

## Total antioxidant in term ferric reducing ability of plasma in pregnant with gestational diabetes mellitus

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### الخلاصة

كان الهدف من هذه الدراسة تقييم حالة مضادات الأكسدة مجموع (TAS) في الثلث الثاني من الحمل للنساء الحوامل المصابات بداء السكري الحملي (GDM) ؛ ضعف السكري الحملي (IGDM) ومقارنتها مع النساء ذوات القدرة الطبيعية على التعامل مع الكليكوز (NGT).

زيادة نشاط الجذور الحرة في السكري الحملي (GDM) قد يؤدي إلى مجموعة من المضاعفات المدمرة والتنكسية للأم والجنين. يمكن قياس مستويات مضادات الأكسدة من خلال قياس قدرة الحديد في البلازما (FRAP).

وجرى تقييم 30 امرأة حامل في الثلث الثاني من الحمل في هذه الدراسة. تم تقسيمهم إلى ثلاث مجموعات المجموعه الاولى تضم 10 نساء بقدرة طبيعه على التعامل مع الكليكوز(NGT)،المجموعه الثانية تضم 10 نساء يعانين من ضعف الحمل السكري (IGDM) ، والمجموعه الثالثه تضم 10 نساء مع وجود داء السكري الحملي (GDM). تمت قياس قدرة الحديد في البلازما (FRAP)، بالإضافة إلى مؤشر كتلة الجسم.

كانت التغيرات في FRAP في GDM تختلف كثيرا عن المجموعه الاولى. وبالتالي، يمكن لمستويات الغلوكوز المرتفعة تحفز الاكسدة في GDM. كان متوسط تركيزات المضادة للأكسدة في المجموعات الثلاثة (414.7 للمجموعه الثالثه، 452.73 للمجموعه الثانية، 858.4 للمجموعه الاولى) مل مول/ لتر.

في هذه الدراسة كان هناك ارتباط كبير ( $R^2 = 0.4685$ ) بين مؤشر كتلة الجسم ( $KG/M^2$ ) والجلوكوز في الدم (ملغم / دل) بعد 2 ساعة اختبار الجلوكوز (2 - OGTT). وأجريت أيضا تأثير مستوى الجلوكوز في بلازما الدم في النساء الحوامل في الفصل الثاني مع GDM IGDM أو على مستوى الوضع البلازما المضادة للأكسدة (TAS) ، بالإضافة إلى العلاقة بين مستوى السكر في البلازما ومضادات الأكسدة في حالة مجموع النساء الحوامل أو مع GDM IGDM.

### Abstract

The aim of this study was assessment of total antioxidant status(TAS) in the second trimester pregnant women with gestational diabetes (GDM); impaired gestational diabetes mellitus (IGDM) and normal glucose tolerance (NGT).

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Increased free radical activity in gestational diabetes (GDM) can lead to a host of damaging and degenerative maternal and fetal complications.

Hence antioxidant levels in the term ferric reducing ability of plasma (FRAP) of GDM could be given.

30 pregnant women in second trimester were evaluated in this study. They were divided into three groups: 10 pregnant having normal glucose tolerance (NGT group), 10 pregnant having impaired gestational diabetes mellitus (IGDM group), and 10 pregnant having newly diagnosis gestational diabetes mellitus (GDM group). Ferric reducing ability of plasma (FRAP) were estimated, in addition to BMI. The changes in FRAP in GDM was significantly different from controls. Hence, elevated glucose levels can induce oxidative stress in GDM. The average concentrations of total antioxidant in term FRAP were (414.7, 452.73, 858.4)  $\mu\text{mole/L}$  for NGT, IGDM, and GDM respectively. In this study there was a significant correlation ( $R^2 = 0.4685$ ) between BMI ( $\text{KG/M}^2$ ) and serum glucose ( $\text{mg/dl}$ ) after 2-hour oral glucose tolerance test (2-OGTT).

