Time Dependent Changes in Circulating Biomarkers during Diabetic Pregnancies: a Perspective Case Study

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Abstract: The aim of study was to estimate the changes of circulatory biomarkers in normal pregnancy and diabetic pregnancy in each trimester. The results showed imbalance between ROS and antioxidants. Increase oxidative stress throughout the pregnancy and compared that stress level with known diabetic pregnant group. In normal pregnancy the MDA (1.82 ±0.16µmol/ml) levels were high but the level are much higher in diabetic pregnant (4.48 ±0.32µmol/ml) group in 3rd trimester. Similarly the levels of sialic acid and neuraminidase are higher in diabetic pregnant group than normal pregnant group. As well as total cholesterol, HDL and TG are increased in diabetic pregnancy as compare to normal pregnancy. GSH, SOD and catalase (antioxidant stress markers) increased during pregnant group as compare to diabetic pregnant group. So all the these results showed that ROS production increased in pregnancy and antioxidative stress enzymes balance was disturbed towards destruction even more in diabetic pregnancy.

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Introduction

Pregnancy is a physiological status that alters renal, respiratory, cardiovascular, hematological, gastrointestinal, endocrine and genitourinary system. Pre-existing maternal diabetes is highly associated with increase in the risk of metabolic complications in mother as well as baby, the perinatal mortality (3.1%) and stillbirth rates (2.1%). These changes occur by the metabolic state of diabetes. Many complications may develop during pregnancy, so regular and frequent laboratory testing is required for monitoring the progress of pregnancy (Pilsczek *et al.*, 2008).

Pregnancy is a condition which can increase the oxidative stress, and disturb the balance between the prooxidant and antioxidant and leads to potential damage. During the pregnancy dynamic changes occur in multiple body systems, that's why increase basal oxygen consumption, and in increase in energy use by different organs including fetoplacental circulation, it is highly vascular organ and expose in high maternal oxygen partial pressure, and generate more reactive oxygen species (Casanueva *et al*., 2003).

In normal pregnancies, the formation of free radicals and lipoperoxidation increase gradually till the end of the pregnancy, as compared to non-pregnant woman. In the same time, the antioxidant production capacity increases during the pregnancy, this can lead to an oxidative balance that is maintained throughout the pregnancy (Hung JH, 2007).

In Pregnancy and diabetics there are susceptible to oxidative stress and antioxidant defense mechanism can be altered in the response of elevated levels of oxidative stress. To date, limited data on diabetes mellitus suggest that increase in the production of lipid peroxidation and decrease in the antioxidant enzyme activity. Extremely high level of free radicals causes damage of cellular protein, lipid and nucleic acid, which damage the cell membrane and at last cell death (Maritim *et al.*, 2003).

Materials And Methods

Biochemical analysis

I- The Total cholesterol (TC), triglyceride (TG) and high density lipoprotein (HDL) were estimated by the human kit method.

II- Malondialdehyde (MDA) was estimated by Ohkawa *et al*., (1979) method.

III- Glutathione (GSH) was estimated by Moron *et al*., (1979) method.

IV- Superoxide dismutase (SOD) was estimated by Kakkar *et al.*, (1984) method.

V- Catalase was estimated by Aebi method (1986).

VI- Neuraminidase in plasma was measured by spectrophotometric method of Ziegler and Hutchinson, (1972).

VII- Sialic acid in plasma was measured by spectrophotometric method of Aminoff, (1961).

Statistical analysis

The Mean±SD and correlations (Pearson) between the value of different parameters of diabetic pregnancies and healthy controls were determined by statistical package of SPSS version 16. The significance of the difference between comparing groups was taken at least equal or P<0.05.

