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RESEARCH ARTICLE

SCREENING OF SERUM AMYLASE AND LIPASE STATUS IN PREGNANT WOMEN

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ABSTRACT

Background: Pregnancy is a dynamic process characterized by dramatic physiological changes. The knowledge of blood parameters is important for assessing the physiological status and health of an individual. Pancreatic enzymes - lipase and amylase play an important role in lipids and carbohydrates metabolism.

Aims and Objectives: This study is aimed at screening the status of amylase and lipase in healthy pregnant female attending anti-natal routine at Vasundhra Hospital and Fertility Research Centre, Jodhpur - India. The level of lipase and amylase were estimated.

Materials and method This is a cross-sectional, hospital based study involving 50 subjects, out of which of 35 were pregnant and 15 nonpregnant all aged from 18 to 55 years, age – matched pregnant and nonpregnant women of jodhpur (devoid of diabetes, urinary tract infections, renal or liver disorders), attending anti-natal Department of Vasundhra Hospital and Fertility Research Centre, India, during the period of February 2016 to May 2016. The data were collected following standard procedures and statistical analysis was done using ANOVA and Pearson correlation.

Results and Discussion: All the adults age ranges at 18-55. There is statistically insignificant different in serum level of pancreatic enzymes in pregnant women (117.22±20.52: 44.44±16.13) when compared with nonpregnant ones (120.09±13.37: 37.63±3.34) for lipase and amylase respectively, as such, pancreatic enzymes were found to be within the normal ranges and there are no marked differences between the study groups ($p>0.05$).

Conclusion: Majority of the screened pregnant women attending VHFRC while conducting this study are at no risk of developing acute pancreatitis, because the levels of the pancreatic enzymes estimated were at normal level as such there is no risk of pancreatic disease.

INTRODUCTION

Pregnancy is a period marked by profound changes in a woman's hormonal status and metabolism (Zavalza-Go´mez *et. al.*, 2008).Biochemical parameters reflect these adaptive changes and are clearly distinct from the non-pregnant state (Tran, 2005). With prevalent increase inproblems associated with pregnancy, which is detrimental to the mother and foetus, a lot of effort has to be brought forward in combating the problems. Some of these underlying problems are associated with body systems and their coordination. Others are related specifically to some organs. Gastric function status during pregnancy can be adequately assessed by the levels of serum amylase and lipase.

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Laboratory testing of serum amylase and/or lipase levels are central to the diagnosis of acute pancreatitis (AP) as direct indication of its manifestation (Jasdanwala and Babyatsky, 2015). AP is an inflammatory condition of the pancreas which is usually reversible and resolves without causing any structural disruption. But the severity of the disease varies from a mild self-limiting illness to a catastrophic event causing multi-organ failure, sepsis leading to death (Murgod *et al.,* 2014). AP is most often diagnosed clinically and by blood tests rather than expensive radiological methods (Murgod *et al.,* 2014). Although earlier there are some findings which suggested trypsin and lipase serum assays to be more reliable indices of pancreatic exocrine funcion (Benini *et al.,* 1987), laboratory testing of serum amylase or lipase levels or both are central to the diagnosis of AP, as they are easy, quick, inexpensive, reliable and probably the only objective criteria available at the bedside at the time of initial presentation

(Jasdanwala and Babyatsky, 2015). Lipases (triacylglycerol acyl hydrolases, EC 3.1.1.3) catalyze the hydrolysis and the synthesis of esters formed from glycerol and long-chain fatty acids (Sharma *et al.,* 2001). Amylase or α-Amylase, (1,4-α-D-Glucan glucanohydrolase (EC 3.2.1.1) is one of the enzyme that is produced by exocrine pancreas and salivary gland that hydrolyses starch and usually is rapidly cleared by kidney. The isozymes comprise ptyalin or S-type, (S-AMY) and P-type, (P-AMY) (Bindu *et al.,* 2013). Twenty percent of pancreatic enzymes are excreted by the kidney thus patients with end stage renal disease have elevated levels of serum pancreatic enzymes (Bindu *et al.,* 2013). A diagnosis of AP can be made if serum lipase and/or amylase levels ≥3 times the upper limit of normal (Banks and Freeman, 2006). Lipase is more specific than amylase and stays elevated longer than amylase due to its longer half-life in serum resulting from renal tubular reabsorptions (Jasdanwala and Babyatsky, 2015). Therefore, in smaller hospitals where limited lab and radiological facilities are available, estimation of serum lipase is preferable over serum amylase in diagnosis of AP (Batra *et al.,* 2015).