The influence of plasma glucose level in the second trimester pregnant women with GDM or IGDM on the level of plasma antioxidant status (TAS) was also conducted in addition to the correlation between plasma glucose level and total antioxidant status in pregnant women with IGDM or GDM.

The research was conducted at AL-Diwaniya Governorate, Al-Qadisiya university, College of medicine, department of clinical chemistry.

## Introduction

Gestation diabetes mellitus (GDM) is a condition in which women without previously diagnosed diabetes exhibit high blood glucose levels during pregnancy<sup>1</sup>, most women with GDM have normal glucose tolerance post-partum up to 60 % will develop type 2 diabetes mellitus with 15 years<sup>2</sup>. This occurs in a range of 1-14 % of pregnancies and associated with an increased maternal mortality<sup>3</sup>. Women with type 1 diabetes<sup>4,5</sup> and type 2 diabetes<sup>6</sup>

should have ophthalmologic assessments before conception, during the first trimester, as needed during pregnancy and within the first year postpartum<sup>7</sup>.

Differential criteria for diagnosis of GDM affects the denominator for the assessment of proportion of women affected<sup>8</sup>. Reliance on fasting glucose screens with failure to perform oral glucose tolerance tests reduces the sensitivity of identifying subsequent diabetes<sup>(9,10)</sup>. Clinical trials now provide evidence for the impact of multiple interventions to prevent the progression to type 2 diabetes in women with a history of GDM<sup>(11)</sup>. Both lifestyle modification and pharmacological therapies (have been shown to reduce diabetes development by 50% or more<sup>(12)</sup>. The diagnosis of GDM should initiate a long-term intervention and diagnostic process to minimize the risk of developing diabetes or to diagnose it as early in the course of disease as possible<sup>13</sup>.

The ferric reducing ability / antioxidant power (FRAP) assay<sup>(14,15)</sup> is recently developed, direct test of "total antioxidant power" other test of total antioxidant power used to date are indirect method<sup>(16,17)</sup> that measure the ability of antioxidant in the sample to inhibit the oxidative effects of reactive species purposefully generated in the reaction. In inhibition assay, antioxidant action induced a large phase; exhaustion of antioxidant power is denoted by a change in signal, such as the rate of oxygen utilization, fluorescence, or chemiluminescence.

Measurement of these signals, requires specialized equipment, and such as tests can be time-consuming, technically demanding and may lack sensitivity<sup>18</sup>.

In contrast to other tests of total antioxidant power, the FRAP assay is simple, speedy, inexpensive, and robust. The FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method.

Unlike the main indirect radical scavenging tests designed to measure total antioxidant power, the FRAP assay does not use a lag phase type of measurement in the FRAP assay, sample pretreatment is not required, stoichiometric factors are constant, linearity of maintained over a wide range, reproducibility is excellent, and sensitivity is high<sup>(19)</sup>. The FRAP assay does not need

highly specializes equipment or skills, of Critical controlled of limiting and reaction conditions.

The FRAP assay can be performed using automated, semiautomated, and manual version, and in a modified version known as the ferric reducing/ antioxidant power, and ascorbic acid concentration (FRAPSC)<sup>20</sup> assay supplied the indices of antioxidants status-the total reducing (antioxidant) power, the absolute concentration of ascorbic acid, and relative contributed of ascorbic acid to total antioxidant power of sample-virtually simultaneously concept of FRAP assay. A biological antioxidant has been defined any substances that when present at low concentrations compared to those of oxidisable substrate, significantly<sup>21</sup> delays or prevents oxidation of that substrate. However, unless an antioxidant prevents the generation of an oxidizing species. The difference is that the oxidizing species reacts with the antioxidant instated of the substrate<sup>(22,23)</sup>.

### Procedure

Blood sampling was preformed conveniently at the morning during the diagnostic OGTT. The subjects were instructed by the followings :

1. Early coming at 8.00 - 9.00 a.m ,after an overnight fasting.
2. No smoking, coffee or tea at the morning of the test.
3. No abnormal indulgence or abstinence from ordinary diet and no performance of strenuous exercises for three days before the test.

