# Activity of Several Purine Metabolic Enzymes in Sera of Pregnant Women

**Noor Khalid Zaidan [1], Wisam Mahmood Mohammed [1]**

[1] *Assist. Prof., Dr., College of applied Sciences - University of Fallujah, Iraq*

## Abstract

This study aims to determine the activity of several purine metabolic enzymes in the sera of pregnant women. These enzymes show potential as indicators to follow the progress of pregnancy. The samples of this study were collected from 75 healthy pregnant women. The age range of the sample study was 17–43 years and 25 samples were used as control (non-pregnant women in the normal physiological state). Purine metabolic enzyme activity was compared among the three groups with each other and with control. Results appeared high significantly with $p \leq 0.05$ in group III compared with group I of adenosine deaminase and the control group . Pregnant women showed increase significantly level of 5-nucleotidase (5-NT) and alkaline phosphatase (AlP) enzyme activity, particularly in the groups III of 5-NT and AlP compared with group I of 5-NT and AlP, the control group was found to possess equal 5-NT and AlP .

***Keyword:*** *pregnancy, adenosine deaminase, 5-nucleotidase, and alkaline phosphatase.*

## Introduction

Pregnancy, also known as gravidity or gestation, is associated with the natural physiological changes that involve the feeding and survival of the fetus. Hematological and biochemical parameters reflect these adaptive changes, which are crucial during complications (1). Moreover, the placenta contains enzymes, including adenosine deaminase (ADA), 5-nucleotidase (5-NT), alkaline phosphatase (AlP), and aspartate aminotransferase, which are spread in different body tissues (2). Immunological balancing takes place in pregnancy, in which the maternal immune system has to admit the paternal human leucocyte antigens expressed in the fetus and maintain normal immune efficiency to defend against microorganisms. Immunologically, the human fetus is always treated as an allograft to the pregnant mother. Despite being biological allografts and fetuses not normally being rejected by the maternal immune system, one possible explanation for this phenomenon is that the placental fetal unit is an immunologically distinct site that creates a mechanical barrier that weakens interactions between maternal lymphocytes and fetal tissues and functionally reduces the maternal immune response (3).

The enzymes 5-NT and ADA perform central roles in the adenosine metabolism in healthy persons, with 5′-nucleotidase (5′-NT, EC.3.1.3.5) catalyzing the hydrolysis of the phosphoric ester bonding of 5′-ribonucleotides to the corresponding ribonucleoside and phosphate (4). The main function of the 5′-NT enzyme is the transfer of the group phosphate of AMP to adenosine (5). 5-NT shows cancer progression at multiple levels, with a growing body of evidence suggesting 5-NT implication in neoplastic progression. Moreover, active enzyme activity has been detected in bladder, ovarian, thyroid, and prostate cancers (6).

ADA (EC 3.5.4.4) is important in purine metabolism and involved in the breakdown of dietary adenosine produced from the turnover of nucleic acids in tissues. This ubiquitous enzyme is found in various microorganisms, bacteria, plants, and vertebrates, with a highly conserved amino acid sequence; moreover, it is present in all mammalian cells. ADA, which is highly important for the differentiation of lymphoid cells, has been used for managing several diseases involving changed immunity (7). These enzymes are widely distributed in various organs and body fluids.

The catalytic activity of AlP (EC 3.1.3.1) requires optimum pH (8,9). The dephosphorylation and phosphorylation of serum proteins are regulated by the opposing activities of phosphatases and kinases (10, 11). AlP was determined by Mahesh et al. (2010), who measured the change in activity of alkaline phosphatase in the placenta of diabetic pregnant women; the results showed increased AlP activity in diabetic gestational women (12).

## Methodology

Sera were collected from 75 pregnant women whose ages ranged from 17–43 years (29.0 ± 8. 7, mean ± SD) and residing in Baghdad, Iraq. Complete medical history and physical examinations were conducted by doctors of the advisory clinic in Yarmouk General Hospital.

**Samples collection of the study:** Inclusion criteria of the study exclude women who smoke, with diabetes or any chronic disease, and who drink wine.

**Collection of blood samples:** Blood samples were collected from pregnant women; 5 mL of venous blood was collected into plastic tubes and stored in sterile polyethylene tubes. After serum samples of whole blood were extracted, the sera were used for the determination of enzyme activity and other analyses.

