Chapter 5: Discussion
This study was performed to assess clinical characteristics of GDM mothers in comparison with those of NGT mothers and to investigate the association of dietary intake of antioxidants (vitamin C and HORAC) and plasma vitamin C concentration, with maternal glucose tolerance in pregnancy. The pregnancy outcomes in GDM and NGT mothers and the associations of these with maternal nutritional measurements also were investigated.

5.1. Characteristics of study population:

It was found that the conventional risk factors like older age, higher pre-pregnancy weight, lower physical activity, and family history of diabetes, were associated with GDM.

5.1.1. Age: Higher age was a risk factor for GDM in current study, which is a well-known risk factor for GDM (Hod. M, 2008). Chan LY, et.al, 2002 reported that the risk of glucose intolerance increased by 3folds in subjects older than 30. Also Terence T et.al, 2006 has indicated that the risk of GDM becomes significantly and progressively increased from 25 years onwards. However this factor is non-modifiable.

5.1.2. Pre-pregnancy BMI: GDM mothers had higher pre-pregnancy BMI in this study. Torloni et.al, 2009 showed that the risk of GDM is positively associated with pre-pregnancy BMI, which is similar with findings of current study. In contrast Kongubol A et.al, 2011 found that compared to normal weight women, obese Thai women without metabolic problems were not at increased risk for gestational diabetes mellitus (RR = 0.9 [95% CI 0.6–1.4]), suggesting that associated metabolic abnormalities maybe more sensitive marker in that population.

Since BMI is a risk for GDM in different countries it should be considered as modifiable factor for education of women in child-bearing age about healthy life style habits to reduce weight and prevent obesity and metabolic disorders.

5.1.3. Family history of diabetes:

Family history is usually considered to represent heritable genetic risk. However it is to be noted that it also represents family and social environment.
GDM mothers had higher family history of diabetes as expected. The high prevalence reflects the epidemiological situation in India where diabetes now affects more than 10% of adult population, and is increasingly affecting the young and the poor. Moshe Hod has discussed the role of family history of diabetes is a risk factor for GDM in his famous text book ‘Pregnancy and Diabetes’. Screening policies in most of the clinics include family history of diabetes as an indication for preferring OGTT. However, now it is quite well known that such a policy fails to diagnose many women with GDM. This was highlighted by Chan LY, et.al, 2002 who reported that the risk of glucose intolerance in young women with positive family history is similar to that in the background pregnant population. In India the current standard of practice is ‘universal OGTT’, however implication is still poor.

5.1.4. Parity: In current study GDM mothers were mostly multiparous. Al Rowaily et.al (2010) showed in Saudi Arabia that multiparous women were 8.29 times more likely to have GDM than nulliparous women. This association is likely to be confounded by age, which was obvious when adjustment for age reduced the risk to 2.95 times. Similarly Kahn et.al (2000) found that African American women with higher parity are more in risk of GDM possibly due to higher waist to hip ratio. Nicholson et.al (2006) showed that grand multiparity (five or more live births) is associated with insulin resistance and type 2 diabetes. Thus parity may be a surrogate for number of clinical characteristics which increase risk of diabetes, or may be a risk factor in its own right. This is not easy to resolve, but investigating the effect of restricting the family size might through some light.

5.1.5. Physical activity: In present research GDM mothers had lower levels of physical activity, compared to that of NGT mothers, though both groups could be categorized as sedentary. It is well known that physical activity of urban population in India is low, as shown in the epidemiological studies of diabetes (National Urban Diabetes Survey (NUDS), Ramachandran, (2001) National Indian Diabetes Survey, Seshiah V, et.al, 2001).

Tobias D.K. et.al (2011), found that higher levels of physical activity before pregnancy are associated with a significantly lower risk of developing GDM. Also Dye

Halperin and Feig in their recent review found that pre-pregnancy exercise was associated with a 55% lower risk of gestational diabetes. Physical activity in early pregnancy reduced risk 25%. A case control study by Dempsey et al (2004) also suggests that recreational physical activity performed before pregnancy is associated with a reduced risk of GDM.

Increasing physical activity of young girls before and after marriage and continuing in pregnancy may be an important preventive measure for GDM and other related disorders. This is being tested in some clinical trials across the world.

