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A COMPARATIVE STUDY ON SPECIFIC SERUM IMMUNOGLOBULIN G AND COMPLEMENT C3 LEVELS IN GESTATIONAL DIABETICS AND NORMAL HEALTHY PREGNANT WOMEN AND ITS COMPLICATIONS DURING PREGNANCY.

MATHEW GEORGE[1], LINCY JOSEPH[2], LEENA P.N[3], JEENA VARGHESE*

1. Department of Pharmacology, Pushpagiri College of Pharmacy, Thiruvalla-689107, Kerala, India.
2. Department of Pharmaceutical Chemistry, Pushpagiri College of Pharmacy, Thiruvalla-689107, Kerala, India.
3. Department of Pharmacognosy, Pushpagiri College of Pharmacy, Thiruvalla-689107, Kerala, India.

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Abstract: Gestational diabetes mellitus is one of the most common medical complication and a metabolic disorder that occurs during pregnancy. The complement and circulating immunoglobulins are essential components of humoral immunity. Maternal antibodies Ig G are transported across the placenta which protects the newborn. The Objectives of the present study was to estimate the serum levels of Immunoglobin (Ig) G and complement3 (C3) in Gestational Diabetic, and to compare the above parameters with normal healthy pregnant women. And also note its complications during pregnancy. Total of 60 pregnant women aged between 18 -35 years were participated in the study. About 3ml of residual blood was collected and estimation of parameters was done in semi-automatic analyser. The study showed significant increase in complement C3 levels in Gestational Diabetes, when compared with normal pregnancy. And there was significant decrease in Immunoglobulin G levels in f gestational diabetes when compared with normal pregnancy with p value <0.01. Some noticeable complications were acanthosis nigricans, hypertension, and infections like urinary tract infection. The complications were seen in both test and control group. Pregnant women with gestational diabetics were more chance to develop these complications when compared with normal healthy pregnant women.

Keywords: Gestational diabetes mellitus, immunoglobulin G , complement C3, immunity, complications

Corresponding Author: MS. JEENA VARGHESE

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INTRODUCTION

Gestational diabetes mellitus (GDM) is one of the most common medical complications and a metabolic disorder that occurs during pregnancy. The disease has important health implications for mother and child. Gestational diabetes is a type of diabetes and is defined as impaired glucose tolerance (IGT) with onset or first recognition during pregnancy. Having diabetes means the amount of sugar in your blood is higher than normal. GDM is a disease of the pancreatic β cells, which do not produce sufficient insulin to meet the increased requirements of late pregnancy. [(1) (8)] immunoglobulin (Ig),is a large, Y-shaped protein produced mainly by plasma cells that is used by the immune system to identify and neutralize pathogens such as bacteria and viruses. The antibody recognizes a unique molecule of the harmful agent, called an antigen, via the Fab's variable region. Each tip of the "Y" of an antibody contains a paratope (analogous to a lock) that is specific for one particular epitope (similarly analogous to a key) on an antigen, allowing these two structures to bind together with precision.

The complement system is an ancient component of the innate immune system; some of its major proteins appeared in echinoderms and exist in each deuterostome .The complement system contains more than 30 proteins in various tissue fluids and on the surface of cells, including proteins of the enzymatic cascade, regulators and receptors. [(7)] 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.

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. The essential components of humoral immunity are complement and circulating immunoglobulin[(2)]. 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 [(4)]. 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 immune reactive 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. [(5)]

Recent advances in transplantation immunology have stimulated considerable interest in immunologic aspects of pregnancy. Pregnancy maybe considered as a state wherein the foetus

exists as a well tolerated homograft. It is possible that the foetus escapes the process of rejection due to depression of maternal immune responses. Conversely, a disturbance in immunologic tolerance may lead to abortion. [4]

Suppression of maternal immune response may be one of the factors contributing to continuation of pregnancy, a state in which the foetus exists as a well-tolerated homograft. Pregnancy changes affecting disease severity can be attributed to placental or maternal hormones, increased circulation, increased fluid volume, metabolic rate, hemodilution, circulating fetal cells, or other factors. The C3 level was associated with maternal leukopenia, elevated serum C-reactive protein (CRP) elevation, hematuria, hypertension, and preterm premature rupture of membranes. The C4 level was associated with maternal proteinuria, hematuria, hematologic disease etc.[3]

MATERIALS AND METHOD

STUDY DESIGN: Prospective comparative study.

STUDY POPULATION: Patients reported in department of Gynaecology.

