Original Article

Saliva as an alternative non-invasive biomarker for the estimation of uric acid levels during pregnancy: A longitudinal study

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Abstract

Background: Pregnancy is a physiological condition in which the maternal environment undergoes many changes. Serum uric acid (UA) levels have been used for the early diagnosis of preeclampsia, predictor of reduced birth weight and fetal outcome. UA is also expressed in saliva, and collection of saliva sample is a noninvasive method which will be more acceptable by the patients.

Aims and Objectives: The present study aimed to estimate and compare serum and salivary UA levels in age-matched healthy nonpregnant and healthy pregnant women at different trimesters longitudinally.

Methodology: Forty female participants with age ranging between 20 and 38 years comprised the study population. The study group consisted of 20 healthy nonpregnant women (controls) and an equal number of confirmed cases of healthy pregnant women in the first trimester (cases). The cases were followed in their second and third trimesters for the sample collection.

Results: Both serum and salivary UA levels were significantly reduced in the first trimester of pregnancy than the controls. In the second and third trimesters, the values of serum and salivary UA levels gradually increased and gained values similar to that of nonpregnant women. Salivary UA levels showed a highly significant positive correlation with serum levels in both controls and cases.

Conclusion: Salivary estimation, being a noninvasive procedure, is easily accepted by the patients and carries minimal risk of exposure to the blood-borne pathogens compared to serum estimation. Our findings warrant the use of saliva instead of blood for UA estimation.

Keywords: Correlation, estimation, longitudinal study, preeclampsia, pregnancy, saliva, serum, trimester, uric acid

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Submitted: 28-Oct-2020, **Revised:** 05-Jul-2021, **Accepted:** 06-Jul-2021, **Published:** 11-Jan-2022

INTRODUCTION

The term "Pregnancy" refers to "the fertilization and development of one or more offspring, known as a fetus or embryo, in a women's uterus."[1] It is associated with many alterations in the metabolic, hematological, biochemical,

Access this article online

Quick Response Code:

Website: www.jomfp.in

DOI: 10.4103/jomfp.jomfp_439_20

How to cite this article: Deepashree PG, Madhushankari GS, Nandini DB, Priya NK, Ashwini R, Shruthy R. Saliva as an alternative non-invasive biomarker for the estimation of uric acid levels during pregnancy: A longitudinal study. J Oral Maxillofac Pathol 2021;25:457-62.

immunological and physiological process that assists the nurturing and survival of the fetus.[2] These are changes occurring during pregnancy which are not seen during normal state. Hence, it is critical to appreciate both the normal and the abnormal changes as laboratory results can influence the management of both the mother and child.[3] As pregnancy advances, the uric acid (UA), a prognostic indicator for the maternal complication, is found to be altered.[4]

UA is an end metabolite of purine metabolism that is synthesized by the enzyme xanthine oxidase.[5] As UA causes vascular damage and oxidative stress, hyperuricemia has evoked as a promotor to the development of preeclampsia.[6]

Preeclampsia, a common obstetric disorder characterized by hypertension and proteinuria, causes higher fetal risk than nonprotein uric hypertension of pregnancy. Preeclampsia may progress to eclampsia which is potentially lethal for both the mother and the fetus.[7] The maternal complications include severe hypertension, eclampsia, hemolysis, elevated liver enzymes and low platelet count while the fetal complications include growth restriction, fetal distress and even perinatal death.[5] These adverse effects in the mother and her fetus develop simultaneously or presumably are a consequence of vasospasm, endothelial dysfunction and ischemia.[6]

Preeclampsia can be detected in the early stages of pregnancy by measuring uricemia while monitoring the same will prevent further maternal complications.[5]

Many salivary components reflect variations similar to those seen in the serum. Quantification of salivary components is an easy, noninvasive procedure with reduced risk of transmitting blood-borne pathogens compared to serum estimation.[8,9] Since UA is also present in saliva,[9] its estimation in pregnant women may be useful. Studies estimating the serum and salivary UA levels in normal healthy pregnant women are limited.[10]

Our aim was to estimate and compare the serum and salivary UA levels in healthy nonpregnant women and healthy pregnant women at three trimesters of pregnancy longitudinally.

METHODOLOGY

A total number of 40 women with an age range from 20 to 40 years were the study participants (n = 40). The study group consisted of age-matched 20 healthy nonpregnant women (controls) and an equal number of confirmed cases of healthy women in the first trimester of pregnancy (cases) reporting to the outpatient Department at City Medical Center and M. K. Memorial Hospital, Davangere. Women were followed in their subsequent trimesters for the sample collection. Before the collection of blood and saliva samples, informed consent was obtained and a detailed case history was recorded. Ethical clearance from the institutional review board was obtained for the study.

