Evaluation of Ribonuclease (RNAse) Activity in the Sera of Women with Breast Tumor.

Basma F. Tawfeq[1], Kdiar A. Kdiar [2] ,Sabah H. Kurshid [3]

[1]Kirkuk Health Directory, Iraq Ministry of Health, Kirkuk, Iraq.

[2]Department of Biochemistry, Collage of Medicine, Tikrit University, Tikrit, Iraq.

[3]Department of Biology, Collage of Education for Pure Science, Kirkuk University, Kirkuk, Iraq.

[1]abasma024@gmail.com, [2]Kdiar Abbase@yahoo.com, [3]Sabah korshed@gmail.com

Abstract

In this study 125 patients with breast tumors (61 patients with benign breast tumors, 64 patients with malignant breast tumors) with age range between (20-75) years collected from Center of Early Detection of Breast Cancer in Azady Teaching Hospital in Kirkuk City from November 2016 to end of May 2018 and matched with 50 healthy women as control group. Serum RNAse activity and total protein (albumin, globulin) concentration were estimated in all patients (benign and malignant) and compared to control group.

The present observational study has shown a significant elevation in serum RNAase activity in cases of breast carcinoma and benign breast lesions as compared to control, which can use RNAse as tumor marker for breast tumor.

Keywords: Breast Tumors**,** RNase , Total protein.

تقييم فعالية انزيم الرايبونيوكليسس في أمصال النساء مصابات بأورام الثدي.

بسمة فاضل توفيق[1]، خضير عباس[2]، صباح خورشيد[3]

[1]دائرة صحة كركوك، وزارة الصحة، كركوك، العراق.

[2] قسم الكيمياء الحياتية، كلية الطب، جامعة تكريت، تكريت العراق.

[3]قسم علوم الحياة، كلية التربية للعلوم الصرفة، جامعة كركوك، كركوك، العراق.

[1]abasma024@gmail.com, [2]Kdiar Abbase@yahoo.com, [3]Sabah korshed@gmail.com

الملخص

تضمنت هذه الدراسة 125عينة من النساء المصابات بأورام الثدي (61 عينة مصابة بأورام الثدي الحميد و 64 عينة مصابة بأورام الثدي الخبيثة) تراوحت أعمارهن بين (20– 75) سنة من مركز الفحص المبكر لسرطان الثدي في مستشفى ازادي التعليمي في مدينة كركوك للفترة من تشرين الثاني 2016 ولغاية نهاية شهر ايار 2018 تضمنت الدراسة ايضا 50عينة من الاشخاص الأصحاء بنفس مديات الأعمار كـ مجموعة ضبط، شملت المعايير التي تم تقديرها في مصل الدم: فعالية أنزيم الرايبونيوكليس، وتركيز البروتين الكلي، (الألبومين و الكلوبيولين) لكل من المرضى ومجموعة الضبط.

وقد اظهرت الدراسة ان هناك زيادة معنوية ملحوظة في فعالية انزيم الرايبونيوكليسس لكل من مرضى النساء المصابات بالأورام الحميدة والخبيثة مقارنة بمجموعة التحكم، ذلك يمكن استعمال انزيم الرايبونيوكليسس كمعلمة للكشف عن اورام الثدي.

الكلمات الدالة: الرايبونيوكليسس، البروتين الكلي، اورام الثدي.

1. Introduction:

Tumor, is a term commonly refer to a mass (neoplasm), may be (Benign) not cancerous cannot spread or (Malignant) which cancerous that can grow and spread [1]. Tumor associated markers reflect behavioral changes from tissue to blood, resulting in changes in levels of enzymes, proteins and hormones both in cancerous tissue and blood because of unchecked proliferation of cells [2]. Ribonuclease is a type of enzyme which catalyzes the breakdown of bonds in ribonucleic acid (RNA) which is the chemical material in a cell that codes for different proteins [3], when ribonuclease is present in the cytoplasm, mean RNA is degraded, this process is important to allow the cell to control the amount of protein produced by any RNA and to reuse the nucleotides to create new RNA [4].

Many studies improved the prospects for using RNase in the diagnosis and treatment of diseases which is a very important enzyme for RNA metabolism in almost all organisms, that circulating RNAse has a major potential as a cancer biomarker by distinct alteration in circulating RNA reflect dysregulation of cancer immunity, cell growth, proliferation and stromal interaction [5]. Total protein determination gives some information about the patient's general status regarding nutrition, or organ disease, but further fractionations yield far more clinically useful information[6]. Total human serum protein profile is made up of albumin and globulins are the most abundant molecules found in any cell, acting as a dynamic system with various biological functions [7, 8].