STUDY SETTING:

- Obstetrics and Gynaecology department of pushpagiri medical college hospital, Thiruvalla
- Pushpagiri college of pharmacy, Thiruvalla

SAMPLE SIZE: 60 patients,

30 - Normal healthy pregnant women

30 - Women with gestational diabetes mellitus

Statistical Equation

$$n = 2\,Sp^2\,(Z_{1-\alpha/2} + Z_{1-\beta})^2 \,/\, \mu^2 d$$

$$Sp^2 = S_1^2 + S_2^2 \,/\, 2$$

Where,

$S1^2$ = Standard deviation in the first group

S2 2 = Standard deviation in the second group

μ2 d = Mean difference between the samples

α = Significance level

1-β = Power

STUDY CRITERIA

➢ ***INCLUSION CRITERIA***

- Diagnosed cases of gestational diabetics
- Aged between 18-35 years
- Gestational age > 23 weeks
- Primi /multi gravid

➢ ***EXCLUSION CRITERIA***

- Those with history of type 1DM, type 2DM,Cardiovascular disease
- Patients with all maternal abnormalities, known renal disease hypertension, hepatic dysfunction, alcoholism, dyslipedemia

After getting approval from the Institutional Ethics Committee, a hospital based prospective comparative study was conducted on the Gynecology department of Pushpagiri medical college hospital, Thiruvalla India. It will be a 6 month study in which patients were recruited based on inclusion and exclusion criteria.60 patients were included in the study. Out of this 30 are normal healthy pregnant women and remaining 30 are women with gestational diabetes mellitus. All the patients should be aware of brief introduction of the study. A written informed consent form has to be collected from the patient/care giver. Informations regarding demographic details of the patient ,socio-economic status ,body mass index, family history of Diabetes Mellitus, medication history , and complications should be noted.

Diagnosis Of Gestational Diabetes Mellitus: Diabetes was checked in the Obstetrics & Gynecology in the Obstetric ward .Oral glucose tolerance test is used. The patient is asked to take a glucose drink (75 gms of glucose) and their blood glucose level is measured before and at intervals after the sugary drink is taken.

Collection of samples: From the Biochemistry lab residual blood (about 3ml) was collected. Serum was separated by centrifugation and used for estimation of serum levels of Ig G and Complement C3. The concentration of serum Ig G and Complement C3 was analyzed by using analytical kits in semi-autoanaylzer. Patients were followed up in terms of maternal outcome (hypertension, Acanthosis nigricans etc).

A well designed demographic questionnaire data will be used to collect necessary information such as regarding age, occupation, obstetric history, past history along with family history and personal history related to exclusion criterion, general & systemic examination, gestational age in weeks, blood sugar level(FBS and PPBS),maternal complications.

DETERMINATION OF IMMUNOGLOBULIN G (Ig G)

Firstly, the Ig G calibrator must be reconstituted exactly with 1.0 ml of distilled water and wait for 5 minutes, gently swirl the vial till the solution attains homogeneity, (the concentration(S) On reconstitution with 1.0 ml distilled water is 900mg/dl).Then to prepare 1.0ml working standard (Sw) pipette out 112µl of normal saline and add 888µl of reconstituted calibrator .For preparation of standard, pipette out 500µl of R1 reagent and 5µl of working standard kept for 10 minutes at 37^0C, then add 50µl R2 reagent and kept in semiautomatic analyzer. For the preparation of test first the test specimen diluted with normal saline(1:10) , pipetted out 500µl of R1 and 5µl of sample serum and kept for 10 minutes at 37^0C, then add 50µl R2 reagent and kept in semiautomatic analyser. Measured at 340nm.

- R1-Quantia- Ig G activation buffer
- R2-Quantia-Ig G reagent

DETERMINATION OF COMPLEMENT C3

Firstly, the complement C3 calibrator must be reconstituted exactly with 1.0 ml of distilled water and wait for 5 minutes, gently swirl the vial till the solution attains homogeneity,(the concentration(S) on reconstitution with 1.0ml distilled water is 90mg/dl) .To prepare 1.0 ml working standard (Sw) take 600µl of normal saline and added to 400µl of reconstituted calibrator. For preparation of standard, pipette out 500µl or R1 reagent and 25µl working standard and kept for 10 minutes at 37^0c, then add 50µl of R2 reagent and kept it in the semi automatic analyser. For the preparation of test first the test specimen diluted with normal

saline(1:10) , pipette out 500µl of R1 and 25µl of sample serum and kept for 10 minutes at 37^0C, then add 50µl R2 reagent and kept in semiautomatic analyser. Measured at 340nm.

