A Study of Complement C3 and Immunoglobulin G levels in Gestational Diabetics and their Newborns

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ABSTRACT

Background & Objectives: *Gestational Diabetes Mellitus (GDM) is one of the most common medical complications of pregnancy. Complement 3(C3) is the central component of the complement system which induce inflammatory, immunomodulatory and metabolic responses. This passive immunity acquired by the fetus is crucial for the adaptation of the newborn to the extra uterine environment, providing protection against infections. The Objectives of the present study was to estimate the serum levels of complement3 (C3) & Immunoglobin (Ig) G in Gestational Diabetic and their newborns, and to compare the above parameters with normal pregnant females and their newborns.*

Material & Methods: *Total 90 pregnant females aged between 20-40yrs with regular antenatal care at Cheluvamba Hospital & Mission Hospital, Mysore, were included in the study. Out of 90, 45 were diagnosed with gestational diabetes. About 4ml of blood sample was collected from the mother during the time of labour and 4ml of cord blood was collected from their newborns and serum was analysed for complement C3 and Immunoglobin (Ig) G by immunoturbidimetric method.*

Results & Interpretation: *The study showed significant increase in complement C3 levels in Gestational Diabetes, when compared with normal pregnancy with mean and standard deviation of 190±14.4 and 105±11.2 respectively. There was significant decrease in Immunoglobulin G levels in cord blood of newborns of gestational diabetes when compared with cord blood of newborns of normal pregnancy with mean and standard deviation of 790.4±138.7 and 974.0±144.3 respectively with p value <0.05.*

Conclusion: *Complement C3 estimation in GDM could help to understand the underlying chronic inflammation which in turn affects the growing fetus innate immune system and predisposes the individual for future Diabetes and its complications.*

Key Words: *Gestational Diabetes, Complement C3, Immunoglobulin G, Inflammation, Innate Immunity.*

INTRODUCTION

Gestational diabetes mellitus (GDM) is one of the most common medical complications of pregnancy. The disease has important health implications for mother and child. The prevalence of gestational diabetes mellitus is increasing worldwide and more so in developing countries including India which may range from 2.4 to 21 per cent of all pregnancies[1]. GDM is a disease of the pancreatic β cells, which do not produce sufficient insulin to meet the increased requirements of late pregnancy. Gestational diabetes mellitus can adversely influence intrauterine fetal development and as long-term complication increases the risk of insulin resistance. GDM are at high risk of developing diabetes mellitus later in life[2]. Thus, GDM can be reasonably considered to be a form of pre-diabetes that manifest as metabolic syndrome with cardiovascular event. An estimated 10% of women with GDM have diabetes mellitus soon after delivery. The rest develop diabetes mellitus at rates of 20–60% within 5–10 years. Overt maternal diabetes mellitus can adversely influence intrauterine fetal development. Spontaneous abortions and major congenital anomalies may be induced in the first trimester. Excessive fetal growth, neonatal hypoglycemia, jaundice, polycythaemia and stillbirth may occur during the second and third trimesters [3].

The complement system, a complex protein network initially identified as part of the innate immune system, is as an essential regulator of cell and tissue homeostasis. Complement 3(C3) is the central component of the complement system which induce inflammatory, immunomodulatory and metabolic responses. C3 has been implicated in metabolic disorders, notably adiposity, dyslipidemia, insulin resistance, liver dysfunction and diabetes, and C3 is increasingly recognised as a cardiometabolic risk factor [4]. The complement system consists of a tightly regulated network of proteins that play an important role in host defense and inflammation. Complement activation results in opsonization of pathogens and their removal by phagocytes, as well as cell lysis. C3-Cross-sectional studies have reported strong correlations between plasma levels of complement C3, insulin and glucose. The relation between C3 and incidence of diabetes could reflect a systemic low-grade inflammation and the actions of these cytokines. The relation between diabetes and inflammation could also be associated with hepatic production of glucose. Type 2 diabetes is associated with abnormalities in hepatic glucose production.

Most blood glucose and plasma proteins originate from the liver, and the synthesis is regulated by IL-6 and other cytokines. Deregulation of hepatic glucose production in combination with insulin resistance could contribute to the relation between inflammatory proteins and diabetes [5]. Besides being produced in the liver, like other acute-phase protein, C3 is also synthesized by activated macrophages and adipocytes therefore behaving as an inflammatory cytokine and an adipokine. Its hepatic production is induced by primary wave cytokines, such as interleukin-1 and tumor necrosis factor, which may interfere with insulin receptor functioning and cause insulin resistance[6].