5.1.6. Stress: There are few studies which have investigated relationship between stress and GDM. GDM women had a higher stress score compared to NGT women. This modifiable factor could become part of GDM prevention strategy in future.

5.1.7. Weight gain during pregnancy:

A lot is written about weight gain in pregnancy and risks to the mother and the baby. World over there is a tendency of higher weight gain in pregnant women compared to the older generations. In our study, GDM mothers had higher weight gain during pregnancy compared with NGT. This finding is similar to the findings of Hedderson et al, 2010. Weight gain in pregnancy is again a modifiable risk factor and is being tested to prevent GDM in many clinical trials.

5.2. Nutritional factors:

This was the major focus of our study. As mentioned in methods we used 24 hour recall and SFFQ to assess dietary intake in these pregnant women. We are aware of the limitations of these techniques which could underestimate or overestimate in different clinical situations. However, studying adequate numbers and careful attention during interview helps reduce these errors. Comparison of different methods also helps. We also compared the estimated intakes with ICMR nutritional guidelines which give RDA for some of the components of the diet for pregnant Indian women (2010).
5.2.1. Macronutrients:

Of the macronutrients, GDM women consumed higher amounts of calories and fats but lower amounts of protein and total fiber compared to the NGT women. Our findings are similar to many of these studies in GDM women across the world.

5.2.1.1. Carbohydrate intake: Total carbohydrate intake was higher in GDM compared with non-GDM mothers (332.1 vs. 271.1 grams/day). There is no RDA for carbohydrate intake. However V. Seshiah in his book (diabetes and pregnancy, 2012) has suggested 175 gm daily carbohydrate intake is sufficient during pregnancy.

Zhang Cuilin et.al, 2010 suggest that the quality of dietary carbohydrate (complex carbohydrate with low glycemic index) intake may be more relevant to GDM risks than the total amount of this nutrient.

5.2.1.2. Protein intake: Similar number of women consumed vegetarian and non-vegetarian diets in the two groups. The protein intake was lower in GDM group, and much lesser than the RDA (63.76 vs. 78 g/day). The reasons for lower protein intake are not obvious but could be related to socio economic status, religion, and to personal beliefs.

In a study of pregnant women in Mysore 2010, Khoushabi et.al reported mean protein intake of pregnant mothers as 56.2 g/day. The protein intake in the present study is higher. These differences could be related to various socio demographic factors as described above. Wei Bao et.al 2013, found that higher intake of animal protein, in particular red meat, was significantly associated with a greater risk of GDM. By contrast, higher intake of vegetable protein, specifically nuts, whole grains and legumes was associated with a significantly lower risk, in this ten years follow up study.

They suggested that substitution of animal source protein by vegetable protein, might lower risk of GDM. This needs to be tested in clinical trials with careful attention to their habitual dietary intake. Additional studies will be necessary to determine the role of protein diets in pregnancy with GDM, to explain apparent paradox that the predominantly vegetarian Indian women have a very high risk of GDM.
5.2.1.3. Fat intake: The women in this study reported a very high fat intake. This was much higher than the RDA in both the groups. It is possible that there are methodological issues of over estimation.

There is evidence that high fat intake leads to GDM (Moses RG et.al, 1997). Saldana et.al 2004, in a cohort of 1698 pregnant women from North Carolina also found an association between increased fat intake and the glucose intolerance during pregnancy.

Moses et.al also found that higher fat intake was associated with a recurrent GDM. Wang et.al found a lower intake of PUFA in GDM women, showing the importance of type of fat in the diet. Present study findings are broadly in agreement with their results.

Restriction of dietary fat (<30%) has been advised to prevent GDM which will also reduce cardiovascular risk (Banerji S et. al, 2012).

5.2.1.4. Calories intake: Total calorie intake of GDM mothers was higher compared with NGT mothers as well to the RDA. Park S et.al, 2013 concluded that ‘high energy and saturated fat intakes were common risk factors for GDM and pregnancy outcome such as large for gestational age. Daily reference intakes for energy and fat during pregnancy need to be re-evaluated according to pre-pregnancy BMI’.