At low pH, reduction of a ferric tripyridyltriazine ( $\text{Fe}^{+3}$ , TPTZ) complex to the ferrous form, which has an intense blue color, can be monitored by measuring the change was absorption at 592 nm. The reaction was nonspecific , in that any half-reaction that has a lower redox potential, under reaction conditions, than that of the ferric/ferrous half-reaction would drive the ferric to ferrous reaction. The change in absorbance, therefore, was directly related to the combined or “total” reducing power of the electron-donating antioxidants present in the reaction mixture.

**Reagents**

Acetate buffer: 300 mM, pH 3.6 ( 3.1g sodium acetate trihydrate and 16 ml glacial acetic acid per one liter of distilled water).

TPTZ (2,4,6 tripyridyl – S-triazine) : 10 mM in 40 mM HCl, dissolved at 50 C° in water bath. Made fresh on the day of the assay.

Dilute HCl ( 40mM ): 3.3 ml concentrated HCl (12.1M)(BDH) in distilled water up to 1 liter. Stored at room temperature.

Ferric chloride( 20mM ): Prepared from 60 % FeCl<sub>3</sub> solution (BDH) on the day of assay.

Standard ( 1000 µM Ferrous ions ) : Use FeSO<sub>4</sub> .7H<sub>2</sub>O powder. Mixed the acetate buffer, the TPTZ and ferric chloride in a ratio of 10: 1: 1 to give FRAP Reagent preparation: the working FRAP reagent. Prepared working reagent fresh as required.

**Manual FRAP assay:**

|                 | Test    | Standard | Blank   |
|-----------------|---------|----------|---------|
| FRAP Reagent    | 1.5 ml  | 1.5 ml   | 1.5 ml  |
| Sample          | 0.05 ml | -        | -       |
| Standard        | -       | 0.05 ml  | -       |
| Distilled water | -       | -        | 0.05 ml |

Read absorbance after standing for 6 minute at room temperature and at wavelength (593 nm ) against a reagent blank.

$$\frac{\text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{standard}}} \times \text{FRAP value of standard } (\mu\text{mole/L}).$$

Serum glucose concentration was measured by glucose oxidase method using randox kit

## Results

The mean level of serum glucose in the term GTT was significantly higher in GDM group ( $243 \pm 34.65$ ) ( $P < 0.05$ ) than in IGDM ( $134 \pm 13.73$ ) and control group ( $94.6 \pm 9.25$ ) as shown in figure (1) and table (1).

The mean level of total antioxidant in the term FRAP was significantly higher in IGDM ( $460.3 \pm 71.2$ ) ( $P < 0.05$ ) than in GDM ( $317.8 \pm 32.57$ ) and control group ( $414.7 \pm 7.64$ ) as shown in figure (2) and table (1).

The level of BMI of a healthy pregnant women was significantly higher ( $49.89$ ) ( $P < 0.05$ ) than in GDM ( $29.03$ ) and IGDM ( $29.84$ ) as shown in figure (3) and table (1). There was significant correlation between serum glucose in term GTT and total antioxidant in the term FRAP of pregnant women with GDM as shown in figure (4) and table (2).

