Serum C-Reactive Protein, Uric Acid and Ferritin Levels in Gestational Diabetes as a Screening Test

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OBJECTIVE: To investigate the value of uric acid, ferritin, C-reactive protein (CRP) both alone or in combination as screening tests for gestational diabetes (GDM) confirmed by the gold standard 100-g oral glucose tolerance test (OGTT).
STUDY DESIGN: A total of 320 pregnant women at 28-31 weeks gestation during the period of study from January-May 2001 were recruited to the study from Social Security Hospital. A total of 91 subjects with a high risk of GDM from 320 subjects who were referred for an OGTT were available for the study. Fasting serum uric acid, ferritin, CRP levels were measured at the time of glucose challenge test (GCT). The area under curve (AUC), the receiver operating characteristic (ROC) curves for uric acid, ferritin, CRP was also calculated.
RESULTS: GDM patients had higher CRP levels compared to patients with GCT≥140mg/dl and patients with GCT<140 mg/dl, normal OGTT. There was no significantly difference in ferritin and uric acid levels between these groups. Area under curve was 0.827 for CRP in the prediction of the development of GDM. Two cut-off values were used for CRP, the upper to rule in and the lower to rule out GDM. At a cut-off value of ≥2 mg/dl to rule out GDM in 91 patients, 38 (42%) would not need OGTT with 33 (87%) being false positives, 16 (17.5%) would need OGTT with 8 (50%) being false positive and 75 (82%) would not need OGTT with 12 (16%) being false negative. At a cut-off value of ≥3mg/dl with the maximum specificity to rule in GDM in 91 patients, 16 (17.5%) would need OGTT with 8 (50%) being false positive and 75 (82%) would not need OGTT with 12 (16%) being false negative. When different levels of CRP were added to GCT, at a CRP cut-off of 2.5 mg/dl, 71 would not need an OGTT with only 9 being false negative to rule out GDM in 91 patients
CONCLUSIONS: CRP predicted the presence of GDM. CRP alone or combination with GCT was not a powerful screening test for GDM; even CRP reduced unnecessary OGTT when used together with GCT.
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Key Words: Gestational diabetes mellitus (GDM), Glucose challenge test (GCT), Oral glucose tolerance test (OGTT), C-reactive protein (CRP)

Gestational diabetes mellitus (GDM) is an important clinical condition for mother and the future development of fetus. A continuing controversy revolves around the laboratory procedures used to screen patients for GDM.[1-8] Since now there is not a consensus for the screening of GDM. A screening test should be diagnostically accurate, cost-effective and acceptable to the patient. In the recent Fourth International Workshop conference on GDM, the 50-g 1-h plasma glucose challenge test (GCT) followed by the 100-g 3-hr oral glucose tolerance test (OGTT) was recommended for the diagnosis of GDM.[9] For screening and diagnose of GDM these GCT and OGTT tests are time consuming, increasing the laboratory workload and not easily acceptable by the patients. So in the last few years new potential alternative screening tests are being investigated.

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CRP, the most commonly used and best standardized inflammatory marker was shown to have the potential to be used as a marker in the prediction of diabetes.[10] This relationship was found to be present strongly in women than men.[11] Uric acid level was also found to be higher in subjects at high risk of diabetes with abnormal glucose tolerance.[12,13] We hypothesized that CRP and uric acid might reflect the existence of GDM and be used as parameters for screening GDM.

Similarly, as in non-pregnant subjects with diabetes, mothers who developed gestational diabetes had significantly increased ferritin levels.[14,15] It seems that iron overload is a typical feature of GDM.[16-18] Serum ferritin levels may also have the potential to be used as a marker in the prediction of GDM.

Material and Methods

The subjects were collected from 320 pregnant women at 28-31 weeks gestation under ongoing care in Social Security Hospital during the period of study from January-May 2001. We excluded 20 pregnants with cardiovascular diseases, acute or chronic renal diseases, chronic inflammatory diseases, acute infections, and thyroid disorders, also with low hemoglobin, mean corpuscular volume and proteinuria. Multiple pregnants, type I and II diabetics and smokers were also not included to the study. A total of 91 subjects from 300

subjects who were referred for an OGTT were available for the study. Predominant reasons for requesting an OGTT were a family history of DM, a positive GCT, previous history of GDM, previous macrosomia, large for gestational age neonate, poor obstetric history, glycosuria.