The essential components of humoral immunity are complement and circulating immunoglobulin. Maternal antibodies Ig G are transported across the placenta which protects the newborn. This passive immunity acquired by the fetus is crucial for the adaptation of the newborn to the extra uterine environment, providing protection against infections[7]. Hyperglycemia alters Ig G transfer across the placenta and decreases immunoglobulin levels in maternal blood and colostrum. Maternal diabetes alters the transfer of antibodies through the placenta and colostrums. The reduction in immunoreactive protein production may be related to changes in the metabolism of carbohydrates, lipids, and proteins, as well as in various organ systems caused by the hyperglycemic status of pregnant women [8]. In view of unavailability of data, especially in South India, this study was undertaken to evaluate the importance of estimation of complement C3 and IgG levels in gestational diabetics and their off springs who are the most important target groups to be identified for development of diabetes in future. Hence the present study was undertaken to know the pre-diabetic status and immune status of mother and their newborns.

OBJECTIVES

1. To estimate the serum levels of complement3 (C3) in Gestational Diabetic and their newborns.
2. To estimate the serum levels of Immunoglobulin G in Gestational Diabetic and their newborns.
3. To compare the above parameters with normal pregnant females and their newborns.

METHODOLOGY

A cross sectional study was done among pregnant females with regular antenatal care at Cheluvamba hospital & Mission Hospital, Mysore. Sample size was calculated with confidence level of 95% taking standard deviation of serum IgG levels as 267.5 as coated by Ramdenee et al [8]with allowable error of 30%.The sample size in each group were 45.

Inclusion criteria: All diagnosed cases of gestational diabetes aged between 20-40yrs according to American Diabetic Association (ADA) criteria for 75g, 2-h OGTT were included as GDM[9] and aged matched normal pregnant females with regular antenatal care were selected as controls.

Exclusion criteria: Those with history of type 1DM, type 2DM, Hypertension, Cardiovascular disease and other immunocompromised state were excluded from study.

Data collection: Data regarding age, occupation, diet, gestational age, family history of DM, Hypertension, BMI, Blood pressure, blood glucose levels, and others were collected by using pretested semi-structured questionnaire. Data of newborns like type of delivery, birth weight, any complications and APGAR score were noted from the case sheet. Ethical clearance was taken from the Institutional Time Bound Research Committee. A written informed consent was taken from the subjects.

Sample Collection: About 4ml of blood sample was collected from the mother during the time of labor and 4ml of cord blood was collected from their newborns. Serum was separated after centrifugation from both the samples and was stored under -20ºC until it was analyzed.

Methodology: Serum samples was analysed for Complement C3 and Ig-G by Immunoturbidimetric method using Transasia XL 600 fully automated chemistry analyser. Anti human C3 and Ig G antibodies when mixed with samples containing C3 and Ig-G, form insoluble complexes. These complexes cause an absorbance change, depending upon the C3 and Ig-G concentration of the patient sample which was quantified by comparing with the calibrated known C3 and IgG concentration respectively [10].

STATISTICAL ANALYSIS

The results are expressed as Mean ± Standard deviation. $p<0.05$ was considered statistically significant. Statistical analysis was performed using Epi info software and for test of significances student t' test was used.

DISCUSSION

Gestational diabetes mellitus (GDM) carries a small but potentially important risk of adverse perinatal outcomes and a long-term risk of obesity and glucose intolerance in offspring. The β- cell defects that underlie GDM occur due to obesity and chronic insulin resistance. These lead many clinicians to view GDM as a form of evolving type 2 diabetes mellitus (T2DM). Several inflammatory markers have been associated with the incidence of diabetes, including C-reactive protein (CRP) orosomucoid, sialic acid, and interleukin (IL-6). It has been

proposed that diabetes is a disease of the innate immune system [11].

The present study shows significantly increase in complement C3 levels in Gestational Diabetes, when compared with normal pregnancy with mean standard deviation 190±14.4 and 105±11.2 respectively, which is in agreement with the study done by Gunnar Engstro etal[12] and Schmidt M I etal[13]. Their study showed that C3 was associated with a markedly increased risk of developing diabetes, which remained after adjustments for HOMA-IR, BMI, and several markers of inflammation. This was the first study to relate the incidence of diabetes in relation to C3.

Complement C3 and C4 are the major plasma proteins of the immune system complement pathways. The synthesis of these proteins is increased in response to inflammation and infection. Both C3 and C4 have shown substantial correlations with obesity, and high gene expression of these complement components has been reported in omental adipose tissue in obese men[14]. High C3 levels have been reported in subjects with diabetes and insulin resistance. C3 is mainly produced in the liver in response to proinflammatory cytokines (e.g., IL-6 and IL-1). The functions of the complement system include control of inflammatory reactions and chemo taxis, clearance of immune complexes, cellular activation and antimicrobial defense. High gene expression of C3 is also observed in omental adipose tissue[15,16]. Adipose tissue is an organ with endocrine functions, and proinflammatory cytokines formed in adipose tissue have been associated with impaired glucose uptake and insulin resistance. The relation between C3 and Gestational diabetes could reflect a systemic low-grade inflammation and the actions of these cytokines[17]. The study did not show any significant change in cord blood complement C3 levels of newborns.