5.2.1.5. Total fiber intake: GDM mothers consumed lower amount of total fiber in their daily diet. Unlike our study, Reece E.A. et.al, were unable to find an association between fiber intake and glucose tolerance in pregnancy. Increasing intake of metabolically active dietary fiber could be another preventive measure for GDM.

Halperin and Feig (2014) suggested that dietary approaches may be more successful than exercise in preventing GDM. However the situation in India demands a careful attention to both aspects of energy metabolism to reduce the risk of GDM.

5.2.2. Micronutrients:

5.2.2.1. Vitamin C intake: This was the special focus of this study. Vitamin C is a reducing agent (Srilaxmi B, 2006, Mahan L.K, 2008) that functions in the body as antioxidant, scavenging free radicals. About 60-70 mg vitamin C intake per day will meet the adult requirement. Additional intakes (20-40% increase) are recommended for women during pregnancy and lactation. (Satyanarayana U, et.al 2006). It’s interesting that
the efficiency of enteric absorption of the ascorbic acid is high (80-90%) at low intake but declines markedly at intakes greater than about 1g/day (Mahan L.K, et.al, 2008). Vitamin C is affected by type of cooking, storage, and chopping which reduce vitamin C content of food. Hence availability of vitamin C depends on content but also on processing. Loss of vitamin C during conventional cooking process has been reported to the extent of 52-82% (N.A.S. Institute of Medicine. 1997). Higher dietary intake of vitamin C by 24 hour diet recall compared to SFFQ could be due to that women are more likely to remember their intake of foods in last 24 hour rather than in the distant past.

We found that lower intake of vitamin C was associated with GDM which is a novel finding for Indian women. There are only a few studies of vitamin C intake and GDM. A few studies have reported on circulating vitamin C concentrations in GDM. Our results are consistent with those of Cuilin et.al, 2004 who used similar methods of conventional 24 hour diet recall and SFFQ to assess nutritional intake. They found an association of lower vitamin C intake with GDM. It is important to note that in our SFFQ, we enquired about dietary intake over the period of whole year.

In a meta-analysis of 14 studies (not including GDM) the effect of dietary vitamin C and E supplementation on plasma glucose, HbA1c and insulin concentration was assessed. Vitamin C didn’t have a significant effect on plasma glucose levels and insulin concentrations (Akbar S, et.al, 2011), but led to reduction of HbA1c, suggesting that “antioxidants may have some benefit in protecting against the complications of T2DM”. On the other hand, a 6 week vitamin C supplementation study with a dose of 1000mg/day suggested that vitamin C helps reduce fasting and post prandial oxidative stress and may help in preventing diabetes related complications (Mazloom Z, 2011).

Vitamin C rich foods in this population included Gooseberry, Guava, Lime, Orange, Papaya, Lmeon, Pineapple, Custerd apple, Strawberry, Mango, Pomagranate, Capsicum, Coriander leaves, Cabbage, Bitter guard, Cauliflower, Fenugreek leaves, Tomato, Spinach, Potato, Green chilli, Tamarind pulp, Drum stick leaves, Turnip, Carrot, Amaranth, and Bengal gram sprouts. Intake of these is influenced by various factors like socioeconomic status, personal likes and dislikes, cultural taboos, availability and
seasonality. As can be seen in our study many of these were different in the GDM and NGT mothers.

Further studies in this field are warranted to understand the role of dietary vitamin C for diabetes prevention.

5.2.2.2. HORAC intake: was not associated with GDM and the consumption level was in the same range for GDM and NGT subjects, may be due to intake of spices as daily source of antioxidant which was approximately same for both groups. This is the first study to assess the association of HORAC foods intake with GDM in Indian women.

Most studies of antioxidant in pregnancy have been heterogeneous and with no conclusive recommendations. Possible reasons for the conflict in results of antioxidant studies, has been explained in detail by Benzie, 2005 as follows:

'1. Antioxidants are likely to work with each other, more of one may increase the need for another.

2. The action of an antioxidant within a heterogeneous food matrix maybe different from that in pure supplement form.

3. A high intake of antioxidants may help to promote health when taken regularly over decades but may have little discernible effect over a few months or years.

4. Benefits of increased antioxidant intake maybe seen only in those with marginal or depleted antioxidant starts at baseline.