The aim of this study was to evaluate the activity of RNAse and total protein concentration (Albumin and globulin) in cases of benign and malignant breast lesions with compared to that with of healthy individuals (control group) as early marker of breast tumor.

2. Materials and Methods:

2.1 Subjects and Methods:

A total number of 125 subjects participated in the present study which included 61 cases of benign and 64 cases of malignant lesions of the breast were newly diagnosed and did not undergo any type of therapy collected from November 2016 to end of May 2018. All were in the age ranged between (20 to 75) years. Study was conducted on patients from Center of Early Detection of Breast Cancer in Azady Teaching Hospital in Kirkuk City.

Cases were histopathologically or cytologically diagnosed as benign and malignant lesions of the breast by physician taking informed consent, a careful history was taken and thorough clinical examination was conducted in all the cases. The blood was allowed to coagulate at room temperature before being centrifuged , the sera were separated and stored at (-20) C^o until used. Serum RNAase activity were determined by ELISA method (kit from United States Biological-Company) and Biurt method used to estimation of total protein(albumin and globulin) in all studies groups.

2.2 Statistical analysis:

Statistical analysis was carried out by SPSS program version (16.0). The differences between groups were tested by the student t-test, and P-value was considered significant if it is ≤ 0.05, highly significant if ≤ 0.001, and non-significant if it is ≥ 0.05.

3. Results:

In the present study maximum cases of malignant lesions were in (40 - 49) years of age group(i.e. 50%) followed by (50 – 59) years of age group (i.e. 15.62%) and there were only 1 cases less than < 30 years of age group. The maximum number of cases of benign lesions were in (30 – 39) years of age group (i.e. 40.98%), followed by (20 – 29) years of age group i.e. (33.8%) while in 70-79 years of age group there were no cases of benign lesion Table 1.

The RNAse activity were presented in Table 2 as mean ± S.D that in patients with benign tumor was 115.4 ±12.55U/L, while in patients with malignant tumor was 386±18.77 U/L and control was 20.34 ± 16.66U/L. These results revealed the presence of a highly significant increase (P<0.001) the activity of enzyme in the sera of women with benign and malignant breast tumors as compared with that of the control group.

Total protein, albumin and globulin concentrations as well as albumin/globulin ratio of study groups and control are showed as(mean ±SD) in Table 2. The results represented that there were a highly significant decrease (P<0.001) in total protein concentration in the sera of women with benign and malignant breast tumors as compared to control .

The results of albumin indicated that there was a highly significant decrease (P<0.001) in patients with malignant breast tumors and control group, while there was non-significant difference in this concentration between patients with benign breast tumors and control group. A

significant increase in globulin concentration in the sera of patients with benign and those with malignant breast tumors in comparison with that of the control group (P<0.05). Finally, [Albumin]/[globulin] ratio in the sera of studied groups was calculated, the results indicated that there were non-significant difference (P>0.05) in this ratio between patients with malignant breast tumor group, benign tumor group as compared with control group ,all these results were illustrated in Table 2.

Table 1: Age Groups , % Number in Benign and Malignant Breast Tumor.

Residence	Breast Tumor		Total
	Benign	Malignant	
Age (years) Mean ±SD	36.58 ± 10.991	55.036 ± 12.276	/
<20	2	0	2
	3.27 %	0 %	1.6 %
20-29	18	1	19
	29.5%	1.56%	15.2%
30-39	25	8	33
	40.98 %	12.5%	26.4%
40-49	11	32	43
	18 %	50%	34.4%
50-59	4	10	14
	6.55%	15.62%	11.2 %
60-69	1	6	7
	1.63%	9.37%	5.6%
70-79	0	7	7
	0%	10.93%	8.74%
Total	61	64	125
	100%	100%	100%

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Table 2: Activity of RNAase and concentration of total protein(albumin, globulin and albumin/globulin ratio) in sera of (control, benign and malignant breast tumors) groups.

Parameters	Study Groups		
	Control Mean ± S.D	Benign Mean ± S.D	Malignant Mean ± S.D
Serum RNAase (U/L)	20.34 ± 16.66	115.4 ±12.55**	386±18.77**
Serum total protein (g/dl)	6.59 ± 0.728	5.94 ± 0.876**	5.6 ± 0.784**
Albumin(g/dl)	3.54 ± 0.360	3.53 ± 0.35	3.19 ± 0.51**
Globulin (g/dl)	3.11±0.945	3.44±0.874*	4.1 ±0.939*
Albumin/Globulin ratio	1.738 ± 0.80	1.532 ± 0.872	1.442 ± 0.738

* Significant difference in comparison to control group at ($P<0.05$).