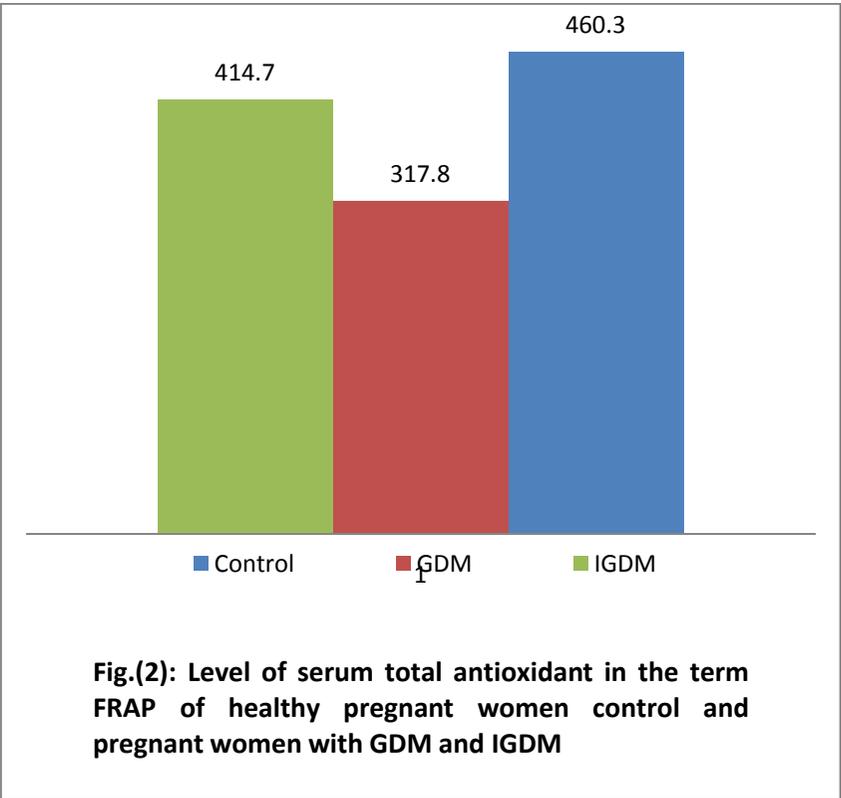
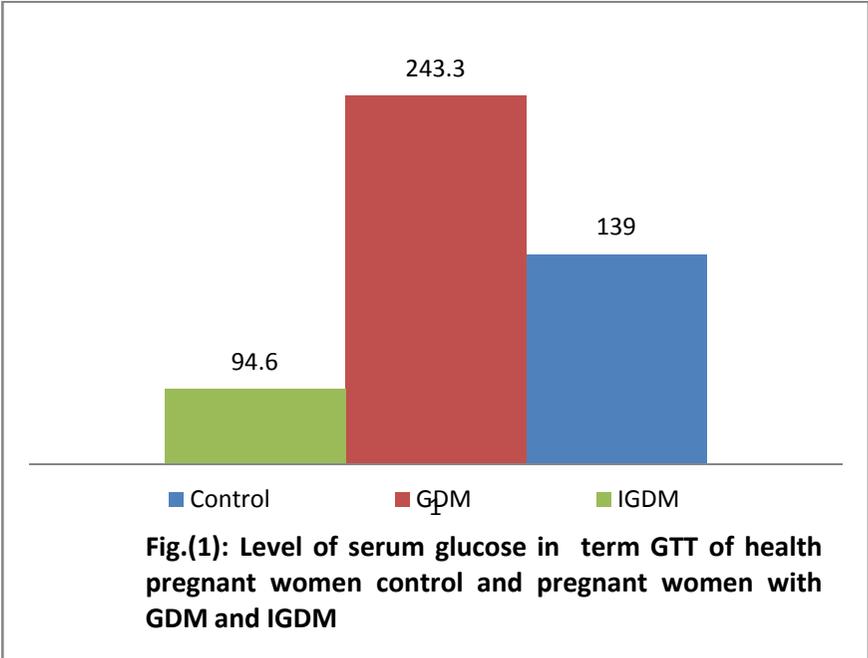
There was also a significant correlation between serum glucose in the term GTT and BMI for pregnant women with GDM as shown in figure (5) and table (2).