Women in the first trimester of pregnancy comprised the cases. Participants with other conditions that may alter the serum and salivary UA levels such as obesity, hypertension, cardiovascular diseases, renal diseases, alcohol use and tobacco use, advanced periodontitis, active oral inflammation, hypoparathyroidism, hyperparathyroidism and medication use such as aspirin and glucocorticosteroids were excluded from the study.

Collection and analysis of blood samples

Under aseptic conditions, 2 ml of venous blood was collected in a sterile vial without ethylenediaminetetraacetic acid. The samples were carried immediately to the laboratory in a vaccine carrier. The blood sample was transferred into a sterile test tube and allowed to clot. The test tube was then centrifuged at 3000 rpm for 5 min to obtain the supernatant. The serum was used for the UA estimation using a semiautomatic biochemical analyzer (Transasia biomedicals Pvt. Ltd, India).

Collection and analysis of saliva samples

Patients were requested not to drink or eat 90 min before the salivary sample collection. Patients were instructed to rinse their mouth with water before sample collection. Five milliliter of unstimulated whole salivary sample was obtained in a sterile container by spitting for 10 min. The samples were carried immediately to the laboratory in a vaccine carrier. The sample was centrifuged at 3000 rpm for 10 min, and the resulting supernatant was used for UA estimation by a semiauto biochemical analyzer.

Uric acid estimation

UA estimation in the serum and saliva samples was done by the enzymatic colorimetric method. One milliliter of UA reagent was taken in a separate test tube and 25 μl of serum or saliva was added to this. The sample was mixed and kept in the incubator for 10 min at 37°C. Then, using a semiautomatic biochemical analyzer, reading was recorded in mg/dl. Reference values for UA were 3.5–7.2 mg/dl for males and 2.6–6.0 mg/dl for females according to the kit manual (Labcare diagnostics Pvt. Ltd, India). This was considered as the normal value.

Statistical analysis

The data were tabulated and results were subjected to appropriate statistical analysis. Paired *t*-test was used for intergroup comparison. Pearson's correlation coefficient was done to assess the association between UA levels in serum and saliva.

RESULTS

The present study comprised of 40 participants with 20 nonpregnant women as controls and 20 pregnant women at different trimesters as cases in the age range of 20–40 years.

Serum and salivary uric acid in controls and cases at different trimesters

The mean serum level in controls was 4.18 ± 0.86 mg/dl and in cases, in the first, second and third trimester, it was 3.46 ± 0.70 mg/dl, 4.0 ± 1.05 mg/dl and 4.59 ± 1.00 mg/dl, respectively. The mean salivary UA level in controls was 3.19 ± 1.03 mg/dl and in cases, in the first, second and third trimester, it was 2.12 ± 0.79 mg/dl, 2.66 ± 0.67 mg/dl and 3.38 ± 0.70 mg/dl, respectively [Figure 1].

Intergroup comparison of serum levels in controls and cases at different trimester

The mean serum level in controls was 4.18 ± 0.86 mg/dl and in cases, in the first trimester, it was 3.46 ± 0.70 mg/dl. The difference was statistically significant between the two groups ($P = 0.007$) [Figure 2].

The mean serum level in controls was 4.18 ± 0.86 mg/dl and in cases, in the second trimester, it was 4.0 ± 1.05 mg/dl. The difference was not statistically significant ($P = 0.57$).

The mean level in controls was 4.18 ± 0.86 mg/dl and in cases, in the third trimester, it was 4.59 ± 1.00 mg/dl. The difference was not statistically significant ($P = 0.17$).

Intergroup comparison of salivary levels in controls and cases

The mean level in controls was 3.19 ± 1.03 mg/dl and in cases, in the first trimester, it was 2.12 ± 0.79 mg/dl. The difference was found to be statistically highly significant ($P = 0.001$) [Figure 3].

The mean salivary UA level in controls 3.19 ± 1.03 mg/dl and in cases, in the second trimester was 2.66 ± 0.67 mg/dl. The difference was not found to be statistically significant ($P = 0.06$) [Figure 3].

The mean salivary UA level in controls was 3.19 ± 1.03 mg/dl and in cases, in the third trimester was 3.38 ± 0.70 mg/dl. The difference was not found to be statistically significant ($P = 0.50$).

