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Abstract - *Background :* Ribonuclease (RNase) are widely distributed in various organs and body fluids, including serum, urine, saliva, and cerebrospinal fluid. Small amounts of extracellular ((exocrine)) forms of this enzyme are secreted by the normal human pancreases into the gut, have observed increased levels of serum RNase in a series of patients with cancer of ovary. They have suggested that this might represent increased enzyme synthesis by proliferating tumor cell within the ovary.

Objective : The aim of this study was to evaluate acid and alkaline RNase activities in serum of women presented with benign and malignant ovarian tumors with respect to these of healthy women.

Method : A total of twenty nine women patients (15 women with benign ovarian tumor and 14 women with malignant ovarian tumors) were included.. Their age were 28-60 years the two groups were compared with a group of age matched (16 healthy women).

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Method : A total of twenty nine women patients (15 women with benign ovarian tumor and 14 women with malignant ovarian tumors) were included.. Their age were 28-60 years the two groups were compared with a group of age matched (16 healthy women).

Five milliliter of blood samples were obtained from patients by vein puncture just before surgery, as well as the from healthy women. Protein concentration was determined for patients and healthy individuals, and RNase activity for both the acid and alkaline forms were estimated by spectrophotometric methods with yeast RNase as the substrate.

Results : Results revealed that patients group with ovarian malignancy had significant increase ($p<0001$) in both serum alkaline and acid RNase activity when compared with patients of benign tumors and the control group. There were significant ($p<0.05$).increases in serum alkaline and acid RNase specific activities, in women with ovarian cancer when compared with women of benign tumors and the control group.

Conclusion : Estimation of alkaline and acid RNase activity is a promising approach for the detecting of ovarian cancer.

I. Introduction

Tumors of the ovary are common forms of neoplasia in women. Among cancers of the female genital tract, the incidence of ovarian cancer ranks below only carcinoma of cervix and the endometrium. Ovarian cancer accounts for 6% of all cancer in the female and is the fifth most common form of cancer in women in the U.S.A (1-). I n addition, because many of these ovarian neoplasms cannot be detected early in their development, they account for a disproportionate number of fatal cancers, being responsible for almost half of the deaths from cancer of the female genital tract (6-8).

Ovarian cancer is the second of the seventh most common malignant tumors among the women in Iraq. The Iraqi cancer registry estimated a threefold increase in the incidence of this disease during the last two decades.(9)

Ribonuclease (commonly abbreviated as RNase) is a type of nuclease that catalyzes the degradation of RNA into smaller component. It has been detected, identified and characterized in several organs and animal body fluids(10). The ribonuclease activity of the three human body fluids, serum, CSF, and urine, is chromatographically heterogenous (11). Serum, for instance, contains at least six species of RNase activities. These species activities have been categorized in two major classes distinguished by their optimal pH for deploymerisation of RNA; acidic RNase (pH 6.5) and alkaline RNase (pH 8.5). The acidic RNase originates from liver or spleen(12), while alkaline RNase originate from pancreas and liver, distributed in cytosol and mitochondria (2).

Levels of serum RNase activities have been noticed to increase in several diseases, such as malignant neoplasia (13,14), renal insufficiencies (15), pancreatic disorders and leukemia (3,11,16). Changes in serum RNase activities have been thought of as potential diagnostic tools of these diseases.

The aim of present study was undertaken to examine further the reliability of serum RNase measurement as an aid to the diagnosis of human ovarian cancer.

II. Material and Methods

Two groups of ovarian tumor patients (15 patients with benign tumors and 14 patients with malignant tumors), the patients age were 28-60 years, these two groups were compared with a group of age matched (16 healthy women).

Patients were admitted to Oncology unit in AL-Sadder Medical city, in Najaf, and Oncology unit in Medical city Hospital, in Baghdad. The patients were

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newly diagnosed and not underwent any type of therapy. Patients suffered from any disease that may interfere with our study were excluded.

Five milliliter of blood samples were obtained from patients by vein puncture just before surgery, as well as from healthy women. Blood samples were left for 30 min at room temperature for coagulation, serum were aspirated by centrifugation at 3000 xg for 10 min, and stored in sterilized tubes at -20 C^0 until processed.

Protein concentration was determined Biuret method using Biomaghreb kit, with a standard procedure (4). RNase activity was assayed by a spectrophtometric method with yeast RNA as the substrate (17). Acid ribonuclease activity was estimated according to Smith, etal method (18) by using acetate buffer (0.1 M, pH: 5.0), alkaline RNase activity assay is estimated by using tris .HCl buffer (0.2M, pH=8.5) and phosphate buffer (0.1 M, pH: 6.7) according to Umeda. et. al method (19).

Acid RNase was calculated according to the following equation: Total activity (U/L)=$\Delta A / t \times V_t / V_S \times 1000$. ΔA/ min = change in measuring absorbance (300 nm) with time. V_t = total volume. V_S = the volume of serum used.

Alkaline RNase was calculated by the following equation: Total activity (U/L) = $\Delta A / t \times V_t / V_S \times 10^6$. Where ΔA = sample absorbance at 260 nm - blank absorbance.

V_t = total volume. V_S = volume of serum used. t = The incubation time (min).

