

## Biochemical study for Alanine amino peptidase isoenzymes of abortion cases in Tikrit , Iraq

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### Abstract

The study was included (66) undiagnosed aborted women at different stage of pregnancy compared with (40) healthy subjects as control groups .

A high significant increase ( $P \leq 0.05$ ) in The activity of alanine aminopeptidase in urine of single & recurrent abortion first & second trimesters were shown ( $5.3 \pm 0.33$ ) ( $10.85 \pm 0.05$ ) U/L and ( $14.5 \pm 0.39$ ) ( $22.7 \pm 0.72$ ) U/L respectively compared to healthy subject ( $5.1 \pm 0.24$ ) ( $5.4 \pm 0.48$ ) and ( $4.3 \pm 0.295$ ) U/L for normal pregnant (1<sup>st</sup> trimesters) , (2<sup>nd</sup> trimesters) and non pregnant women . AAP was purified from urine of aborted women by gel-filtration using sephadex (G-50) and two isoenzymes of AAP I,II were separated from urine samples by using DEAE-cellulos .

### Introduction

Aminopeptidase N (APP) is a ubiquitous enzyme present in several human organs, tissues and cell types. Alanine aminopeptidase is (3.4.11.2) described as a multifunctional ("moonlighting")<sup>[1]</sup> protein with enzymatic as well as other functions, including antigen presentation and a receptor for some human viruses (e.g. corona viruses)<sup>[2]</sup>. The AAP is identical with cell differentiation CD13 molecule which expressed on early progenitors of granulocytes and macrophages and on all stages of these lineages<sup>[3]</sup>, which plays essential role in endothelial cell function<sup>[4, 5]</sup>.

Then induction of apoptosis in activated lymphocytes so they providing a mechanism for maternal immune tolerance to the fetus<sup>[6,7]</sup>. The enzymes excreted in the urine as part of secreted exosomes, which illustrates the potential value for diagnostic, prognostic, and pathophysiologic discovery, as urinary biomarkers of various diseases<sup>[8]</sup>. Several examples of important diagnostic enzymes are findings in the urine due to injury of individual organs and reflect the capabilities localize the site of damage in diagnosing the disease<sup>[9]</sup>.

AAP has broad specificity and is most efficient in the pH7 to 8 the enzyme preferentially releases N-terminal hydrophobic amino acids from oligopeptides, the order of preference for cleavage was alanine the favoured amino acid<sup>[10]</sup>. Proteinases regulate all aspects of life functions, from fertilization through development to cell death. A variety of pathological events involve dysregulation of proteinases activity, including (cardiovascular, reproduction, cancer, diabetes, and Alzheimer's disease). Peptidases also have been shown to play important roles in blood pressure regulation during pregnancy, and a number of articles evaluate the involvement of peptidases such as the novel oxytocinase subfamily of aminopeptidases and other endopeptidases such as neprilysin and its relatives in reproductive processes and particularly pre-eclampsia<sup>[11]</sup>, many enzymes have been found to increase during normal pregnancy, so evaluation of urinary alanine aminopeptidase during abnormal pregnancy, have been found useful in the management of pregnancy.

### Materials and Methods

A patients group of 66 pregnant women with history of single abortion or recurrent spontaneous abortion (RSA) attended Obstetrics and Gynecology department at Tikrit teaching hospital in Tikrit were taken for this study, and compared with control group of 40 women, consist of 25 normal pregnant women in first and second trimester, and 15 non pregnant women.

Fresh 10 ml urine samples were collected from normal and patient women and centrifuged at (1000xg) for 2 min then the activity of alanine aminopeptidase was determined.

### Methods

#### The Activity of Urine Alanine aminopeptidase

Using (Alanine-4-nitroanilid derivatives) as a substrate and measuring the liberated p-nitroanilid according to method described by Jung and Scholz<sup>[12]</sup> then by plotting the standard curve between optical density (OD) of p-nitroanilid (y-axis) with respect to the corresponding concentration values in  $\mu\text{mol/L}$ .

#### Protein Concentration

The total serum protein concentration was determined by following Lowry's method<sup>[13]</sup>, using bovine serum albumin (BSA) as a standard protein.

#### Partially Purification and separation of AAP isoenzymes in urine of normal pregnancy and abortion

Alanine aminopeptidase was purified via two stages as follows:

involved gel filtration chromatography on Sephadex G-50 followed by ion exchange chromatography on DEAE-cellulose according to Jung and Scholz<sup>[12]</sup>.