The diagnosis of AP should not solely be based on the arbitrary value of three or four times greater than normal of pancreatic enzymes, but interpreted together with other clinical presentation, which are typical abdominal pain and Computed Tomography (Toouli *et al.,* 2002). Lipase level increases within 4-8 hours, peaks at 24 hours and stays longer in circulation – for 1-2 weeks (Hepburn, 2012). On the other hand, serum amylase activity typically increases within 2–12 h of onset of symptoms, has a 24 h peak and remains elevated for 3–7 days (Hepburn, 2012). The serum amylase and lipase are also elevated in patients with end stage renal disease in absence of pancreatitis (Vaziri *et al.,* 1988). The highand marked levels of amylase and lipase are also noted in advanced chronic kidney diseases patients and in patients undergoing peritoneal dialysis (Caruana *et al.,* 1989).

Montalto *et al*. (1997) found that increase in serum pancreatic enzyme during chronic renal pathology is slight but frequently occurs. This work is designed for screening of serum levels of amylaseand lipase in healthy pregnant and nonpregnant women attending Vasundhra Hospital and Fertility Research Centre (VHFRC) Jodhpur-India, and calculating the possible correlations between the parameters measured.

MATERIAL AND METHODS

Chemicals, Reagents and Kits

All the chemicals and reagents used for this work were analytically graded and purchased from reputable chemical laboratories. Calibration and quality control products were provided by Laboratories concerned. Kits for determination of serum lipase and amylase are provided by *VIN* Biomedicals, procured from Batra Agencies, Sardarpura, Jodhpur, India.

Study Area

The present study was conducted mainly in female subjects who attended anti-natal medicine outpatient Department at Vasundhra Hospital and Fertility Research Centre (VHFRS), Jodhpur, Rajasthan, India. The study was conducted from February 2016 – May 2016.

Experimental Design

The subjects were categorised into two groups based on the following categories:

Group I: Pregnant women
Group II: Nonpregnant women

After categorization, a sample of blood was collected for the biochemical analysis. Estimation of lipase and amylase were done and comparison between the groups concern was made.

Demographic Characteristic of The Study Subjects

There were 50 female subjects total in number recruited for this study, out of which of 35 were pregnant and 15 nonpregnant all aged from 18 to 55 years. All are self-reported with no any other disorders. All the pregnant female subjects were attending the ante-natal medicine outpatient Department of Vasundhra Hospital and Fertility Research Centre (VHFRS), Jodhpur, Rajasthan, India.

Ethical Consideration

The Ethical and Research Committee of the Jodhpur Medical College and Hospital approved the study protocol as a part of the post graduate curriculum, and informed consent was obtained from all the subjects before the collection of the blood samples.

Exclusion and Inclusion Criteria

Subjects with history of intake of thyroid drugs, hypertensive, diabetes mellitus, obesity, all other causes for electrolyte abnormalities, renal disorders, malabsorption syndrome, hepatic disorders and so on were excluded from this study. Subjects on diuretics, not fitting into criteria and any other systemic disease those with other possible abdominal conditions, were also excluded. Those included are healthy pregnant patients, infertility (confirmed and suspected), those with or without convulsions, all aged between 18 – 55yrs.

Sample Collection

All serum samples were collected in the laboratory of the outpatient department of Vasundhra Hospital and Fertility Research Centre (VHFRS), Jodhpur, Rajasthan, India. 5ml venous blood was collected from the antecubital vein using plain test tube. The blood was allowed to clot and then subjected to centrifugation at 3000×g for 5 minutes for separation. The serum samples were collected for the analysis of biochemical parameters: serum lipase and amylase.