**Highly significant differences in comparison to control group at ($P<0.001$).

4. Discussion:

The risk of developing breast cancer increases with age that most lesions were in reproductive age group, the disease is uncommon in women under the age of 40 years and there were no cases of benign lesion of age group (70-79), while (i.e. 40.98 %) in age group 30-39 of benign lesion; the mean age of malignant and benign was (55.036 ± 12.276) and (36.58 ± 13.991) respectively these results are similar for many studies in breast tumor, BhattacharyaS *et al* [9] were found breast cancer cases in age range between 40 – 49 years (i.e. 52.5%) followed by and there were no cases in <20 years of age group.

Many studies have tried to evaluate role of tumor markers in diagnosis of breast carcinoma, the present study showed high level of the RNAse activity in patients groups (malignant and benign) when compared to control group this agree with many authors, that they reported level of serum RNase activities have been noticed to increase in several

diseases such as malignant neoplasia [10] renal insufficiencies [11] pancreas disorder and leukemia [12].

De Lorenzo et al [13]. found abnormal elevation in the sera of serum RNase activity which is serve as reliable biochemical marker in the carcinoma of the pancreas in the presence of the normal renal function that RNases represent an extensive group of the essential enzymes for a cell which function provide necessary balance between processes of synthesis, operation and destruction of various classes of RNA in the cells on various phases of a cell cycle.

The elevation in RNase activity reported in the present studies, which may be due to one of the following reasons: generally it is known that cancer cells are characterized by uncontrolled increase in the number of cells and their sizes, which means that there will be an increase in the synthesis of different proteins and this explains the need for high RNase activity[14], in addition to this the alteration in cell permeability of tumor cells membranes where a number of changes in the biochemical characteristic of malignant cell surface have been observed, these include the appearance of new surface antigen proteoglycans, glycolipids, and mucins, and altered cell-cell and cell-extracellular matrix communication[15], such changes may lead to transport of different enzymes from tumor cells or the surrounding tissues to the blood and one of these enzymes may be RNase, and its excessive entry into serum rather than to diminish its urinary excretion [16].

Throughout this study the decrement the total proteins concentration in the sera of patients with benign and malignant breast tumors which can be explained by the fact that breast cancer is one of the condition that initiates the acute phase response, and that can affect by increase the levels of specific hepatic proteins (e.g. positive acute phase proteins)[17]. At the same time, there are group of proteins in the body were reported to decrease in its concentration because of the enhancement in their catabolism rather than their synthesis, such proteins known as negative-phase proteins like albumin and prealbumin[18]. Therefore, the hypoproteinemia that observed here can be results from the net balance between the protein synthesis of positive acute phase protein, and catabolism of negative acute phase protein furthermore the release of protein from the necrotic cells into the blood[19]. Generally, cancer patients have lower serum albumin concentration than healthy individuals[20].

Rizzoli *et al*., 2001 reported that nutritional deficiency and weight loss in association with psychological stress and lower quality of life may cause reduction in serum albumin concentration. Moreover this decrease in albumin concentration in the sera of patients with malignant breast tumors due to increased breakdown of protein as may be due to tissue damage or inflammation association with cancer or may take place because of its action as a sacrificial antioxidant, when the radical reactions continue on albumin surface and causes a damage to the albumin molecule[21]. In addition to that a reduction in the globulin concentration observed in the sera of patients with benign and malignant breast tumors agrees with Lis CG, 2005[22].

This decrease may be due to increase breakdown of protein due to tissue damage or inflammation associated with cancer. Moreover such decrease in globulin concentration may be due to that the disorders of immunoglobulin synthesis which is associated in some syndromes with defective cell-mediated immunity, in which there is a deficiency of immunoglobulin. The results of [albumin]/[globulin] ratio in the sera of the studied group's revealed non-significant differences in this ratio among control group, benign and malignant breast tumors groups. This results disagree with Seth *et al*., 2016 [23], who showed that [albumin]/[globulin] ratio decreased in sera of patients with breast cancer at different stages of the disease (stage I–stage IV), their results showed that the decrease in this ratio was 50% in comparison to their control group.

5. Conclusions:

The present observational study has shown a significant elevation in serum RNAse activity in cases of breast tumor.

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