#### Gel filtration

Gel filtration technique Sephadex G-50 was used to separate APP from urine and the elution started by adding (25) ml of Tris-buffer then collected (6) tubes of eluted fractions contain (5) ml for each fraction the flow rate equals (2.5) ml/min then solution stand at 4°C<sup>[14]</sup>.

#### Ion-Exchange Column Chromatography

Ion-exchange chromatography technique was used to partially purification of AAP isoenzymes from the isolated AAP in previous step, with using DEAE-

cellulose column, and the elution was performed with using Tris-HCL buffer (pH 7.8) and Nacl gradient . The enzyme was eluted with Tris-HCl buffer solution 22ml (50mM, pH 7.8) . Avolume of 2ml of 12 fractions were collected, then enzyme which was retained on the column, was eluted with a linear concentration gradient of sodium chloride (0.1 -0.4) M .

**Sodiumdodecyl sulfate -Polyacrylamide Gel Electrophoresis (SDS-PAGE)**

The purity of different alanine aminopeptidas isoenzymes partially purified in urine samples was detected using polyacrylamide gel electrophoresis according to method of [15] with some modification .

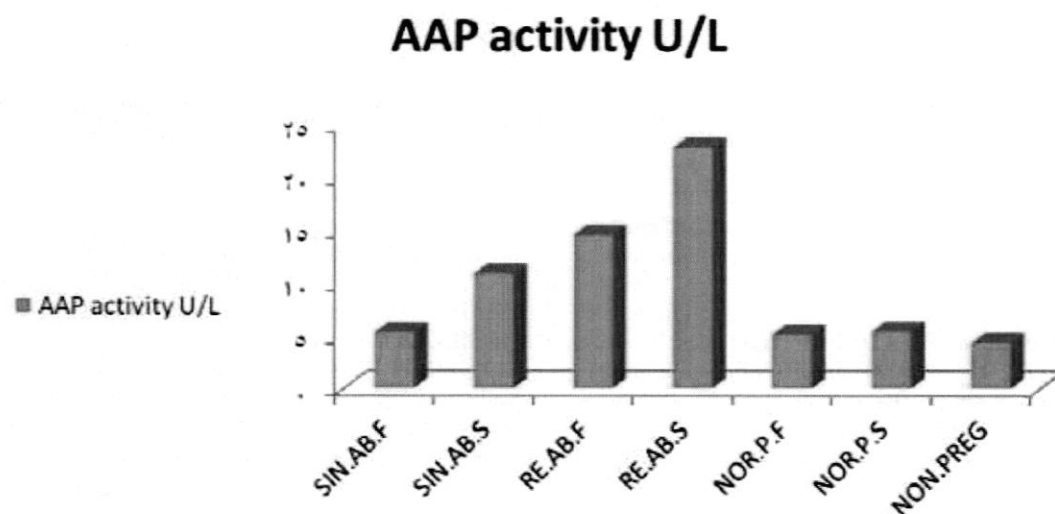
**Results and Discussion**

The results of statistical analysis showed highly significance increase in AAP activity ( $P \leq 0.01$ ) when compared between the groups of women with abortions (single and recurring) and control groups (healthy subjects and non-pregnant women) . The

level of (AAP) in urine of aborted women showed a significant increase when compared to control group (non-pregnant women) ,the highest level of these activities in women with recurrent abortion in the second trimester ( $22.7 \pm 0.72$ ) U/L, while the activity was ( $14.5 \pm 0.39$ ) U/L in women with recurrent abortion in the first trimesters. In the single abortions in the first and second trimesters of pregnancy AAP activity has reached the level of ( $5.28 \pm 0.33$ ) ( $10.85 \pm 0.05$ )U/L, respectively, compared to level of non-pregnant control groups ( $4.3 \pm 0.295$ )U/L and healthy subjects in the first and second trimesters ( $5.1 \pm 0.24$ ) ( $5.4 \pm 0.48$ ) U/L, respectively .The results of statistical analysis showed that this increase was highly significance ( $P \leq 0.01$ )when compared between the groups of women with abortions (single and recurring) and control groups (healthy subjects and non-pregnant women) , as shown in the Table (1) and Fig (1) .