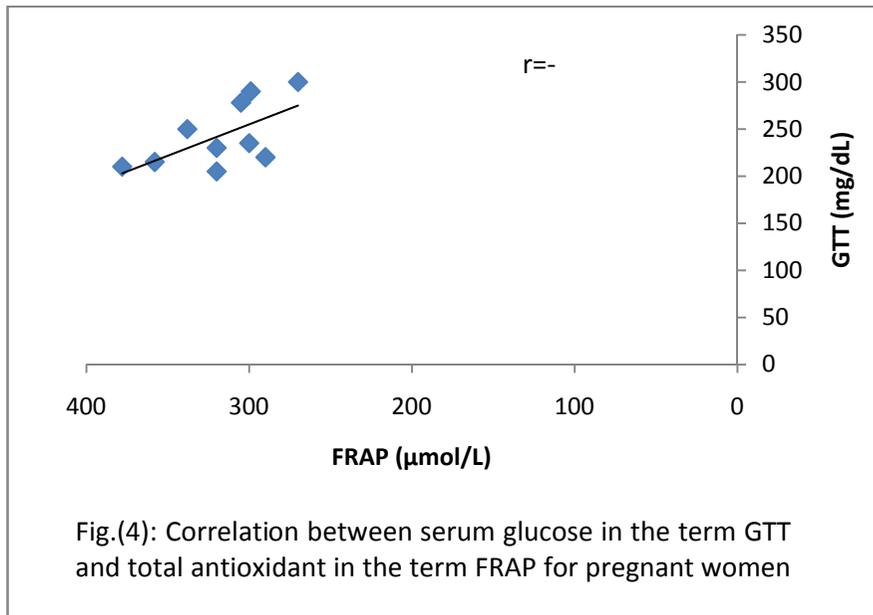
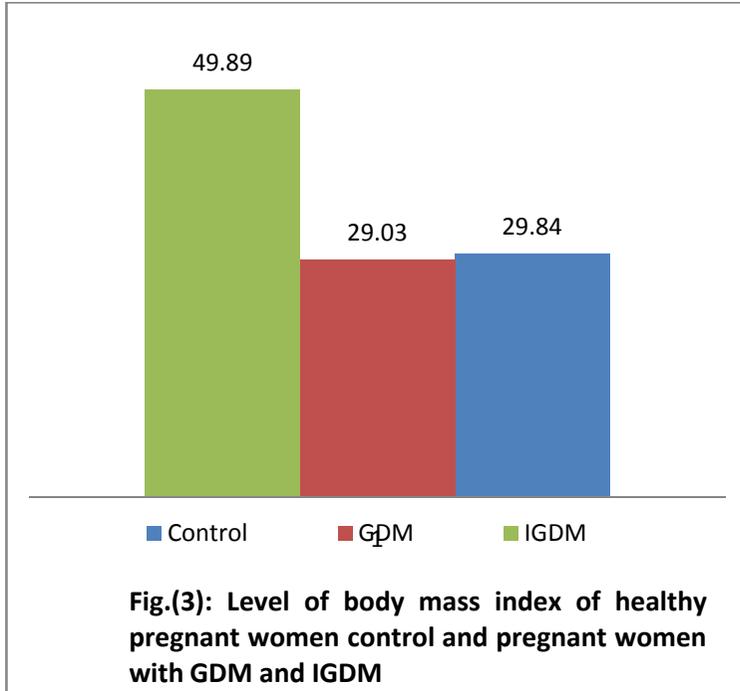
**Table (1): The levels of glucose, FRAP, and BMI in plasma of pregnancy women with GDM, IGDM, and control  $P \leq 0.05$**