All the 91 patients underwent to a standard 50-g GCT and 100-g OGTT interpreted by the World Health Organization criteria.[9] GCT was performed by measuring plasma glucose level 1 hour after ingestion of 50-g glucose load. A value of ≥140 mg/dl identifies a subgroup of women at risk for GDM.[9] GDM diagnose was based on an abnormal OGTT as recommended by the American Diabetes Association.[9] Informed consent was obtained from all pregnant women participating to the study.

After an overnight fasting, blood samples were collected from each patient for the measurement of serum ferritin, CRP and uric acid levels. The serum was stored at -700C until analyzed.

Serum glucose levels were measured by an enzymatic method (Biocon, Germany) in Technicon RA-XT autoanalyzer. Ferritin levels were measured by using fluorescence polarization immunoassay in TOSOH autoanalyzer by the reagents supplied by the manufacturer. CRP is measured by latex immunoturbidimetric assay (Scil Diagnostic, Germany) and uric acid measurements were done by using uricase method by the reagent supplied by the manufacturer in Aeroset autoanalyzer (TOSHIBA, Japan).

Statistical analyses were performed using a commercial computer package (SPSS 10 for Windows, SPSS Inc., Chicago, IL.). Sensitivity, specificity, positive and negative predictive values (PPV+, PPV-), likelihood ratio of positive test (LR+) for different test threshold values in each group was calculated by using standard definitions.[19] Efficiency [100 (true positives+true negatives/n] and the area (AUC) under the receiver operating characteristic (ROC) curves were also calculated.

We arranged the available data to develop a screening profile for GDM by our proposed application of the rule-in and rule-out strategy (as recommended by Henderson). Henderson offers two cut-off values for each test.[19] The higher threshold cut-off value having an increased specificity was used to rule in the disease. To rule out the disease the lowest threshold cut-off value having an increased sensitivity was used.

Results

In the study 47 patients of the 91 had a value of ≥140mg/dl for GCT which means a positive screening test. Twenty (23%) of the 91 patients had gestational diabetes. Forty-four patients had both negative screening test and normal OGTT.

The 91 subjects were divided into three groups according to their GCT levels. These groups were arranged as group I (patients with GCT <140 mg/dl and normal (OGTT), group II (patients with GCT≥140mg/dl) and group III (patients with GDM). The ferritin, CRP and uric acid levels of the groups were shown in Table I. Group II and group III had significantly ($p<0.05$) increased serum CRP levels compared to group I, but there was no difference in the levels of ferritin and uric acid between all groups.

Table 1. The ferritin, CRP and uric acid levels of the group I patients with GCT<140 mg/dl and normal OGTT, group II patients with GCT≥140mg/dl and group III patients with GDM

	GroupI	Group II	Group III
	n=44	n=47	n=20
Ferritin (ng/ml)	10.6±9.7	10.5±6.8	11.2±6.9
CRP (mg/dl)*	1.1±1.0	2.5±0.6	2.8±0.7
Uric acid (mg/dl)	3.3±0.9	3.5±0.8	3.7±0.7

Values are given as mean±SD. *: P<0.05 Difference between group I and II, group I and III.

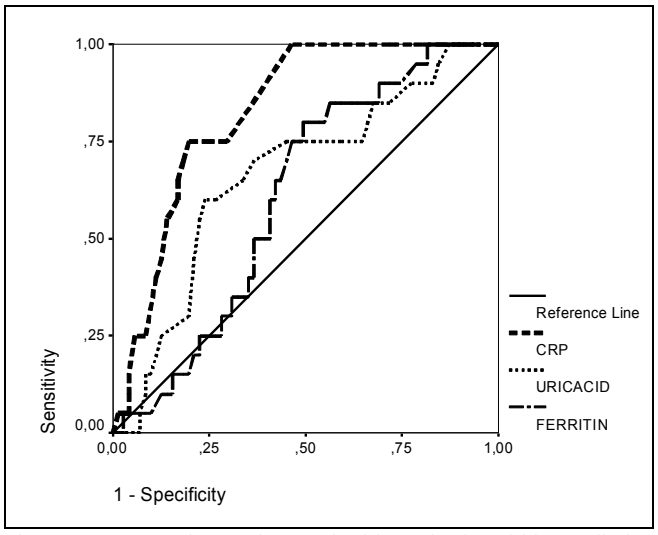

Figure 1. ROC analyses of CRP, ferritin and uric acid in prediction of gestational diabetes. The higher the area under curve (the greater the curvature away from the 50% line), the greater predictive power.