**Table (1) Alanine aminopeptidase level in urine of women with spontaneous abortion, single and recurrent**

Study groups	Number	Mean± SE U/L	Significance
<b>Single abortion</b>	<b>20</b>	<b>5.3±0.33</b>	<b>Highly significance <math>P \leq 0.05</math></b>
-First trimesters	10	10.85±0.05	
<b>Recurrent abortion</b>	<b>24</b>	<b>14.5±0.39</b>	<b>Higly Significance <math>P \leq 0.01</math></b>
-First trimesters	12	22.7±0.72	
<b>Normal pregnancy</b>	<b>15</b>	<b>5.1±0.24</b>	
-First trimesters	10	5.4±0.48	
<b>Second trimesters</b>			
<b>Non-pregnant</b>	<b>15</b>	<b>4.3±0.29</b>	



**Figure (1) Activity of alanine aminopeptidase in urine of women with spontaneous abortion, single and recurrent**

The observed results revealed that AAP are involved in local immune responses of endometrial cell

attachment, maturation / Differentiation , and surface peptidase suggest an important role of endometrial

cell in implantation processes . More recently, CD13 antigen, which has been investigated immunologically, was demonstrated to be identical to AAP<sup>[17,3]</sup>.

The purification scheme of Alanine aminopeptidase (AAP) from the urine of women with spontaneous abortion single and recurrent is represented in Table (2) .

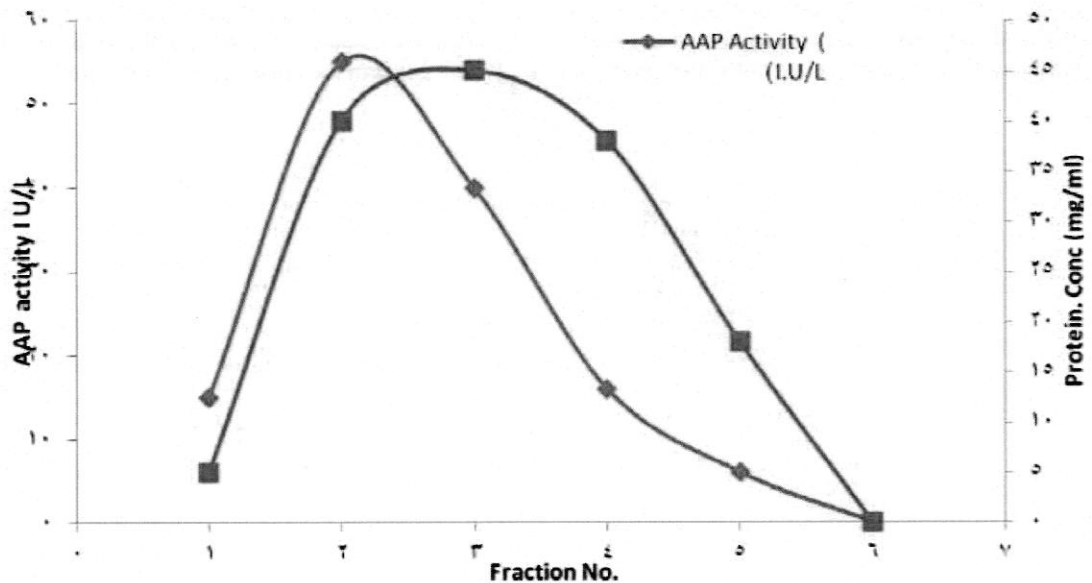
The procedure involved gel filtration chromatography using Sephadex G-50 . The starting specific activity in the crude urine was 0.003mu/mg . protein, and the separation with sephadex G-50 column revealed the presence of one peak of AAP activity with specific activity 0.018 mu/mg.protein.as shown in Fig (2) .

For further purification the fraction of AAP separated above was applied on ion-exchange column (DEAE-Cellulose) which revealed the presence of two peaks of AAP activity ; isoenzyme I eluted with Tris-HCL buffer (pH 7.8) and isoenzyme II eluted with Tris-HCL pH (7.8) with using gradient Nacl conc. (0.1-0.4) Mol/L. as shown in Fig (3) .

So after ion-exchange chromatography on DEAE-Cellulose column , the specific activity of isoenzymes I,II were increased to 0.28 and 0.6 mu/mg. protein respectively , which represented 93 and 200 fold purification over the crude urine with 56% and 43% recovery respectively as shown in Table (2) .