Intragroup comparison of serum and salivary levels in different trimesters of pregnancy in cases

The mean serum levels of cases at the first, second and third trimester were 3.46 ± 0.70 mg/dl, 4.0 ± 1.05 mg/dl and 4.59 ± 1.0 mg/dl, respectively. This infers that as the pregnancy progressed, serum UA levels increased [Table 1]. The mean salivary levels of cases at the first, second and third trimester were 2.12 ± 0.79 mg/dl, 2.66 ± 0.67 mg/dl and 3.38 ± 0.70 mg/dl, respectively. This infers that as the pregnancy progressed, UA levels in saliva also increased [Table 2].

No significant difference in the serum UA levels between the first and second trimesters ($P = 0.165$) and the second and third trimesters ($P = 0.127$) was observed. However, between the first trimester and third trimester, there was a statistically highly significant difference ($P = 0.001$) [Table 1].

There is no significant difference in the salivary UA levels between the first and second trimesters ($P = 0.061$). However, between the first and third trimesters, a highly significant difference was seen ($P = 0.000$). Moreover, between the second and third trimesters, a significant difference was seen ($P = 0.007$) [Table 2].

Correlation of levels in serum and saliva among cases and controls

In the first trimester, as the serum levels increased, the salivary levels also increased with $r = 0.506$, which was statistically significant.($P < 0.05$) In the second trimester, as serum UA levels increased, salivary UA levels also increased with $r = 0.726$, which was statistically highly significant ($P < 0.001$). In the third trimester, as serum UA levels increased, salivary UA levels also increased with $r = 0.695$, which was statistically highly significant ($P \leq 0.001$) [Table 3]. A linear positive correlation was noticed between the serum and salivary levels in the pregnant women at different trimesters [Figure 4].

DISCUSSION

Pregnancy is a normal state of physiology that assists the nurturing and survival of the fetus.

Many changes occur during pregnancy such as renal function, carbohydrate and protein metabolism and hormonal pattern. These changes may be assessed by biochemical estimation which helps in differentiating from the nonpregnant state. It is critical to appreciate both

normal and abnormal changes as laboratory results can influence the management of both mother and child.[3] During normal pregnancy, as pregnancy advances, the UA, a prognostic indicator for the maternal complication, is found to be altered.

UA is the end byproduct of purine metabolism and is synthesized by the enzyme xanthine oxidase. It can promote inflammation, oxidative stress and vascular damage that could promote hypertension, vascular disease and renal disease. Higher oxidative stress and reactive oxygen species production have been proposed as a contributing source of hyperuricemia noted in preeclampsia apart from renal dysfunction.[11]

Saliva is often called "the mirror of health of the organism" since it reflects the current physiological condition of the body.[9] In recent years, saliva is a well-known diagnostic fluid in the clinics and for the research purpose. The advantages of obtaining saliva are ease of collection, quick availability, painless noninvasive procedure compared to blood collection.[9,12,13] This makes saliva, a unique and ideal specimen of choice for investigation and diagnosis of many physiologic and pathologic conditions.

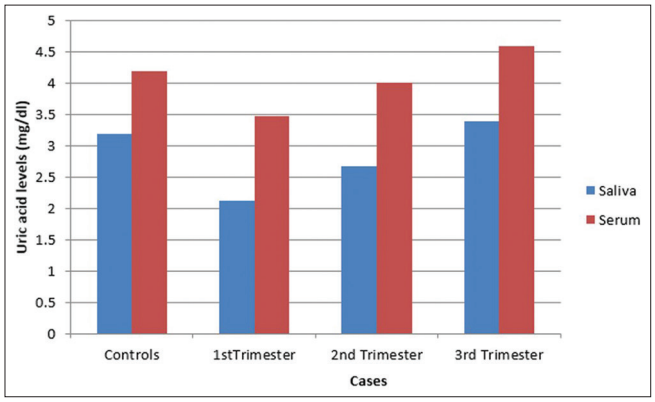

Figure 1: Graph showing serum and salivary uric acid levels in controls and cases at different trimesters

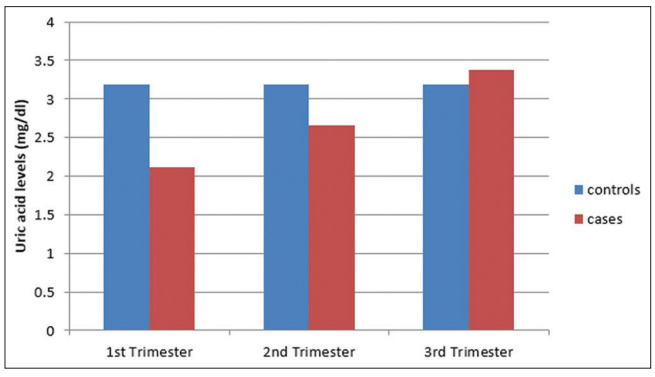

Figure 3: Graph showing the comparison of salivary uric acid levels in controls and cases at different trimesters