Area under curve was 0.827 for CRP in the prediction of the development of GDM. AUC for ferritin and uric acid were 0.607 and 0.663 respectively as shown in Figure 1.

Discussion

In some studies uric acid levels were found to be higher in non-pregnant subjects with abnormal glucose tolerance test and at high risk of DM.[13] Contrary to diabetic non-pregnant subjects, in our study pregnant women who developed GDM did not have significantly increased serum uric acid levels. It is impossible to establish a relation between increased GDM risk and serum uric acid concentration.

In literature there are studies investigating the association of increased ferritin levels and GDM.[16-18] To our knowledge there is not any study investigating the value of ferritin in prediction of GDM. In our study even in GDM group, we

could not determine a significantly higher ferritin levels. The novel aspect of our study is that ferritin and uric acid can not be used as a predictive parameter in the diagnosis of GDM.

To the best of our knowledge this is the first report on serum CRP levels in gestational glucose intolerance. According to our results serum CRP levels in pregnant women with GDM was statistically higher than healthy pregnant women.

Studies regarding with CRP and the risk of developing diabetes showed that women with elevated CRP levels had significantly increased risk for the development of diabetes and it was independent of adiposity and other risk factors.[20] The cause for the increase of CRP level is unclear.[21-23] CRP as an inflammatory marker is produced and released by the liver under the stimulation of cytokines IL-6, IL-1 and TNF-alpha. Cytokines promote de novo synthesis of hepatic fatty acid and interfere with lipoprotein lipase activity. Hepatic uptake of insulin is inhibited with spontaneously elevated portal free fatty acids.[24] The elevated production of free fatty acids might hypothetically increase hepatic secretion of glucose as well as contribute to elevated peripheral insulin concentration. Cytokines also may impede insulin stimulated glucose uptake.

In our study, ROC analyses for CRP showed that CRP might be used as a parameter in the prediction of GDM development. Though CRP seems to be a predictor for GDM, the different CRP cut off values are not good enough to be used in clinic as a parameter for the screening of GDM.

The conventional screening test for gestational diabetes mellitus is the measurement of plasma glucose 1 hour after 50-g glucose ingestion. Any data about the PPV, sensitivity and specificity for GCT which is routinely used in the clinics are not available for our population. In the literature the sensitivity, specificity and PPV values for GCT were reported as 88-89%, 85-87% and 21.5% respectively as confirmed by 100-g OGTT.[25] According to our results if the cut off value of CRP was accepted as ≥2 mg/dl, the sensitivity was 100%, the specificity 53.5% and PPV 37.7%. If the cut off value was considered as ≥3mg/dl the sensitivity was 40% and the specificity 88.7%, PPV 50%. The sensitivity at ≥2.5mg/dl was 70%, and the specificity and PPV were 81.7% and 91.9% respectively. The results of our study demonstrate that any cut off value of CRP does not have any advantage compared to GCT.

As a screening test using only CRP will not help us to get a better sensitivity, specificity, but a better PPV. So we can speculate that combination testing with parallel testing GCT and CRP may help us in preventing to perform unnecessary OGTT. For diagnosis of GDM, parallel testing, using GCT ≥140 mg/dl and a cut-off value of ≥2 mg/dl for CRP, showed that in 44 patients (48%) OGTT could be avoided but still OGTT was unnecessary for 27 (38%) patients. If GCT≥140 mg/dl and a cut-off value of ≥3 mg/dl for CRP is used as the cut-off value, 79 (87%) unnecessary OGTT would be avoided with 12 (15%) being misclassified as false negatives. If ≥2.5 mg/dl was used as the cut-off value 68 (75%) unnecessary OGTT would be avoided but we would miss 6 (9%) patients with GDM.

The important aspect of our study is that, CRP predicted the presence of GDM. But CRP alone or in combination with GCT was not a powerful screening test for GDM; even CRP reduced unnecessary OGTT when used together with GCT. Further studies in large groups are warranted to determine if CRP can be used as a predictor for GDM alone or in combination

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