**Table (2) Partially purification of alanine aminopeptidase (AAP) from urine of women with spontaneous abortions single and recurrent**

Step	Eluet (ml)	Protein Conc. µg/ml	Total Protein	Activity mu/ml	Specific Activity mu/mg	Degree of Purification (FOLD)	Yield %
1-Crude urine	10	400	4000	13	0.003	1	100
2-Sephadex G-50	5	170	8500	15.8	0.018	6	82
3-DEAE CELLULOSE							
ISOENZYM I	2.0	40	80	22.9	0.28	93	56
ISOENZYME-II	2.0	25	50	30	0.6	200	43



**Figure (2) partially purification of alanine aminopeptidas (AAP) from aborted women by using gel filtration method**

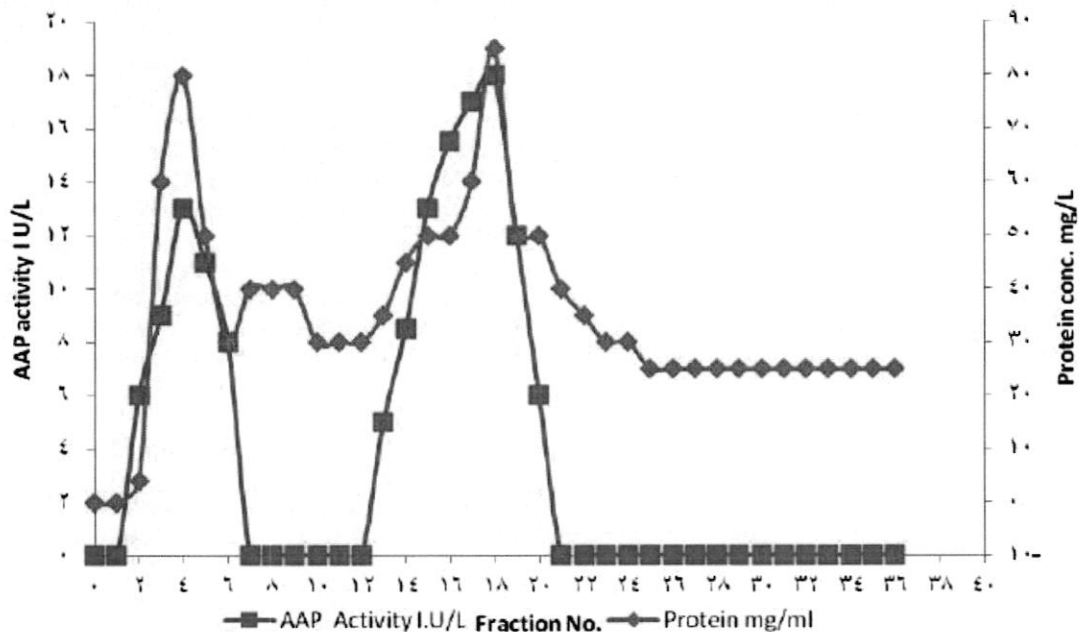
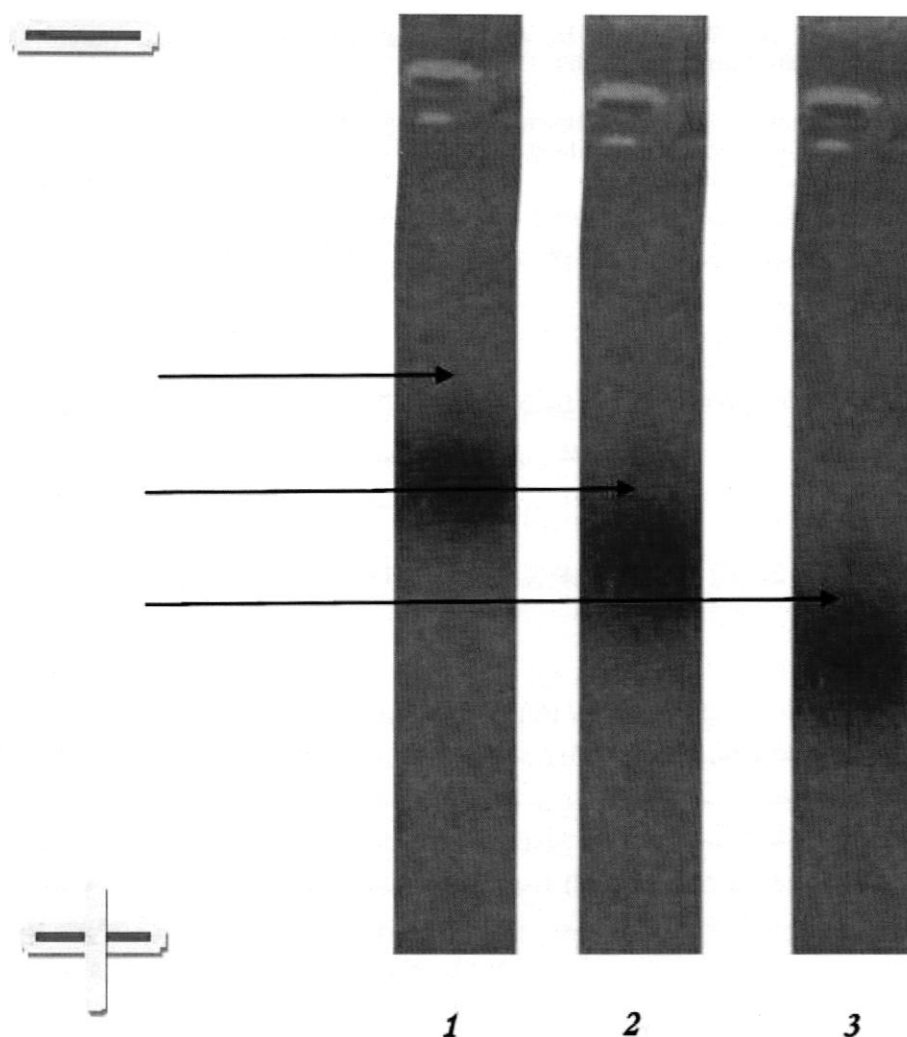