5. Antioxidant action per se may not be the key mechanism of action of protection, for example, immune-modulatory, anti-inflammatory, anti-proliferative and pro-apoptosis effect of dietary agents (antioxidants or otherwise) maybe more relevant to overall effects in terms of disease risk'.

5.3. Blood biomarkers:

5.3.1. Plasma vitamin C:

As already mentioned ours is one of the few studies to measure dietary vitamin C intake and also circulating vitamin C levels in normal glucose tolerant and gestational diabetes women. We found that plasma vitamin C concentration in GDM mothers was much lower than that in the NGT group (45.9 vs. 95.2μmol/l). There is no universal
agreement on ‘deficient’ levels of vitamin C, therefore we are not able to comment on this aspect. However the distribution of vitamin C levels in the GDM women was substantially shifted to the left compared to that in the NGT women. The role of processing of food on availability of vitamin C has already been discussed. Despite this limitations we found a high correlation between dietary intake and plasma vitamin C concentrations ($r=0.78$, $p<0.001$). It is to be noted that our estimates of dietary vitamin C intake include those obtained from supplements. Plasma vitamin C concentration in people who regularly consume vitamin C supplements were 60-70% higher than those who do not take supplements, 70-80 and 45-50 μmol/l respectively (Machlin, L.J., 1991).

Another consideration is the plasma volume expansion and changes in erythrocyte mass during pregnancy which affects circulating levels of different nutrients. Srilaxmi (2007) have cited in her book that ‘Blood volume expands by 50% resulting in a decrease in hemoglobin levels, blood glucose values and serum levels of albumin, other serum proteins and water soluble vitamins. The decrease in water soluble vitamin concentration makes determination of an inadequate intake or a deficient nutrient state difficult.

Plasma vitamin C concentrations were strongly and inversely related to BMI, such that overweight and obese women had low vitamin C concentrations. This finding is similar to that described for number of other nutrients (vitamin B12, vitamin D etc). It is important to remember that obesity can therefore add as a confounding factor in various associations of vitamin C levels, and therefore due care has to be taken in statistical analysis and interpretation. In addition there were strong, inverse relationships between plasma vitamin C concentration and stress level as well as pre-pregnancy physical activity. Therefore, the associations of vitamin C with metabolic parameters need to take these confounding in to consideration.

On this background, Cuilin Z et.al. (2004) found results consistent with findings of present study. They discovered that plasma ascorbic acid concentrations were inversely associated with the risk of GDM.

An additional factor is that high glucose levels increase oxidant stress and therefore reduce levels of antioxidants, i.e. a reverse causality. This explanation needs to be kept in mind to interpret the vitamin C and glucose associations. Machlin, (1991) found that
diabetic patients have an increased turnover rate and decreased vitamin C status compared to non-diabetic individuals, which is coherent with this thinking.

There is scarce information available for plasma vitamin C level of GDM mothers worldwide. In view of a potential causal role and therefore a potential for prevention, there is a need for further investigations of the role of vitamin C in the etiology of type 2 diabetes by supplementation trials.

5.4. Outcomes:

In current research, GDM mothers had statistically significant higher rate of premature delivery and C-section. Their neonates experienced higher rate of LGA, admission to NICU, hypoglycemia, and respiratory distress syndrome (RDS). Plasma vitamin C concentrations were inversely associated with premature delivery, but there was no association with neonatal birth weight, and other outcomes.

5.4.1. Prematurity: In present research GDM mothers had lower gestation days at delivery, which means higher rate of premature deliveries. There was a direct association between plasma vitamin C levels and gestation days at delivery. The results remained significant even after adjustment for confounders (age, total family income, pre-pregnancy BMI, pre-pregnancy physical activity, stress, GDM).

Only one study has reported association between maternal vitamin C level and pre-term delivery. In that study vitamin C supplementation did not reduce spontaneous preterm birth (Hauth JC et al, 2010). Hedderson et al. (2003) showed that “The risk of spontaneous preterm birth increased with increasing levels of pregnancy glycemia”. It will be relevant to study the contribution of micronutrient nutrition (vitamin C and others) to this association.