It is not possible to define the enzyme`s activity in terms of international units because the molecular weight of the serum polynucleotide is unknown. (Reddi) (20). The activities of both alkaline and acidic RNase were determined and expressed as units as well as specific activities (units/ mg serum protein).

III. Results

The activities (mean ± SD) of both alkaline and acid RNase were determined and expressed in unit/L as well serum acid and alkaline RNase specific activities were estimated and expressed as U/mg in twenty nine women suffered from ovarian tumors and compared with sixteen healthy. The two enzymes activities were differentiated with respect to the optimal PH of maximal activity.

Statistical analysis was performed using t-test to examine the difference in the mean of the studied parameters between control and patients groups. All values are expressed as mean ± SD.

The results in table 1 revealed that the patients group with ovarian cancer had significant ($p<0.05$) increase in both serum alkaline and acid RNase activities when compared with those of the healthy women. Otherwise, there were significantly ($p<0.001$) increased serum acid and alkaline RNase specific activities in women with malignant tumors when compared with healthy.

The acid / the alkaline RNase activity ratio show no significant differences among benign and control group, while there were a significant ($p< 0.05$) decrease when the ratio of malignant group was compared with that of control group.

Table 1 : Comparison of RNase activities in a control group and women with benign and malignant ovarian tumors.

Parameters	Control (n= 16) Mean ± SD	Benign tumor (n=15) Mean ± SD	Malignant tumor (n=14) Mean ± SD
Total protein (g/ dL)	7.3±	6.8±0.5	6.06± 0.4
Alk. RNase (U/L)	84.68± 1.3	95.2 ± 2.6	250.2 ± 2.8 **
Alk. RNase Specific activity (U/mg)	± 0.05	1.4 ± 0.3	4.17 ± 0.16 *
Ac. RNase (U/L)	11.5± 0.9	11.4± 1.3	38.18± 2.1 **
Ac. RNase Specific activity (U/mg)	0.158 ± 0.03	0.168 ± 0.07	0.303 ± 0.08 *
Ac/Alk. Specific RNase activity ratio	0.136±	0.12±	± 0.009 **

* $p<0.001$, ** $p<0.05$, Alk: Alkaline, Ac : Acid.

When the two groups of patient with benign and malignant tumors were compared together, there were significant ($p<0.001$) increases in alkaline and acid RNase. The specific activities of alkaline and acid RNase activity significantly ($p<0.001$) increased in women with malignant tumors when compared with those of women of benign tumors.

The acid/alkaline RNase ratios were observed refereed to be significantly ($p<0.05$) decreased when the ratio of malignant group was compared with that of benign group.

Table 2 : Serum alkaline and acid RNase activities of two groups of patients benign and malignant tumors.

Parameters	Benign tumor (n= 15) Mean ± SD	M alignant tumor (n=14) Mean ± SD
Alk. RNase specific Activity (U/mg)	1.4 ± 0.12	4.17± 0.16 *
Ac. RNase specific Activity (U/mg)	0.168 ± 0.0	0.303± 0.08 *
Ac./Alk. RNase specific Activity ratio	0.12±	0.07± 0.009 **

* $p<0.001$, ** $p<0.05$, Alk.: Alkaline, Ac: Acid.

IV. Discussion

There has been increased interest in recent years in the examination of serum parameters which could provide a sensitive and reliable means of monitoring the presence or progression of neoplasms in humans. Numerous reports have appeared in which significant increases in the level of ribonuclease were observed in the sera of cancer patients (20). Holzmaun etal (21) reported that 60% of patients with malignant diseases demonstrated serum RNase levels that were significantly higher than those of normal individuals. A report by Reddi and Holand (22) also indicated the effectiveness of serum RNase as an indicator of malignancy ingeneral, but most notably in the case of ovarian cancer. Moreover, Gerdes etal (23) have suggested that the serum RNase level is a reliable tumor marker in the detection of ovarian malignancy.

The elevation in serum RNase activity observed in the ovarian cancer patients was in agreement with many other studies in different kinds of cancer, including multiple myeloma (24), liver cancer (25), a denocarcinoma cell line(26), Leukemia (27), and renal failure (28). Levy and Ratline (29), noticed an increased serum RNase activity in patients with cirrhosis, trauma, leukemia, AL-Shammaree (30), also found an elevation in serum RNase activity in uterine cancer when compared with the control group, and the ratio of acid/alkaline RNase was decrased in malignant uterine tumor when compared with the ratio of control group.

Although many human tissues express ribonuclease, the reason of the elevation of serum RNase activity is unknown (25). It is not clear whether the increase are associated with lack of a host defense mechanism, production by malignant cells, a secondary destructive process in other cells or tissues, or other conceivable mechanisms.

One suggestion for such, high serum RNase levels was that it might be due to excessive entry of RNase into the serum rather than to diminish urinary excretion of the enzyme (24).

Another suggestion was that the increases in activity could reflect factor other than an increase in RNase concentration in serum. Metal ions especially zinic, copper, and manganese affect RNase activity by interacting both with the substrate to cause activation and with the enzyme resulted in activity inhibition. Putrescine (chemical compound breakdown for amino acids) stimulate RNase activity, and prevent aggregation of RNase. Hence, variation in concentration of serum polyamines have the potential of altering serum RNase activity (25, 26).

V. Conclusion

The current investigation suggested the use of the estimation of RNase activity a promising parameter to predict ovarian cancer.

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