Figure (3) partially purification of alanine aminopeptidase (AAP) from urine of aborted women by DEAE cellulose

It is found that the activity (AAP) in the urine of aborted women has increased after a process of gel filtration using gel SephadexG-50 and this is due to the elimination of undesired substances that reduce the activity of the enzyme in the urine including urea, amino acids and ammonia<sup>[14]</sup> by using SephadexG-50 we noted a single peak of enzyme as shown in Figure (2) . By using DEAE cellulose we noted two peaks of

isoenzyme as shown in Figure (3) . The results of this study agreed with (AL-salihi 2011) observed<sup>[16]</sup> . Samples from the purification steps (Gel filtration &DEAE-Cellulose) were analyzed electrophoretically on SDS-PAGE Fig (4) the isoenzyme pattern contains a single distinct band Which confirm the purity of that isoenzyme pattern contains a single distinct band . Which confirm the purity of that isoenzyme .



**Fig (4) :The electrophoresis of purified AAP: 1-AAP purified by gel filtration 2- isoenzym (I) AAP purified by DEAE 3- isoenzym (II) of AAP purified by DEAE**

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## دراسة كيموحيوية لمتناظرات أنزيم الألدن أمينوبيبتايديز لحالات الأجهاض في تكريت

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### الملخص

الألدن أمينوبيبتايديز AAP وأجريت المقارنة مع مجموعة السيطرة المتكونة من (40) امرأة تضمنت (15) امرأة ذوات حمل طبيعي خلال الأشهر الأولى من الحمل و(10) امرأة ذوات حمل طبيعي خلال الأشهر الثانية من الحمل و(15) امرأة غير حوامل وبالفتات العمرية نفسها وقد أوضحت نتائج هذه الدراسة ارتفاعاً معنوياً بنشاط أنزيم الألدن أمينوبيبتايديز ( $P \leq 0.05$ ) في إدرار النساء المجهضات كافة المفردة ( $5.3 \pm 0.33$ ) ( $0.05$ ) و( $10.85 \pm$ ) وحدة عالمية/لتر والمتكررة عند مختلف مراحل الحمل ( $22.7 \pm 0.72$ ) ( $14.5 \pm 0.39$ ) وحدة عالمية/لتر خلال الأشهر الثلاثة الأولى من الحمل والأشهر الثلاثة الثانية من الحمل على التوالي قياساً بمجاميع السيطرة ( $5.4 \pm 0.48$ ) ( $5.1 \pm 0.24$ ) وحدة عالمية/لتر وغير حوامل ( $4.3 \pm 0.295$ ) وحدة عالمية/لتر. وشملت الدراسة فصل AAP جزئياً من الإدرار بواسطة كروماتوغرافيا الترشيح الهلامي (G-50) ثم تم فصل متناظران ل AAP بطريقة كروماتوغرافيا التبادل الأيوني (DEAE-cellulose) .