Results

Malondialdehyde (MDA) levels of normal pregnant women were (1.82 µmol/ml) than diabetic pregnant women (2.72µmol/ml) in 1st trimester (table 1). In the 2nd trimester the normal pregnant women were (2.34mmol/l) from which is the diabetic pregnant women (3.34 µmol/ml).While in the 3rd trimester the mean MDA level in normal pregnant women were (3.43µmol/ml) than diabetic pregnant women (4.48µmol/ml). So the MDA level increase 53% from 1st to 3rd trimester in normal pregnancy and in diabetic pregnancy it is increases 60%. Patil *et al*., (2007) also reported that the MDA levels also increased in pregnant women as compared to control. This increase was gradual with the progression of pregnancy from 1st to 3rd trimester; according to Patil *et al*., (2006) there was a consistent significant increase in lipid Peroxidation (MDA) in all the trimesters as compared to non-pregnant controls (P< 0.001) Suhail *et al*., (2010) discussed the antioxidative status; it was evaluated in terms of MDA contents in all the patients groups. Thus oxidative level in pregnant increased significantly as compared to non-pregnant, and in gestational diabetics.

Super Oxide Dismutase (SOD) level of normal pregnant women were (4.60ng/ml) and in diabetic pregnant women (0.91ng/ml) during 1st trimester (table 1). While in the 2nd trimester the mean level of SOD in the normal pregnant women were (5.59ng/ml) and in diabetic pregnant women (0.74ng/ml). In the 3rd trimester the mean SOD level in normal pregnant women were (2.99ng/ml) than diabetic pregnant women (0.88ng/ml). The correlations between different trimesters are non-significant. So the SOD level increase from 1st to 3rd trimester in normal pregnancy is 154% and 103% in all trimester in diabetic in pregnant groups. According to Patil *et al.*, (2007) antioxidants SOD were found to be lowest in 3rd trimester of pregnancy as compared to 1st trimester. Chaudhari *et al*., (2003) describe a lower SOD activity in diabetic pregnancy as compared to normal pregnancy.

1st trimester the mean reduced glutathione (GSH) level of normal pregnant women were (6.54mg/dl) than diabetic pregnant women (2.81mg/dl). On the 2nd trimester the mean level of GSH in the normal pregnant women were (7.22mg/dl), diabetic pregnant women shows (2.86mg/dl). While in the 3rd trimester the mean GSH level in normal pregnant women were (6.41mg/dl) than diabetic pregnant women (3.30mg/dl). The main difference between 1st and 3rd trimester in normal pregnancy is 102% while in diabetic pregnancy it increase 87% (table 1). The correlation between 1st trimester with 2nd and 3rd trimester are non-significant in diabetic pregnant group.

Catalase levels in 1st trimester of normal pregnant women were (2.38µmol/mol of protein) than diabetic pregnant women (1.38µmol/mol of protein).While in the 2nd trimester the mean level of catalase in the normal pregnant women were (2.76µmol/mol of protein) and in diabetic pregnant women (1.62µmol/mol of protein) and in the 3rd trimester the mean catalase level in normal pregnant women were (2.30µmol/mol of protein) than diabetic pregnant women (0.61µmol/mol of protein). The difference between 1st trimester and 3rd trimester in normal pregnancy is 103% and in diabetic pregnancy 226% (table 1). According to the Patil *et al.*, (2007) results antioxidants catalase was found to be lowest in 3rd trimester of pregnancy as compared to 1st trimester.

Table 1:Time Dependent Changes In MDA, SOD, GSH and Catalase Levels (Mean±SD) In Diabetic and Non Diabetic Pregnant Women

Trimesters	Groups	MDA	SOD	GSH	Catalase
		(µmol/ml)	(ng/ml)	(mg/dl)	(µmol/mol of protein)
1st	NP	1.82 ±0.16	4.66 ±1.02	6.54 ±1.02	2.38±0.38
	DP	2.72 ±0.79	0.91 ±0.84	2.81 ±0.46	1.38 ±0.70
2nd	NP	2.34 ±0.53	5.59 ± 6.77	7.22 ±0.85	2.76 ±0.62
	DP	3.34 ±0.45	0.74 ± 0.58	2.86 ±0.79	1.62 ±0.99
3rd	NP	3.43 ±0.28	2.99 ± 0.40	6.41 ±0.91	2.30 ±0.50
	DP	4.48 ±0.32	0.88 ± 0.62	3.30 ±0.95	0.61 ±0.49
	P value	.000*	.331	.000*	.000*

