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Original research article

Chromatographic screening of aminoaciduria in pregnant women attending a tertiary care center

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Abstract

Background: Aminoaciduria is one of the most common urinary abnormalities during pregnancy. The aim of this study was to assess aminoaciduria among pregnant women by using paper chromatography, which is performed by standard procedure.

Materials and Methods: A total of 60 urine samples were analysed, out of these, 20 were from pregnant women and 40 were from non-pregnant women.

Results: The mean age of the study participants was 27.6 + 4.25 years. There was no statistically significant difference between the mean age for the pregnant (27.05 + 4.32) and the non-pregnant group (26.88 + 4.24) (p-value>0.05). The mean RF values of the urine samples tested from the pregnant women were 0.5, 0.6, and 0.8, respectively.

Conclusions: The present study shows that pregnancy stimulated an increased excretion of amino acids in urine due to increased GFR and low threshold of reabsorption, but the degree of stimulation varied for amino acids.

Keywords: Chromatography, renal threshold, aminoaciduria, pregnancy

Introduction

Aminoacids in the blood are filtered through glomerular membranes but normally are reabsorbed in the renal tubules by saturable transport systems. The mechanism of reabsorption is an active transport system dependent on membrane-bound carriers and the intraluminal Na+ concentration. When the transport mechanisms become saturated or are defective, aminoacids spill into the urine, resulting in a condition known as Aminoaciduria [1]. Three types of aminoaciduria have been identified: 1. Overflow aminoaciduria: It occurs, when the plasma concentration of one or more aminoacids exceeds the renal threshold (capacity of reabsorption). 2. Renal aminoaciduria: This occurs, when plasma concentrations are normal, but the renal tubular reabsorption system has a congenital or acquired defect. 3. No-threshold aminoaciduria: It occurs, when excessive amounts of an aminoacid, arising from an inherited metabolic block is present in urine, but plasma concentrations are essentially normal because all aminoacids are excreted, note that no-threshold aminoaciduria, such as homocystinuria, are not due to congenital or acquired kidney defects, but are due solely to saturation of the normal renal tubular reabsorption system [2].

In the urine of normal adults, glycine usually is the dominant fraction. renal threshold of many substances is lowered during pregnancy, amino acids, such as lysine, tyrosine, histidine, and phenylalanine are present in urine [3]. Aminoaciduria may be primary or secondary: Primary cause is due to: An inherited enzyme defect, also called as an inborn error of metabolism [2]. The defect is located either in the pathway by which specific amino acid is metabolized or in the specific renal tubular transport system by which amino acid is reabsorbed. The secondary cause is due to: 1. Diseases of an organ, such as liver, which is an active site of amino acid metabolism. 2. Generalized renal tubular dysfunction. 3. Protein-energy malnutrition. 4. Lowered renal threshold in pregnancy.

This present study was done to assess aminoaciduria among pregnant females by using paper chromatography, which is performed by standard procedure. This is demonstrated by the separation of amino acids and determining their RF values using paper chromatography. Early possible detection of any abnormal parameter will help in the diagnosis of aminoaciduria which will help in the prevention of complications.

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The following amino acids are detected in the urine of pregnant women:

1. Arginine 2. Phenylalanine. 3. Tyrosine. 4. Histidine. 5. Lysine. 6. Methionine. 7. Tryptophan. 8. Proline [4-6]

Materials and Methods

The present study was carried out in the Department of Biochemistry, Shadan Institute of Medical Sciences, Hyderabad. The relevant data is gathered from the Department of obstetrics, Shadan Hospital and Research Centre during the year 2010. The present study includes 20 cases of pregnant women and 40 normal females who serve as the control group. The study was conducted following the ethical guidelines and institutional ethics committee approval was obtained for data collection. A fully informed voluntary consent was taken from study participants and those not willing for consent were not included in the study.

Materials

1. Stationary phase: Whatman 3mm paper 2. Mobile phase: solvent system 3. Aminoacid solution 4. Ninhydrin reagent 0.2%

Method

Determination of the presence of aminoacid in urine by ascending chromatography. this can be conveniently performed on 250x250 mm sheets in plastic frames using glass tanks (Shandon, universal frame chromo tank) [7-8].

