopeptidase activity in women with breast cancer and the control group.**

Study groups	No.	Alanine aminopeptidase activity (ng/ml) (Mean±SD)	T. test	P. value
Breast cancer women	42	71.73±1.5	29.8	0.001 highly significant
Control group	44	15.29±0.9		

The mean serum level of aggrecan was found among women with breast cancer (41.81±11.5 ng/ml) compared to control group which was 7.27±3.6 ng/ml. The result

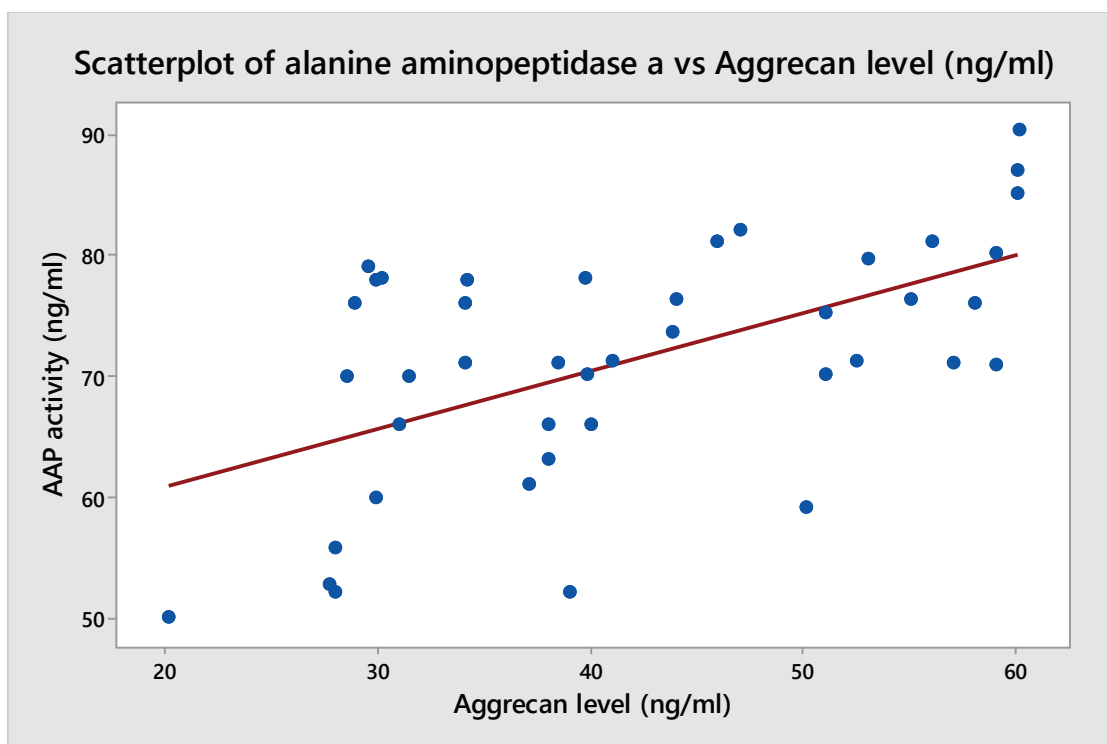
was highly significant at a P value of P - 0.001, as shown in the following table.

**Table (5): Level of aggrecan in women with breast cancer and the control group.**

Study groups	No.	Aggrecan level (ng/ml) Mean ± SD	T. test	P. value
Breast cancer women	42	41.81±11.5	18.8	0.001 Highly significant
Control group	44	7.27±3.6		

This study reveals that there was a moderate positive correlation between serum alanine aminopeptidase

activity and serum aggrecan in women with breast cancer (R<0.7), figure (1).



**Figure (1): Correlation between serum alanine aminopeptidase activity and serum aggrecan in women with breast cancer (R:0.6)**

## Discussion

This study reveals that the peak age of women with breast cancer was between 44 - 53 years this finding was in agreement with AL-Hashimiet *al*<sup>(19)</sup> reported that the median age at diagnosis was 49 and the mean age was 52 years, also this study was in agreement with Majid *et al*<sup>(20)</sup> who reported that the breast cancer increased in post-menopausal Iraqi women, and the rate of incidence in Arabs and its Kurdish region were generally stable between 2000 to 2009.

This study also shows that the breast cancer was more common in women from urban area more than rural as seen in the table (2). This result was agreement with Majid *et al*<sup>(20)</sup>, that found in his study that the urban exceeding the rural rate by 2:1, for both Kurdish and Arabic women. This finding could be attributed to the migration of some peoples in Iraq from the rural to the rural area in the last decades.

Body mass index consider one of the modifying risk factor for breast cancer.<sup>(21)</sup> In the present study it was found that there were an increase in the weight of women with breast cancer more than that of the control

group. The mean of BMI in breast cancer women that show in table (3), this finding was consistence with Platet N. *et al*<sup>(22)</sup> and Rebbeck R. *et al*<sup>(23)</sup> who reported a high prevalence of overweight and obesity in women with breast cancer. Epidemiological studies have shown that postmenopausal obesity causes an increase in the risk of breast cancer,<sup>(24)</sup> since the obesity has a close relationship with increased levels of insulin and insulin like growth factors.

This study show that there was a significant difference between breast cancer women and the control group concerning serum level of alanine aminopeptidase which was found to be significantly higher in women with breast cancer comparing with the control. These results were in agreement with Martinez *et al*<sup>(25)</sup> who found an increase in the activity of this enzyme in neoplastic tissue when compared with unaffected tissue. Also these results were in agreement with Mina-Osorio *et al*,<sup>(26)</sup> and Taylor *et al*<sup>(27)</sup> who found an increased AP activity in breast cancer tissue *vs* surrounding normal tissue. Wickstrom *et al*<sup>(28)</sup> and Bauvois B. *et al*,<sup>(29)</sup> noticed an increased APN activity in the serum of women with breast cancer *vs* its activity in serum of healthy women. These findings could be attributed to that tumors usually

associated with a rapid overgrowth of cells along with cell destruction.

The finding of a significant difference between BC women and the control group concerning serum aggrecan level at a P value of less than 0.01. This result was in agreement with the finding of Nikitovic *et al*<sup>(30)</sup> who reported that the proteoglycans are important constituents of the intracellular or extra cellular matrix, that have been associated with cancer pathogenesis. These findings could be attributed to that tumors usually associated with a rapid overgrowth of breast cells along with increase production of this hormone .

The positive correlation between alanine aminopeptidase and aggrecan in this study, could be attributed to that both of these biochemical parameters were increased in serum of women with breast cancer in this study.

### Conclusion

This study reveals an increase in serum levels of alanine aminopeptidase and aggrecan in women with breast cancer.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest:** Non

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