**Determination of total protein:** The sensitive biuret method by Janairo[13] was used to determine the total protein in blood serum, with bovine serum albumin as the standard. Absorption of the colored solution was measured at $\lambda = 545$ nm.

**Determination of the different enzymatic activity:** ADA activity was determined using the method by Giusti and Galanti [15]. ADA activity was expressed using U/L. AlP activity was determined by the calorimetric method. 5¢-NT activity was measured by following Wood and Williams's method (16). This method is based on measuring the liberated phosphate (a blue color will develop from a reaction with stannous chloride/molybdenum solution), which is produced upon the hydrolysis of AMP by 5¢-NT to adenosine. Given that AlP also hydrolyzes this substrate, the amount of phosphate released by incubating AMP with the serum will be the result of the activity of 5¢-NT and ALP. Therefore, nickel chloride was used to inhibit the activity of 5¢-NT, and the difference between that in the absence of nickel ions and in its presence will reflect the 5¢-NT activity.

## Results and Discussion

The results in Figure (1) reveal that serum ADA level in the pregnant group increased significantly ($p \leq 0.05$) in groups II and III compared with that of the control group.

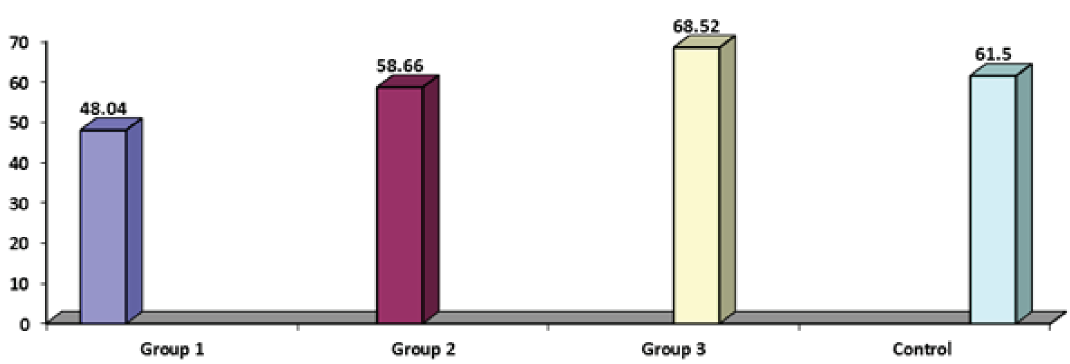


**Figure (1): Adenosine deaminase activity in the sera of pregnant women in the three groups and control group**

In this study, the pregnant women were divided into three groups. The first trimester group included pregnant women in the beginning of their first month to the end of the third month; the second trimester group included the women beginning their third month to those ending their sixth month; and the third trimester group included women from the beginning of their seventh month to those at the end of the ninth month.

Changes in hematological and biochemical parameters were compared among the three trimesters and between the pregnant women trimester groups and control group. Significant difference (at level $p \leq 0.05$) was found in the total account of WBCs, with the highest value being observed in the third trimester group at 10188.00 ± 1659.39 cell/mm$^3$ and the highest value in the control group being 7896.00 ± 1834.27 c/mm$^3$. Hemoglobin (Hb) and packed cell volume (PCV) showed a significantly decrease in the third trimester group (9.77 ± 1.32 g/dL) (30.40 ± 3.89%) compared with the first and second trimester groups (11.15 ± 1.78 and 10.63 ± 1.08 g/dL ; 34.44 ± 5.41% and 33.55 ± 3.58%, respectively). Biochemical parameters tests were found in women's sera, and the results showed non significant differences (at level $p \leq 0.05$) in the concentration of total proteins; moreover, a marked decrease in the level of albumin concentration was observed especially in the second and third trimester groups (0.72 ± 4.90 and 4.69 ± 0.72 g/dL) compared with the control group (non-pregnant women) (5.55 ± 0.56 g/dL); whereas non significant difference was observed in the globin and glucose levels. The results of this study recorded a significantly elevation in the activities of ADA, 5- NT, and AlP (68.52 ± 23.97, 4285.59 ± 2371.17, and 98.80±37.08 U/L, respectively), especially in the pregnant women in their third trimester.