Principle

Paper chromatography is a simple and widely used technique, which is employed in separating closely related compounds from a mixture. It is based on the principle of partitioning compounds (aminoacids) to be separated between two solvent phases.

Reagents

1. Solvent: n-butanol: acetic acid: water12: 3: 5 (v/v) ratio. Mix, allow to settle, and discard the lower layer (use it for saturating the chamber), the upper layer is used as a solvent. 2. Visualizing agent: 0.5% ninhydrin in acetone. 3. Standard amino acid solution: 0.2% solution of amino acid.

Preparation of reagents: 1. Solvent system: for 200 ml butanol-120 ml acetic acid - 30 ml water 50 ml 2. Ninhydrin reagent: Dissolve 250 mg up to 100 ml acetone. (if required 10 mg can be included to enhance colour produced with iminoacids). Ninhydrin reagent (1,2,3- tri keto hydrindene hydrate) reacts with alpha-substituted amino acids to form a coloured product. Reference mixture: Dissolve selective amino acid up to 10 ml distilled water. use 1 microlitre of reference mixture. Whatman filter paper contains 90% cellulose, these cellulose fibres tightly capture moisture through hydrogen bonds.

Sample

Urine: 5ml of a clean, early morning, the fasting specimen was collected from each study participant.

Procedure

Urine (not desalted) containing 4micro grams of creatinine is applied as a small spot at the origin, drawn 25mm from the bottom of a 250 x 250 mm sheet of Whatman 3mm paper (a) pattern for ascending chromatography, or 10cm from the bottom of a length of 3mm paper for descending chromatography. Develop overnight in the freshly prepared solvent. Dry the papers at room temperature and locate amino acids by dipping them in the ninhydrin reagent followed by heating at 75°c for 5 min. Most amino acids produce red-purple spots, proline and hydroxyproline stain yellow, or blue if isatin is used in the ninhydrin reagent. Secondary staining of chromatograms can be used to detect histidine or citrulline and tryptophan standard amino acid mixtures can be run as markers to allow semi-quantitative estimation of concentration [7-9].

Calculations

$$\text{Retention Factor (RF) value} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent.}}$$

Standard RF values: (1). Arginine: 0.20 (2) Phenylalanine: 0.68 (3) Tyrosine: 0.45 (4) Histidine: 0.11 (5) Lysine: 0.14 (6) Methionine: 0.55 (7) Tryptophan: 0.66 (8) Proline: 0.43.

Results

Data Analysis: SPSS software (version 14) was used for analysis. Continuous data were handled using mean ± standard deviation. Categorical variables were analyzed using chi‑ square test. All analyses were two tailed, and $p< 0.05$ was considered to be statistically significant.

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A total of 60 urine samples was analysed, out of these 20 were from pregnant women and 40 were from non-pregnant women. The mean age of the study participants was 27.6 ± 4.25 years. There was no statistically significant difference between mean age for the pregnant (27.05 ± 4.32) and non-pregnant group (27.88 ± 4.24) (p-value>0.05). All the participants in both groups were apparently and clinically reported not to have any diseases.

Aminoaciduria: 20% of pregnant women reported having aminoaciduria. There was no amino acid detected in the urine samples tested from non-pregnant women. Out of the 4 samples that tested positive for aminoaciduria 2 (50%) had Arginine and another 2 (50%) had histidine.

Table 1: Paper Chromatography results for aminoaciduria among pregnant women

S. No.	Pregnancy (yes/No)	Age	Month of gestations	Amino acid detected	Rf Value
1	Yes	23	8	Nil	N/A
2	Yes	20	8	Nil	N/A
3	Yes	26	8	Nil	N/A
4	Yes	27	8	Nil	N/A
5	Yes	30	8	Nil	N/A
6	Yes	25	8	Arginine	0.11
7	Yes	22	8	Nil	N/A
8	Yes	30	8	Nil	N/A
9	Yes	25	8	Nil	N/A
10	Yes	29	8	Nil	N/A
11	Yes	26	9	Histidine	0.11
12	Yes	32	9	Nil	N/A
13	Yes	30	9	Nil	N/A
14	Yes	33	9	Nil	N/A
15	Yes	34	9	Nil	N/A
16	Yes	20	9	Arginine	0.11
17	Yes	23	9	Nil	N/A
18	Yes	29	9	Nil	N/A
19	Yes	24	9	Nil	N/A
20	Yes	33	9	Histidine	0.10