The primary antioxidant constituents of saliva are UA, albumin and ascorbic acids.[8] Among them, the UA acts as a dominant nonenzymatic antioxidant present in saliva[8] which is directly affected by the systemic oxidative stress.[12]

The suggestion of human salivary UA being imported from plasma is since the salivary UA correlates with levels in plasma.[12] As saliva exchanges, a few substance existing in human serum including UA makes it an ideal reason for its use as a potential specimen for diagnosis.[8]

A thin layer of epithelial cells separates the circulatory system from the salivary ducts and this is where the exchange occurs between serum and saliva.[9] This interchange of substances occurs due to diffusion across the cell membrane by passive diffusion directed by the concentration gradient and active transport.[9]

In our study, when the mean serum levels between cases and controls were compared, the mean serum UA levels in the cases during the first trimester (3.46 ± 0.70 mg/dl) and second trimester (4.0 ± 1.05 mg/dl) were decreased in comparison to the controls (4.18 ± 0.86 mg/dl) with the $P = 0.007$ and $P = 0.57$, respectively [Figure 1]. This finding was in agreement with other studies.[6,14-16] This

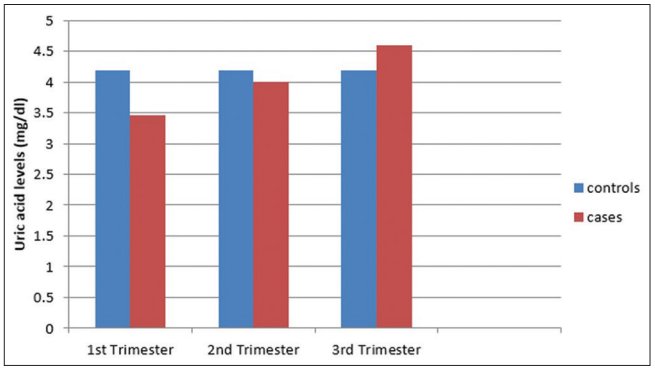

Figure 2: Graph showing the comparison of serum uric acid levels in controls and cases at different trimesters

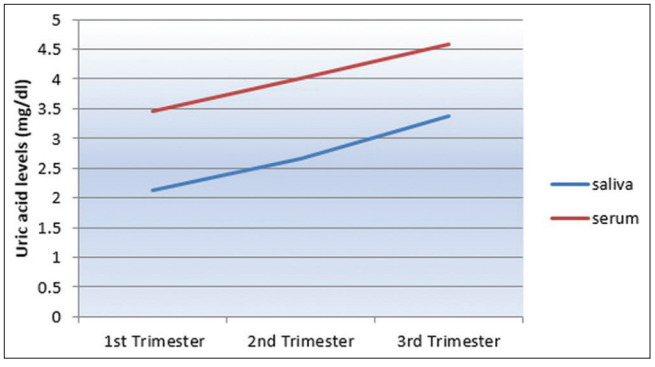

Figure 4: Graph showing the correlation between serum and salivary uric acid levels at different trimesters

Table 1: Intragroup comparison of serum uric acid levels in different trimesters of pregnancy

	Pregnancy	UA levels (mg/dl)
Serum	1st trimester	3.46±0.70
	2nd trimester	4.0±1.05
	3rd trimester	4.59±1.0
	F=0.001, *P*=0.001, (HS) 3≥2≥1 or 3>1	
Intragroup comparison	1st trimester versus 2nd trimester	0.165 (NS)
	1st trimester versus 3rd trimester	0.001 (HS)
	2nd trimester versus 3rd trimester	0.127 (NS)

P=0.05. S: Significant. NS: Not significant, HS: Highly significant, UA: Uric acid

Table 2: Intragroup comparison of salivary uric acid levels in different trimesters of pregnancy

	Pregnancy	Mean salivary UA levels (mg/dl)
Saliva	1st trimester	2.12±0.79
	2nd trimester	2.66±0.67
	3rd trimester	3.38±0.70
	F=15.049, 3>2≥1	
Intragroup comparison	1st trimester versus 2nd trimester	*P*=0.061 (NS)
	1st trimester versus 3rd trimester	*P*=0.000 (HS)
	2nd trimester versus 3rd trimester	*P*=0.007 (S)

P=0.05. S: Significant. NS: Not significant, HS: Highly significant, UA: Uric acid

Table 3: Correlation between serum and salivary uric acid levels at different trimesters