**Table (1):Demographic data of all parameters for all groups**

| | **Mean ± SD** | | | | |
|---|---|---|---|---|---|
| Parameter | **G1 (1–3 month)<br>N = 25<br>Pregnant woman** | **G 2 (4–6 month)<br>N = 25<br>Pregnant woman** | **G3 (7–9 month )<br>N = 25<br>Pregnant woman** | **Control G.<br>N = 25<br>Non-pregnant woman** | **p-value p < 0.005** |
| Hb g/1000 mL | 11.15 ± 1.78 | 10.63 ± 1.08 | 9.77 ± 1.32 | 11.15 ± 1.78 | 0.00 |
| WBCs c/mm3 | 9690.00 ± 1808.37 | 9061.11 ± 1205.32 | 10188.00 ± 1659.39 | 7896.00 ± 1834.27 | 0.00 |
| PCV % | 34.44 ± 5.41 | 33.55 ± 3.58 | 30.40 ± 3.8944 | 40.68 ± 3.49 | 0.00 |
| T. protein g/100 mL | 6.93 ±0.62 | 7.05 ±0.65 | 7.28 ± 1.018 | 7.63 ± 0.83 | 0.456 |
| Albumin g/100 mL | 4.79 ± 0.61 | 4.90 ± 0.72 | 4.69 ± 0.72 | 5.55 ± 0.56 | 0.00 |
| Globulin g/100 mL | 2.15 ± 0.80 | 2.14 ± 0.96 | 2.63 ± 0.96 | 2.08 ± 1.03 | 0.201 |
| Glucose mg/100 mL | 101.16 ± 22.36 | 106.22 ± 21.06 | 111.20 ± 23.33 | 105.15 ± 18.85 | 0.433 |
| ADA (U/L) | 48.04 ± 15.72 | 58.66 ± 17.30 | 68.52 ± 23.97 | 61.50 ± 17.04 | 0.03 |
| 5-NT | 2554.09 ± 1352.85 | 3369.88 ± 1039.35 | 4285.59 ± 2371.17 | 2761.04 ± 1375.61 | 0.00 |
| ALP | 57.18 ± 13.01 | 62.80 ± 15.20 | 98.80 ± 37.08 | 59.35 ± 20.99 | 0.00 |

Results in Figure (2) reveal that serum 5-NT level in the pregnant group increased significantly ($p \leq 0.05$) in groups II and III compared with that of the control group.

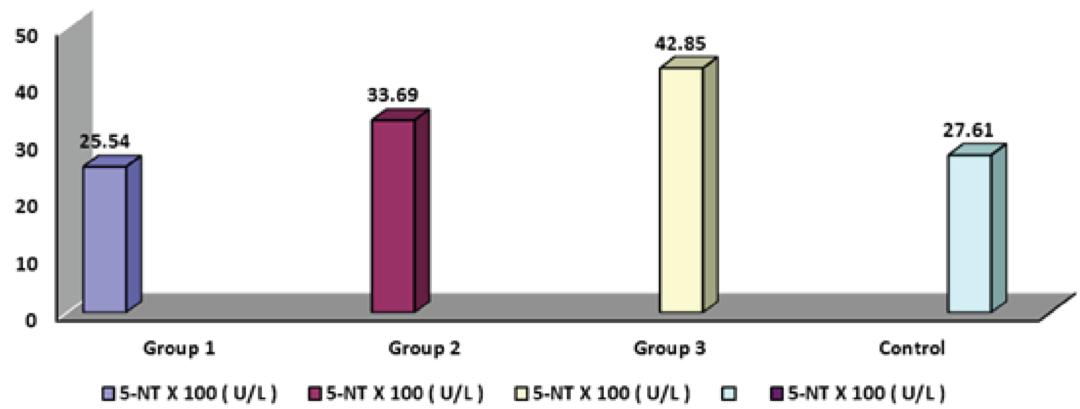


**Figure (2): 5-Nucleotidase activity in the sera of pregnant women in the three groups and the control group**

Figure (3) reveals that AlP activity in the pregnant group was significantly different ($p \leq 0.05$) in groups II and III compared with that in the control group.