NP(normal pregnant)
DP(diabetic pregnant)

Neuraminidase level of normal pregnant women were (0.25mg/100ml) and in diabetic pregnant women (0.44mg/100ml) in 1st trimester (table 2). Neuraminidase score in the 2nd trimester the mean level of neuraminidase in the normal pregnant women were (0.25mg/100ml) than the diabetic pregnant women (0.54mmol/l).While in the 3rd trimester the mean level in normal pregnant women was (0.24mg/100ml) diabetic pregnant women (0.48mg/100ml). The average difference 104% in normal pregnancy in all trimester while in diabetic pregnancy it is about 92%. The correlation between 1st trimester with 2nd and 3rd

trimester are non-significant in diabetic pregnant group while significant differences seen in all trimesters with control group.

The sialic acid concentrations level in the 1st trimester of normal pregnant women were (1.54µg/L) than in diabetic pregnant women (0.60µg/L), while in the 2nd trimester the mean level of sialic acid in the normal pregnant women were (1.52µg/L) than diabetic pregnant women (0.59µg/L).While in the 3rd trimester the mean sialic acid level in normal pregnant women were (1.59µg/L) than diabetic pregnant women (0.28µg/L). The average percentage between normal pregnancies from 1st to 3rd trimester is 97% and in diabetic pregnancy in all trimesters are 214% (table 2). The correlation between 1st trimester with 2nd and 3rd trimester was observed statistically non-significant in diabetic pregnant group while significant differences seen in all trimesters with control group.

Cholesterol level of normal pregnant women were (5.12mg/dl) than diabetic pregnant women (8.17mg/dl). In the 2nd trimester the mean level of cholesterol in the normal pregnant women were (5.37mg/dl) than diabetic pregnant women (7.47mg/dl).While in the 3rd trimester the mean cholesterol level in normal pregnant women were (6.31mg/dl) than diabetic pregnant women (9.53mg/dl). The average difference between 1st trimester and 2nd trimester is 81% and in diabetic pregnant group it is about 85% (table 2). The results of Toescu *et al*., (2004) the total cholesterol levels were similar between diabetic and non-diabetic groups. Cholesterol increased progressively throughout pregnancy in all groups.

High density lipoprotein (HDL) concentrations level of 1st trimester in the normal pregnant women were (1.45mg/dl) and in the diabetic pregnant women (1.64mg/dl). While in the 2nd trimester the mean level of HDL in the normal pregnant women were (1.47mg/dl) than diabetic pregnant women (2.24mg/dl).While in the 3rd trimester the mean HDL level in normal pregnant women were (1.56mg/dl) than diabetic pregnant women (3.16mg/dl, table 2). Ekhator and Ebomoyi (2012), the HDL was significantly lower in the first trimester than in the control group, second, or third trimester.

In the 1st trimester the mean triglyceride (TG) level of normal pregnant women was (1.04mg/dl) than diabetic pregnant women (2.33mg/dl). In the 2nd trimester the mean level of TG in the normal pregnant women were (1.26mg/dl) than diabetic pregnant women (2.10mg/dl). While in the 3rd trimester the mean TG level in normal pregnant women were (1.52mg/dl) than diabetic pregnant women (2.80mg/dl). Average difference between 1st and last 3rd trimester was 68% and in diabetic pregnant group were 83% (table 2).