Discussion

Urination is one of many bodily processes that undergo changes during pregnancy. The changes in urine can indicate health issues during pregnancy. Therefore, it is crucial to identify these changes in urinary parameters to help with the diagnosis and treatment of prevalent medical issues during pregnancy, such as hypertension, preeclampsia, gestational diabetes, and UTIs [10].

According to the data, a slight rise in urinary protein is normal due to an increase in the glomerular filtration rate (GFR), which may be greater than the capacity of the renal tubules to reabsorb protein [11]. Aminoaciduria is a potential indicator of preeclampsia [12]. In our study aminoaciduria reflecting proteinuria during pregnancy was reported among 20% of the study participants. However, none of our subjects had neither hypertensive disorder nor preeclampsia or eclampsia, which is contrary to earlier studies [12]. Pregnancy stimulated an increased excretion of amino acids in urine due to increased GFR and low threshold of reabsorption, but the degree of stimulation varied for amino acids [13]. This requires further research to evaluate the prevalence of asymptomatic aminoaciduria among pregnant women with a larger sample for both quantitative and qualitative biochemical analysis of urine.

References

1. Hytten FE, Cheyne GA. The aminoaciduria of pregnancy. BJOG: An International Journal of Obstetrics & Gynaecology. 1972 May;79(5):424-32.
2. Hollerman CE, Calcagno PL. Aminoaciduria-renal transport. American Journal of Diseases of Children. 1968 Feb 1;115(2):169-78.
3. Dunstan RH, Sparkes DL, Macdonald MM, De Jonge XJ, Dascombe BJ, Gottfries J, *et al*. Diverse characteristics of the urinary excretion of amino acids in humans and the use of amino acid supplementation to reduce fatigue and sub-health in adults. Nutrition journal. 2017 Dec;16(1):1-1.
4. Dietzen DJ. Amino acids, peptides, and proteins. In: Rifai N, ed. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 6th ed. St Louis, MO: Elsevier. 2018, 28.
5. Kliegman RM, St. Geme JW, Blum NJ, Shah SS, Tasker RC, Wilson KM. Defects in metabolism of amino acids. In: Kliegman RM, St. Geme JW, Blum NJ, Shah SS, Tasker RC, Wilson KM, eds. *Nelson Textbook of Pediatrics*. 21st ed. Philadelphia, PA: Elsevier; 2020, 103.
6. Riley RS, McPherson RA. Basic examination of urine. In: McPherson RA, Pincus MR, eds. Henry's Clinical Diagnosis and Management by Laboratory Methods. 23rd ed. St Louis, MO: Elsevier; 2017, 28.
7. Block RJ, Le Strange R, Zweig G. Paper Chromatography: A Laboratory Manual. Elsevier; 2013 Sep

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3.
8. Wilson ID. Paper Chromatography. C. Poole, M. Cooke. 2000:397-404.
9. Smith I. editor. Chromatography. Elsevier, 2013 Oct 22.
10. Navarro A, Sison JM, Puno R, Quizon T, Manio LJ, Gopez J, *et al*. Reducing the incidence of pregnancy-related urinary tract infection by improving the knowledge and preventive practices of pregnant women. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2019 Oct 1;241:88-93.
11. Chappell LC, Shennan AH. Assessment of proteinuria in pregnancy. bmj. 2008 May 1;336(7651):968-9.
12. Bibbins-Domingo K, Grossman DC, Curry SJ, Barry MJ, Davidson KW, Doubeni CA, *et al*. Screening for preeclampsia: US preventive services task force recommendation statement. Jama. 2017 Apr 25;317(16):1661-7
13. Miller S, Ruttinger V, Macy IG. Urinary excretion of ten amino acids by women during the reproductive cycle. Journal of Biological Chemistry. 1954;209:795-801.