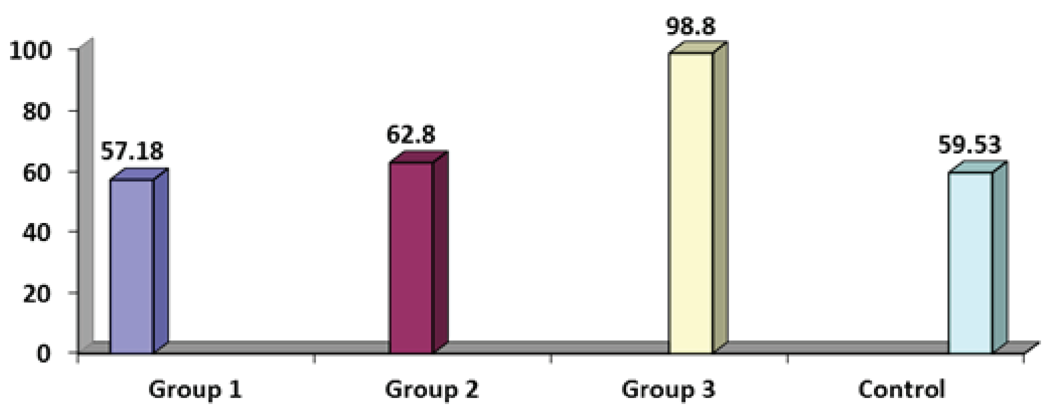


**Figure (3): Alkaline phosphatase in the sera of pregnant women in the three groups and the control group**

Figure (1) refer to the significant variations in the serum ADA activity in the pregnant groups, particularly in group III, compared with those of group I and the control group. The result showed increased ADA activity in pregnant women compared with the control group.

Our result agrees with a previous study [18], which indicated increased adenosine deaminase activity in pregnant women, with the highest levels of ADA found in the placenta during the stages of fetal development. This result suggests an important role of placental ADA during these stages of development. A major function of this enzyme in the placenta may be to prevent the accumulation of substrates that are potentially toxic to the developing embryo. However, these results were not in agreement with the results in this study [19], which showed decreased ADA activity in pregnant women.

Figure (2) refers to the significant variations in the serum 5-NT activity in the pregnant group, particularly in group III, compared with those of group I and control group. The result showed increased 5-NT activity in pregnant women compared with the control group. Our results agree with those in a previous study [20], which reported increased 5′-NT activity in pregnant women. This finding may be due to increased 5-NT activity, which leads to increased adenosine during normal pregnancy and may act as an endogenous compensatory mechanism that diminishes platelet activation and maintains vessel integrity during normal pregnancy.

Figure (3) showed the significantly variations in the serum AlP activity in the pregnant group, particularly in group III, compared with those in group I and control group. Our results agree with those in a previous study [21], which reported increased AlP activity during pregnancy. The AlP levels increase by the third trimester because of placental synthesis, which led to the production of the placental isoenzyme. The activity of 5-NT and AlP significantly increased in the third trimester, being asymptomatic during normal pregnancy and showing supplementary production from placental tissue [13]. The placenta contained AlP and 5-NT, but the spread of 5-NT out of the placenta into maternal circulation may be limited due to the high molecular weight of 5-NT[14].

## Conclusions

Activities of certain metabolic purine enzymes were altered during pregnancy. Activities of the enzymes adenosine deaminase, 5-nucleotidase, and alkaline phosphatase were significantly increased pregnant women, particularly in groups II and III, compared with those in group I and control group.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of applied Sciences and all experiments were carried out in accordance with approved guidelines.

## References

1- Al Tawil SR. Biochemical and hematological profile of normal pregnant women in Gaza Governorate, Gaza strip. Biochemical and Hematological Profile of Normal Pregnant Women in Gaza Governorate, Gaza strip. 2013.

2- Spaans F, de Vos P, Bakker WW, van Goor H, Faas MM. Danger signals from ATP and adenosine in pregnancy and preeclampsia. Hypertension. 2014 Jun;63(6):1154-60.

3- Barton BM, Xu R, Wherry EJ, Porrett PM. Pregnancy promotes tolerance to future offspring by programming selective dysfunction in long-lived maternal T cells. Journal of leukocyte biology. 2017 Apr;101(4):975-87.

4- Sharma J, Menon BK, Vijayan VK, Bansal SK. Changes in Adenosine Metabolism in Asthma. A Study on Adenosine, 5’-NT, Adenosine Deaminase and Its Isoenzyme Levels in Serum, Lymphocytes and Erythrocytes. Open Journal of Respiratory Diseases. 2015 Mar 19;5(02):33.