Table 2:Time Dependent Changes In TC, HDL, TG, Neuraminidase and Sialic Acid Levels (Mean±SD) In Diabetic and Non Diabetic Pregnant Women

Trimesters	Groups	TC	HDL	TG	Neuraminidase	Sialic Acid
		(mg/dl)	(mg/dl)	(mg/dl)	(mg/100ml)	(µg/L)
1st	**NP**	5.12 ±0.82	1.45 ±0.22	1.04 ±0.08	0.25 ±0.08	0.43 ±0.09
	DP	8.17 ±1.60	1.64 ±0.40	2.33 ±0.45	0.44 ±0.21	0.60 ±0.09
2nd	**NP**	5.37 ±0.68	1.47 ±0.22	1.26 ±0.18	0.25 ±0.13	1.52 ±0.16
	DP	7.47 ±1.21	2.24 ±0.70	2.10 ±0.57	0.54 ±0.41	0.59 ±0.54
3rd	**NP**	6.31 ±0.97	1.56 ±0.17	1.52 ±0.14	0.24 ±0.12	1.59 ±0.18
	DP	9.53 ±1.78	3.16 ±0.29	2.80 ±0.34	0.48 ±0.19	0.28 ±0.05
	P value	.000*	.000*	.000*	.000*	.000*

NP(normal pregnant)
DP(diabetic pregnant)

Discussion

Normal pregnancy is characterized by a high energy demand for different body functions and an oxygen demand increase for different body organ, such as feto-placental circulation. Physiologically insulin resistance increase during late pregnancy.

The present study confirms that, the oxidative stress during diabetic pregnancy increases significantly as the pregnancy precede. The oxidative stress markers such as, SOD, MDA, sialic acid, catalase, neuraminidase, GSH as well as cholesterol, HDL, Triglycerides increase in normal pregnancy but statistically significant in diabetic pregnancy. Oxidative stress during diabetic pregnancy leads to recurrent pregnancy loss, spontaneous abortions, premature labor, and caesarian section.

Oxygen is an essential component for life in the cell, it under goes extensive metabolism that can leads in the formation of toxic derivatives. This metabolism is confined to the electron transport chain in the mitochondria that finally results in the generation of ATP, which support the cells for all metabolic processes. The end product of oxygen metabolism includes molecules in an activated electronic state that

have unpaired electrons and these are highly reactive with other molecules which are found in biological systems. These activated molecular species are designated as reactive oxygen species (ROS). These ROS can extensively damage cellular organelles such as mitochondria, nuclear and mitochondrial DNA and cell membrane, and ultimately leads to cell death. For the prevention of ROS induced damage, the cells have an antioxidant system. So there is a delicate balance between ROS production and the antioxidant activity that maintains physiological balance, which leads to cellular homeostasis.

In the diabetic mellitus, the possible sources of oxidative stress might include auto-oxidation of glucose, it shift the redox balance and decrease the antioxidants, like reduced glutathione (GSH) and vitamin E and impaired the activity of superoxide dismutase (SOD) and catalase (CAT). ROS generation by high glucose is causally linked to elevated glucose and other metabolic abnormalities and these are important to the development of diabetic complications.

Table 3: Levels of MDA, SOD, GSH and Catalase among (Mean±SD) Control, Normal Pregnant and Diabetic Pregnant

Groups	N	MDA	SOD	GSH	Catalase
		(µmol/ml)	(ng/ml)	(mg/dl)	(µmol/mol of protein)
Control	10	1.64 ±0.56	4.66 ± 0.70	9.44 ±0.80	4.29 ±0.83
Normal Pregnant	35	2.50 ±0.72	4.69 ± 4.93	6.86 ±0.96	2.55 ±0.57
Diabetics Pregnant	26	3.37 ±0.82	0.82 ± 0.65	2.93 ±0.73	1.35 ±0.89
P value		.000*	.001*	.000*	.000*

Table 4: Levels of TC, HDL, TG, Neuraminidase and Sialic Acid (Mean±SD) among Control, Normal Pregnant and Diabetic Pregnant

Groups	N	TC	HDL	TG	Neuraminidase	Sialic Acid
		(mg/dl)	(mg/dl)	(mg/dl)	(mg/100ml)	(µg/L)
Control	10	4.04 ±0.60	1.06 ±0.21	0.04 ±0.04	0.02 ±0.00	0.43 ±0.09
Normal Pregnant	35	5.56 ±0.90	1.49 ±0.21	1.28 ±0.23	0.25 ±0.11	1.54 ±0.17
Diabetics Pregnant	26	8.08 ±1.59	2.23 ±0.76	2.31 ±0.55	0.49 ±0.32	0.53 ±0.49
P value		.000*	.000*	.000*	.000*	.000*