Instrument and Equipment

Semi-auto analyser (Stat Fax 3300 Awareness Technology, Inc. P.O. Drawer 1679, Palm City, FL 34991, USA), and High Speed Centrifuge, were used to determine biochemical markers in this study.

Biochemical Analysis

First, the calibration and the determination of quality control products for all biochemical markers were performed. After

all, results of quality control products were within the permitted ranges. The lipase and amylase estimation were done using Stat Fax 300 semi-automatic Biochemical Analyser. All serum samples were directly determined without any dilution.

Estimation of Serum Lipase and Amylase

Estimation Serum Lipase: lipase single reagent kit for quantitative determination of lipase in human serum was usedbased on turbidimetric principle. (Tietz and Shuey, 1993)
Principle: lipase catalyses the breakdown of Triolein in the presence of Colipase**,** deoxycholate and calcium ions, hydrolyses the substrate to form glycerol and fatty acids which is measured as rate of decrease in turbidity at 340nm.

$$\text{Triolein} + 3H_2O \xrightarrow{\textit{lipase}} \text{Glycerol} + 3 \text{ fatty acids}$$

Estimation of Serum amylase: α-amylase reagent kit for quantitative determination of amylase in human serum and plasma based on kinetic method using GalG2-α CNP (2-chloro-4-nitrophenol β-1-4 galactopyranosylmaltotrioside) (Tietz, 1995). It is a direct substrate for determinationof amylase activity

Principle:

$$\text{GalG2-}\alpha\text{ CNP} \xrightarrow{\textit{α-amylase}} \text{CNP} + \text{GalG2}$$

Statistical Analysis

Descriptive and inferential statistical analysis has been carried out in the present study. The analysis was done using one-way Analysis of Variance (ANOVA) to find the mean value of each parameter within a group, and the values are expressed in mean±SD. Pearson's correlation was done to see the correlation between serum lipase and amylase. This was done by using Minitab software (version 15.0). Interpretation was done according to p values, where $p < 0.05$ is considered significant, $p < 0.01$ is considered highly significant while $p > 0.05$ is considered insignificant.

RESULTS AND DISCUSSION

RESULTS

In the present study mean age group of study subjects were between 18-55yrs. There were 35 (70%) pregnant, and 15 (30%) nonpregnant women. Mean±SD serum levels of amylase and lipase were presented in Table 1.

Table 1. Average serum levels of lipase and amylase in the pregnant and nonpregnant women

	LIPASE (p= 0.233)	AMYLASE (p = 0.730)	LIPASE:AMYLASE
GROUP I	117.22±20.52	44.44±16.13	2.6:1
GROUP II	120.09±13.37	37.63±3.34	3.2:1

The results were expressed as Mean ± SD. P value < 0.05 is considered significant otherwise is insignificant

Table 2. Pearson correlations (r) between parameters in pregnant women (n = 35) and non-pregnant women (n = 15)

PARAMETERS	Amylase	
Lipase	Pregnant	Non-pregnant
	r = 0.487	r = 0.365
	p = 0.183	p = 0.334

r - Pearson correlation: P-Value
*Correlation considered significant when p<0.05 and ** Correlation considered highly significant when p<0.01: all p values >0.05 are considered insignificant

DISCUSSION

Pregnancy induces physiological changes which are coupled with emotional stress and challenges. It also contributes to changes in hormonal and biochemical status of pregnant women, especially, electrolytes imbalance and depletion of macronutrients (Akinloye *et al.,* 2013; tidy *et al.,* 2014). Biochemical parameters reflect these adaptive changes which are clearly distinct from the non-pregnant state (Tran, 2005). The results of the experiments in the present study were presented in tables at result section above. Table 1 shows the mean levels of serum lipase and amylase for pregnant women attending ante-natal clinic for routine pregnancy check-up at VHFRC Jodhpur and non-pregnant women as controls. Pancreatitis results when proteolytic enzymes (as proenzymes) are activated in the pancreas rather than in the intestinal lumen. They are usually activated by endotoxins, exotoxins, viral infections, ischemia, anoxia, and to some extent direct trauma. Upon been activated, they digest pancreatic and peri-pancreatic tissues and bring about a severe damage. Elevated levels of serum amylase and lipase establish the diagnosis of AP. Thediagnosis of AP was mostly based on clinical evaluation, Computed Tomography (CT) findings and biochemical parameters such as serum lipase and amylase. Theseenzymes are usually utilized in this diagnosis, due to the fact that, they are comparatively easy to measure, though there are other causes of increased amylase and lipase levels. (Choi *et al.,* 2009).