5.4.2. C-section: GDM mother experienced higher rate of C-section in compared with NGT mothers, which is consistent with findings of Gorgal et al 2011, and Donovan et al 2011. The reason is due to macrosomic babies of these mothers, and an iatrogenic intervention by obstetritians because of the fear of unexplained intra uterine deaths.

5.4.3. LGA and neonatal birth weight: There was no association between plasma vitamin C and neonatal birth weight. Even though Lee et al. (2004) found that maternal serum vitamin C levels during second trimester of gestation were correlated with...
birth weight in full term babies. Since vitamin C level affect body iron status, and maternal iron level affect neonatal birth weight, may be that is the indirect reason of above research findings. In another study (PMNS) Rao et.al, 2001, demonstrated a relationship with dietary intake of micro-nutrient rich foods (especially vitamin C and iron) with birth size. A prospective cohort study in south England, among 693 nulliparous singleton pregnant (Mathew F 1999) showed that vitamin C was the only nutrient predictive of birth weight after adjustment for height and smoking, and that each mg increase in vitamin C was associated with 50.8 grams increase in birth weight.

5.4.4. Neonatal admission to NICU: Neonates of GDM mothers experienced higher rate of admission to NICU, this findings was similar with Donovan et.al, 2011. It could be due to higher rate of morbidity in neonates of GDM mothers like hypoglycemia, RDS, etc, or due to a tendency for preventive admission for observation in a tertiary care center.

5.4.5. Neonatal hypoglycemia:
Almost half of the neonates of GDM mothers were detected to have a mild hypoglycemia. This is a well known complication of diabetic pregnancies. In addition regular screening in a tertiary care NICU also increases the prevalence. Not many of the neonates of non-diabetic mothers are screened for hypoglycemia, therefore, background rates are not very clear. Flores-Le-Roux, et.al, (2011) reported a higher rate of hypoglycemia in neonates of GDM mothers, and commented that mild and moderate neonatal hypoglycaemia is common in infants of women with GDM. Two common factors which increase the risk of hypoglycemia include: poor maternal peripartum glycaemic control and LGA.

5.4.6. Neonatal RDS: The rate of RDS was higher in neonates of GDM comaperd with neonates of NGT mothers. In the majority it was mild and very few needed assistance with ventilator. There are very few articles with focus on maternal GDM and neonatal RDS.
5.5. The strength and limitation of current study:

5.5.1. Strengths: To our knowledge this is the first study to examine the relation of dietary vitamin C, plasma vitamin C, and HORAC simultaneously to study the association with GDM and pregnancy outcomes in India.

5.5.2. Weaknesses: Assessing micronutrient status in human samples is challenging due to its unstable nature. Ascertainment of causality was not possible due to observational nature of study. Since diet recall was based on self-report, hence under/over estimation of total intake is possible. We do not expect a systematic error, although 114 of 272 participants refused to take part, since there were no differences in their age, socio economic status, weeks of gestation, BMI in these two groups.

In view of the limitations of design (small size, observational, a single measurement of vitamin C along with glucose levels) it is difficult to comment on the causality of the associations with maternal glycemia and pregnancy outcomes. Therefore this can be looked upon as hypothesis generating.

5.6. Suggestions for further studies:

There is a lot of hype about potential benefits of vitamin C, but not enough good scientific evidence to support a recommendation for or against taking it for diabetes prevention. Further studies in this field are warranted.

Replication of study with larger sample size and prospective serial measurements starting from before pregnancy may help to understand the causality of the relationships. This will guide appropriate interventions to reduce risk of GDM and prematurity.

5.7. Contribution to knowledge / society:

Our study is a preliminary investigation to elucidate the association between maternal antioxidant intake and her risk of gestational diabetes and pregnancy outcomes. We found that dietary intake of vitamin C of GDM mothers was low and much below the RDA. Low vitamin C intake reflected in low vitamin C levels in the blood and was associated with higher plasma glucose and adverse pregnancy outcomes, including prematurity. Even though causality cannot be certain, our study suggests that all women preparing for pregnancy and during pregnancy need to increase the dietary intake of
vitamin C to match the RDA. This could reduce the risk of GDM and prematurity. This needs to be tested in a large prospective study and subsequently in a clinical trial.