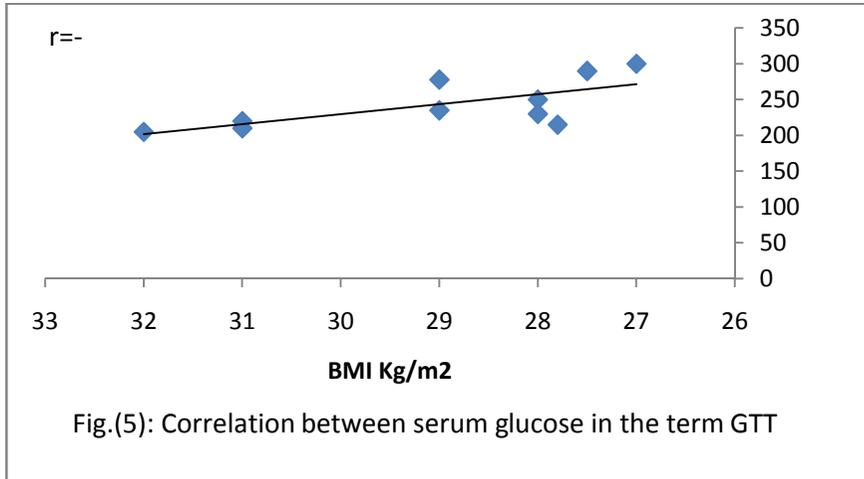
| Variables                  | IGDM, no.=10      | GDM, no.=10       | Control, no.=10   | P-Value |
|----------------------------|-------------------|-------------------|-------------------|---------|
|                            | Mean $\pm$ SD     | Mean $\pm$ SD     | Mean $\pm$ SD     |         |
| BMI                        | $29.84 \pm 6.57$  | $29.03 \pm 1.72$  | $49.89 \pm 12.46$ | S       |
| Glucose (mg/dL)            | $460.3 \pm 13.73$ | $243.3 \pm 34.65$ | $94.6 \pm 9.25$   | S       |
| FRAP ( $\mu\text{mol/L}$ ) | $139 \pm 71.2$    | $317.8 \pm 32.57$ | $414.7 \pm 7.64$  | S       |

**Table (2): The correlation of glucose levels with FRAP levels and BMI in plasma of pregnancy women with GDM, and IGDM  $P < 0.05$ .**

| The correlation of glucose () vs FR AP, and BMI | IGDM no.= 10                |         | GDM, no.=10                |         |
|---|-----------------------------|---------|----------------------------|---------|
|   | Correlation coefficient (r) | P-Value | Correlation coefficient(r) | P-Value |
| vs. FRAP ( $\mu\text{mol/L}$ )                  | -0.67334                    | S       | -0.62570                   | S       |
| vs. BMI   | 0.847961                    | S       | -0.69079                   | S       |







### Discussion

Oral glucose tolerance test, which was necessary for the diagnosis of IGT, was time - consuming and is inconvenient as a screening test in ordinary clinical practice because it require fasting, preparations, and multiple analyses. Therefore, there was a need to identify subjects at high risk for diabetes by using techniques that will be widely accepted in clinical and public health settings, not just in research settings. Some investigators had tried a multivariate model using available clinical and biochemical risk factors for identification of subjects at high risk for type 2 diabetes and have reported a good predictive ability<sup>24</sup>. Therefore, identification of risk factors that were efficient and convenient would contribute to such prediction or identification and thus focusing preventive efforts on people at high risk for diabetes. Moreover, if such risk factors were also modifiable. There are several potential sources of increased free radical production in diabetes including autooxidation of plasma glucose and decreased total antioxidant<sup>25</sup>.

In this study there were significant differ in total antioxidant in term between the healthy pregnant women and GDM , some recent study shows the increase in serum glucose will increase free radical and decrease total antioxidant<sup>26</sup>.

In addition the study present pregnant women with IGDM had higher total antioxidant(FRAP) generated as compared to healthy pregnant women ( $P \leq 0.05$ ); table (1), also significant correlation was present between GTT and BMI in GDM ; Figure(5) .

The FRAP assay is quick and simple to perform and reaction is reproducible and linearly related to the molar concentration of the antioxidant<sup>27</sup> .

### **Conclusion**

Radical scavenging antioxidant are consumed by enhanced levels of free radicals produced during glucose induced oxidative stress this can be measured the FRAP assay which offers a putative index of antioxidant defense of potential use to and within the technological reach of every Laboratory and researcher interested in oxidative stress.

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