From table 1 it was shown that, the primary serum level of lipase and amylase for all the groups were found to be within the normal range, as such none of the two groups screened were found to be at risk of AP. Moreover, lipase/amylase ratiowas also found to be consistent between the pregnant and nonpregnant subjects, thus, confirming the safety of the pregnant subjects. Thelipase/amylase ratio which was proposed by many outstanding studies as an index that can differentiate alcoholic AP from biliary and/or other types of AP. Gumate *et al.* (1991)was among those to propose this index of lipase/amylase ratio as a distinguisher of alcoholic (Lipase/Amylase>2) from non-alcoholic pancreatitis (Lipase/Amylase<2). In another study, Adiga *et al.* (2013) found a significant elevation in serum lipase and amylase in all the AP patients when compared with the control subjects. It was also reported that lipase/amylase ratio is higher in alcoholic AP type when compared with non-alcoholic AP. Their results expressed 4.4 and 2.4 against 1.84 in controls for alcoholic type and non-alcoholic type respectively. Moreover, Devanath *et al.* (2009) also found out that the lipase/amylase ratio than 3.0 could be used to differentiate between the two types of AP. However, on the other hand, some studies like that of Chang *et al.* (2005), have a total contradicting view.

In their research, after using 247 patients in Taiwan, they tested the levels of their lipase/amylase ratio, they find that the serum Lipase/Amylase ratio showed no significant changes among each group despite both enzymes expressed significant elevations. In a different study, Choi *et al*. (2009) shows that Lipase/Amylase can serve as significant index for distinguishing mild acute biliary pancreatitis from non-pancreatitis. However, the critical value of Lipase/Amylase seemed to depend on the diet pattern and cultural background. Nevertheless, in all the studies reported above, the levels of both lipase and amylase found to be elevated significantly to confirm AP. It can also be interestingly deducted that, the ratio seems to be playing an interesting role in differentiation for sub-types of AP, as those studies backing in favour are more recent.

Correlations

Table 2 summarises the details of Pearson correlation (r) between parameter lipase and amylase within each group.Thecorrelationsare common for all, as they areall found to havea positive correlation. This is in consistency with the findings of Madole *et al.,* (2016), as they reported positive correlation of serum amylase with serum lipase in diabetic patients. None of the parameters shows any significances between the groups (p>0.05).

Conclusions

Based on the results obtained in this study, we can conclude that majority of the screened pregnant women attending VHFRC are at no risk of pancreatic disease as the levels of the pancreatic enzymes estimated were at normal level when compared with nonpregnant subjects. Therefore, pancreatic enzymes were less affected by the pregnancy period and its accompanied changes. And also the lipase and amylase have a mutual positive correlation between one another in the subjects recruited.

Recommendations

The limitation of our study is the small sample size because of which we could not standardize the reports; further studies need to be conducted on a larger population of pregnant, nonpregnant and possibly subject with diseases in order to make a clear conclusion about the correlationsbetween the parameters. Secondly, as the hepatobiliary system and the pancreatic system are linked with each other, the concentration of liver function parameters and those of amylase and lipase in the blood are dependent upon one another. Thus, when there is any problem in the hepatic circulation or the pancreatic juice flow, the two processes affect each other, resulting in elevation of the concentrations of these materials in the blood. Thus, a complementary assay of liver function parameters and the enzymes screened here should have been done, and their correlation would have given a clear picture.

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