Table 5: Correlation Matrix Circulating Biomarkers during Diabetic Pregnancies

	MDA	SOD	GSH	Catalase	Neuraminidase	Sialic Acid	TC	HDL	TG
MDA	1	-.354**	-.611**	-.614**	.402**	-.152	.639**	.608**	.664**
		.002	.000	.000	.001	.205	.000	.000	.000
SOD		1	.484**	.352**	-.218	.293*	-.387**	-.288*	-.296*
			.000	.003	.068	.013	.001	.015	.012
GSH			1	.737**	-.559**	.317**	-.699**	-.633**	-.810**
				.000	.000	.007	.000	.000	.000
Catalase				1	-.545**	.166	-.658**	-.514**	-.722**
					.000	.167	.000	.000	.000
Neuraminidase					1	-.150	.473**	.281*	.570**
						.213	.000	.018	.000
Sialic Acid						1	-.339**	-.185	-.198
							.004	.123	.098
TC							1	.516**	.806**
								.000	.000
HDL								1	.564**
									.000
TG									1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Women with diabetes are at high risk of maternal and fetal complications during pregnancy. Because diabetes produces changes in maternal metabolic fuels and because diabetic pregnancy is often associated with

complications, the effects of maternal diabetics on lipid metabolism are unclear (Sobki, 2004). Plasma lipids and lipoproteins changes in diabetic pregnancy have been studies by many researchers (Ersanali, 1997).

The current study was designed to observe the time dependent changes in cholesterol, triglyceride (TG) and high density lipoprotein (HDL) in known diabetic and non-diabetic women groups. In our study the cholesterol levels in diabetic pregnant group were significantly high ($p<0.05$) in all trimesters, actual level being (5.56±0.90) when compare to non-diabetic pregnant group (8.08±1.59). Hyperlipidemia is a common feature in pregnancy and consists primarily of triglycerides with smaller in cholesterol (Montelonge, 1992).

Regarding the triglyceride (TG) non-diabetic pregnant group and diabetic pregnant group did not show any significant difference though diabetic subjects were with higher mean values (2.31±0.55) than non-diabetic pregnant group (1.28±0.23). HDL also elevated in all trimesters in diabetic pregnancy (2.23±0.76) along with other lipids, while in non-diabetic it is not increase in all trimesters (1.49±0.21) and significantly low. Sialic acid is an chronic inflammatory component is involve in its pathogenesis so the sialic acid levels are related to previously diagnosed diabetic mellitus pregnancy not to GDM. Study demonstrates sialic acid levels increased more in non-diabetic pregnancy (4.69±4.93) when compare to the diabetic pregnant women group (0.88±0.65). That current increased sialic acid levels are significantly associated with cholesterol, SOD, GSH and MDA. The results show that as the pregnancy progress the reduced glutathione levels increased in each trimester especially in 2nd trimester and total increase is (6.86±0.96) but in diabetic pregnancy it increase but not as in non-diabetic (2.93±0.73). The relationship between hyperglycemia and oxygen free radicals is supported by results demonstrating and an increase in oxidative stress reduced the levels of reduced glutathione.

Activity of antioxidant enzyme SOD, which detoxifies the superoxide anion radical, were found to be decreased significantly in diabetic pregnancy group (0.82±0.65) as compared to normal pregnancy (4.69±0.65). This decrease in SOD activity may result from decrease enzyme production or enzyme inactivation. Catalase is an antioxidative enzyme localized in cytosol and peroxisomes. Catalase protects the cell against hydrogen peroxide toxicity through catalyzing dismutation reaction of H_2O_2. In oxidative stress conditions, an uncontrolled increase in reactive oxygen species is observed. According to our study the catalase activity significantly increased during normal pregnancy (2.55±0.577) as compare to the diabetic pregnant group (1.35±0.89).

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Conflict Of Interest

The authors declare that they have no competing interest

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