Serum	Saliva		
	1st trimester (*r*, *P*)	2nd trimester (*r*, *P*)	3rd trimester (*r*, *P*)
1st trimester	0.506, <0.05 (S)	-	-
2nd trimester	-	0.726, <0.001 (HS)	-
3rd trimester	-	-	0.695, ≤0.001 (HS)

P=0.05. S: Significant. *r*: Correlation coefficient, HS: Highly significant

finding is due to the alterations in the renal handling of UA which results in an elevation in the clearance of UA by the kidneys secondary to increase glomerular filtration rate and filtered load with reduced tubular reabsorption and the uricosuric action of estrogen during pregnancy.[17]

Intergroup comparison showed that the mean serum level in the cases during the third trimester (4.59 ± 1.00 mg/dl) was higher than the control group (4.18 ± 0.86 mg/dl) and was statistically insignificant ($P = 0.17$) [Figure 2] which was also documented in other studies.[5,14,15]

The serum levels in the second trimester (4.0 ± 1.05 mg/dl) were raised when compared to the first trimester (3.46 ± 0.70 mg/dl) of pregnancy [Table 1] which was in agreement with many studies.[5,13-15,18] This is because, during this stage of pregnancy, significant transfer of UA from the growing fetus to the maternal bloodstream begins to occur,[16] and there will be increased tubular reabsorption with falling renal clearance of UA.[5,11,15,17]

Contrary to our results, the studies done by Prakash *et al*.[6] and Boyle *et al*.[16] found that serum levels reduced in cases during the second trimester in comparison to the cases of the first trimester of pregnancy which they suggested was due to further increased UA clearance by the kidneys.[16] This contrary finding is probably caused by the variation in the time of pregnancy when the sample was collected. The sample was collected in the later days of the second trimester in our study whereas they could have collected the sample in the early days of the second trimester.

Intragroup comparison between the first trimester and third trimester showed a statistically highly significant difference ($P = 0.001$) and no difference between the first and second as well as second and third trimesters [Table 1].

On intergroup comparison, the mean salivary level in cases during the first trimester (2.12 ± 0.79 mg/dl) and second trimester (2.66 ± 0.67 mg/dl) was decreased compared to the controls (3.19 ± 1.03 mg/dl) [Figure 3]. There existed a highly significant difference among cases in the first trimester and controls ($P = 0.001$), and no difference was found between the cases in the second trimester and the controls ($P = 0.06$). The mean salivary level in cases in the third trimester (3.38 ± 0.70 mg/dl) was increased when compared to the control group (3.19 ± 1.03 mg/dl) and found to be statistically insignificant ($P = 0.50$).

Intragroup comparison of mean salivary UA level revealed statistically highly significant difference ($P = 0.000$) between the first and third trimesters, a significant difference ($P = 0.007$) between second and third trimesters and no difference between the first and second trimesters [Table 2].

A significant positive correlation was detected between serum and saliva UA levels in both cases and controls [Figure 4 and Table 3]. Studies estimating salivary UA levels in pregnancy are limited in published literature for comparison. We could not find any study conducted longitudinally in the published literature in India. Thus, the present study is the first longitudinal study conducted in India to correlate serum and salivary UA levels in the same healthy pregnant women at all trimesters.

Similar to our findings, Singh *et al*. recently found that salivary UA had a linear correlation with serum UA levels in women with preeclampsia.[19] However, their study was a cross-sectional study. The authors suggested that salivary UA levels could serve as an index for severity of preeclampsia and also provided a salivary UA cutoff value of 3.350 mg/dl which was predicted to be 78% sensitive and 73% specific.[19]

CONCLUSION

A significant positive correlation was detected between UA levels in serum and saliva in both the study groups, suggesting that saliva reflects the changes in serum levels and could be reliably used as an alternative to serum for clinical monitoring UA levels in pregnancy and for preventing the complications associated with it.

Future scope and limitation

Further studies with a large population need to be undertaken to validate salivary UA in predicting preeclampsia at the early stage and avert adverse outcome. The participants were not segregated concerning to diet which can be considered in future studies.

Acknowledgment

We thank the Outpatient Department of City Medical Center and M.K. Memorial Hospital, Davangere, Karnataka, India, for their assistance in carrying out the study, Dr. Naveen Kumar P.G., Department of Public Health Dentistry for statistical analysis, and Mr. Kumar, laboratory technician, College of Dental Sciences, Davangere, Karnataka, India, for the technical help.

Financial support and sponsorship

This study was financially supported by College of Dental Sciences, Davangere, Karnataka, India.

Conflicts of interest

There are no conflicts of interest.

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