5- Santos CA, Saraiva AM, Toledo MA, Beloti LL, Crucello A, Favaro MT, Horta MA, Santiago AS, Mendes JS, Souza AA, Souza AP. Initial biochemical and functional characterization of a 5′-nucleotidase from Xylella fastidiosa related to the human cytosolic 5′-nucleotidase I. Microbial pathogenesis. 2013 Jun 1;59:1-6.

6- Sadej R, Skladanowski AC. Dual, enzymatic and non-enzymatic, function of ecto-5’-nucleotidase (en, CD73) in migration and invasion of A375 melanoma cells. Acta Biochimica Polonica. 2012 Nov 16;59(4).

7- Bahadır G, Döventaş YE, Turkal R, Koldaş M, Basınoğlu F, Dane B, Altunkaynak E. Serum adenosine deaminase and its isoenzyme activities in pregnancy. Journal of the Turkish German Gynecological Association. 2011;12(4):209.

8- Khodadadi I, Vahedi MS, Abdi M, Daneshkhah N, Rahbari R, Menbari S, Ahmadi D, Ahmadi A, Lahoorpour F, Hakhamaneshi MS, Javid S. Evaluation of adenosine deaminase (ADA) isoenzymes activity and tumor necrosis factor-α (tnfα) concentration in chronic heart failure. EXCLI journal. 2014;13:58.

9- Lee SJ, Hwang HS, Kim BN, Kim MA, Lee JW, Park YW, Kim YH. Changes in serum adenosine deaminase activity during normal pregnancy. Journal of Korean medical science. 2007 Aug 1;22(4):718-21.

11- Salahat MA, Ibrahim AI. Prevalence of anemia among Jordanian pregnant women and the effect of early pregnancy on alkaline phosphatase activity. Jordan Journal of Biological Sciences. 2012 Mar;147(617):1-6.

12- Mahesh M, Neha G, Rajesh TS, Somashekhar R, Puttaiah ET. Isolation and characterization of extracellular thermostable alkaline phosphatase enzyme from Bacillus spp. International Journal of Applied Biology and Pharmaceutical Technology. 2010;1(1):21-33.

13- Gowda S, Desai PB, Hull VV, Math AA, Vernekar SN, Kulkarni SS. A review on laboratory liver function tests. The Pan african medical journal. 2009;3.

14- Anitha G, Mahaboob R, Obulesu G. Study of adenosine deaminase levels in the diagnosis of tuberculous pleural effusion. Intern. Arch. Of Integrated Medicine. 2016;3(7):353-7.

15- Hameed RM, Mehdi WA, Mehde AA. Biochemical Significance of Ecto-5'-Nucleotidase, Xanthine Oxidase, and Glutathione S-Transferase Determinations in Sera of Cigarette and Water Pipe Young Men Smokers. Medical Journal of Babylon. 2019;16(1):70-6.

16- Allaithi LA, Al-Azawi WM. Study of the effect of salvia officinalis leaves extract and xenical drug on some of the biochemical and histological parameters in the rats induced with hyperlipidemia. Plant Archives. 2019;19(2):1111-22.

17- Guo F, Yang S, Zhang Y, Yang X, Zhang C, Fan J. Nomogram for prediction of gestational diabetes mellitus in urban, Chinese, pregnant women. BMC Pregnancy and Childbirth. 2020 Dec 1;20(1):43.

18- Chowdhury SR, Kamal AH, Barua S, Ullah SA. The Management of Pre-Existing Diabetes in Pregnancy: A Review for Primary Care. Journal of Army Medical College Jashore. 2020 Jan;1(1):21-31.

19- Yoneyama Y, Suzuki S, Sawa R, Otsubo Y, Power GG, Araki T. Plasma adenosine levels increase in women with normal pregnancies. American journal of obstetrics and gynecology. 2000 May 1;182(5):1200-3.

20- Ali DM. Effect normal pregnancy and duration on liver enzymes tests. Global Journal of Medical Research. 2015 Apr 23.

21- Bacq Y, Zarka O, Brechot J, Mariotte N, Vol S, Tichet J, Weill J. Liver function tests in normal pregnancy: a prospective study of 103 pregnant women and 103 matched controls. Hepatology. 1996 May;23(5):1030-4.