The study showed statistically significant decrease in Immunoglobulin G levels in cord blood of newborns of gestational diabetes when compared with cord blood of newborns of normal pregnancy with mean standard deviation 790.4+138.7 and 974.0+144.3 respectively. Similar observations have been reported by earlier studies. The lower levels of cord serum IgG is probably due to depression of Ig G synthesis or formations of immune complexes[18, 19.]

The study showed an association with increasing age, higher parity, family history of diabetes, literary and among above poverty line population. These results are in support of study done by Rajput et al & Seshiah V et al.[19,20]. Hence based on the results derived from the present study it could be inferred that an increase in serum Complement C3 in gestational diabetics could predict the ongoing inflammatory changes which could explain a state of pre-diabetes in them which in turn affects the immune system of newborn. Thus, GDM can be reasonably considered to be a form of pre-diabetes that manifest as metabolic syndrome with cardiovascular event. This Complement C3 estimation in GDM could help to understand the underlying chronic inflammation which in turn affects the growing fetus innate immune system and predisposes the individual for future Diabetes and its complications. By understanding the genetic and pathophysiological underpinning these differences may be useful in developing targeted approaches to prevent both GDM and diabetes mellitus after GDM in mothers and their offspring.

RESULTS

In the present study the baseline characteristics of the subjects are shown in Table 1. The mean age of participants was 25.62 ± 3.42 yr. The occurrence of GDM was seen to be higher in women aged between 26-30y, literate, belonging to above poverty line [APL], BMI of ≥25 kg/m^2 & in mothers of newborn with birth-weight more than 3.5 kgs.

Table 1: Baseline characteristic of study population

Characteristics	Gestational Diabetes	Normal Pregnancy
Age(years)		
18-20	5	3
21-25	10	22
25-30	20	10
>30	10	10
Parity		
0	10	7
1	15	21
2	15	09
≥3	05	08
Education		
Graduate	3	7
Above Middle School	24	20
Primary School	12	10
Illiterate	6	8
Socio Economic Status		
BPL	20	23
APL	25	22
BMI in Kg/m^2		
<25	15	25
≥25	30	20
Birth Weight of New Born		
<3500gm	10	25
>3500gm	35	10

Table-2 shows significantly increase in complement C3 levels in Gestational Diabetes, when compared with normal pregnancy with mean and standard deviation of 190±14.4 and 105±11.2 respectively

Table 2: Maternal and cord sera C_3 levels in Gestational Diabetes and Normal Pregnancy

Group	Subjects	C_3 levels in Maternal serum (mg/dl) Mean ±S.D	C_3 levels in Cord serum (mg/dl) Mean ±S.D
Gestational diabetes	45	190±14.4	102±13.6
Normal Pregnancy	45	105±11.2	98±10.3
p value		<0.05	>0.05

Test of significance: Students t test, p value <0.05 is significant.

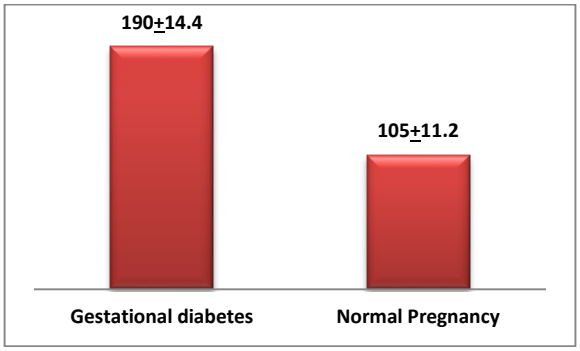

Figure 1: Mean Serum Complement C_3 levels in Gestational Diabetes and Normal Pregnancy

Table 3 shows significant decrease in Immunoglobulin G levels in cord blood of newborns of gestational diabetes when compared with cord blood of newborns of normal pregnancy with mean and standard deviation of 790.4+138.7 and 974.0+144.3 respectively

Table 3: Maternal and cord sera Ig G levels in Gestational Diabetes and Normal Pregnancy

Group	Subjects	IgG levels in Maternal serum (mg/dl) Mean ±S.D	IgG levels in Cord serum (mg/dl) Mean±S.D
Gestational diabetes	45	1298.2+175.2	790.4+138.7
Normal Pregnancy	45	1348.5+184.6	974.0+144.3
p value		>0.05	**<0.05**

Test of significance: Students t test, p value <0.05 is significant.

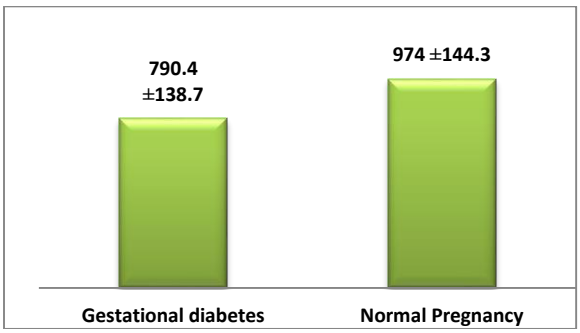

Figure 2: Mean Cord sera IgG levels in New Born of Gestational Diabetes and Normal Pregnancy

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