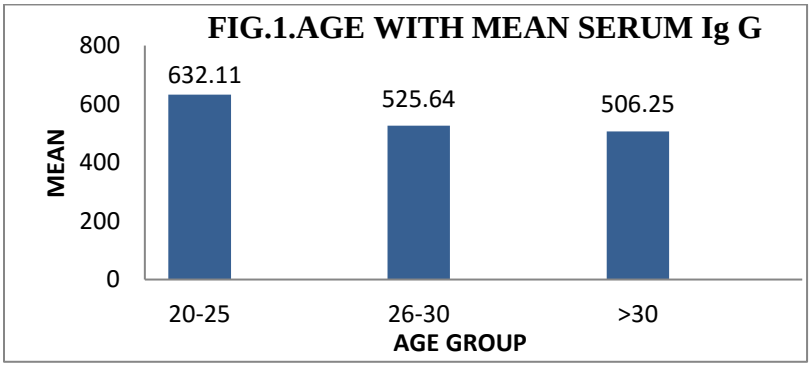
- R1- Quantia-C3 activation buffer ,R2-Quantia –C3 reagent

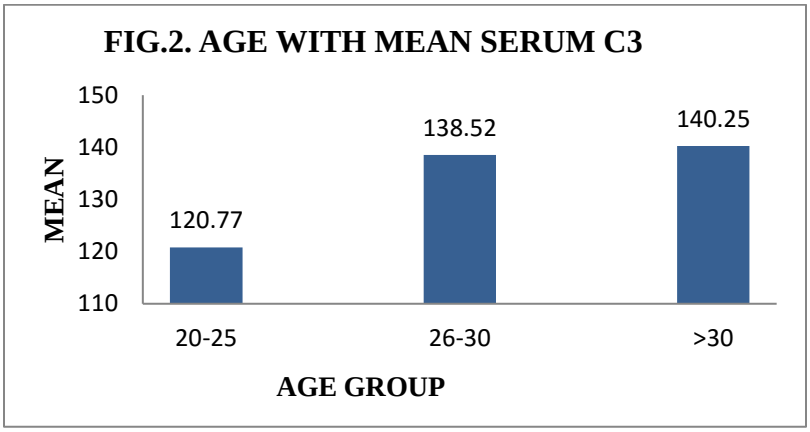
RESULTS AND DISCUSSIONS

In the 6 months study, a total of 60 pregnant women were participated. Out of this, 30 were normal healthy pregnant women and 30 were pregnant women with gestational diabetes. Patients were selected as per the inclusion and exclusion criteria. The Ig G and C3 levels were estimated along with other demographic details. The distribution of patients were based on their age, gravidity, period of gestation.BMI, family history of diabetes mellitus, history of GDM in previous pregnancy, economic status.

- In this study, majority of patients found in the age group 25-30. In test population majority of population in age group 25-30 and in control 20-25
- The majority of patients were multigravida (58.33%) and followed by primigravida (41.66%) .
- In body mass index, it was found that majority of patients were healthy (73.3%). Studies shows that BMI is an important risk factor for GDM.
- Period of gestation, majority of patients in the third trimester (28 weeks- up to birth).
- The majority of patients in the study population had family history of diabetes mellitus
- And most of the multigravida had history of GDM in previous pregnancy.

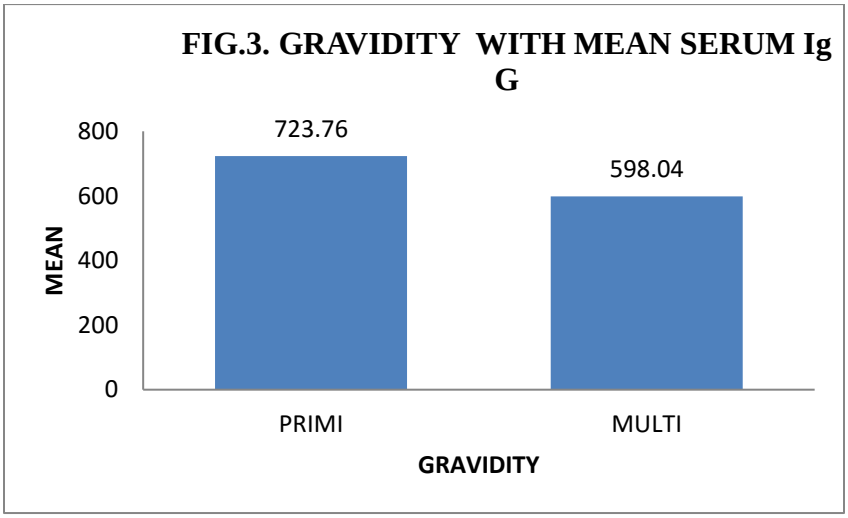
❖ **AGE WITH SERUM Ig G AND C3**

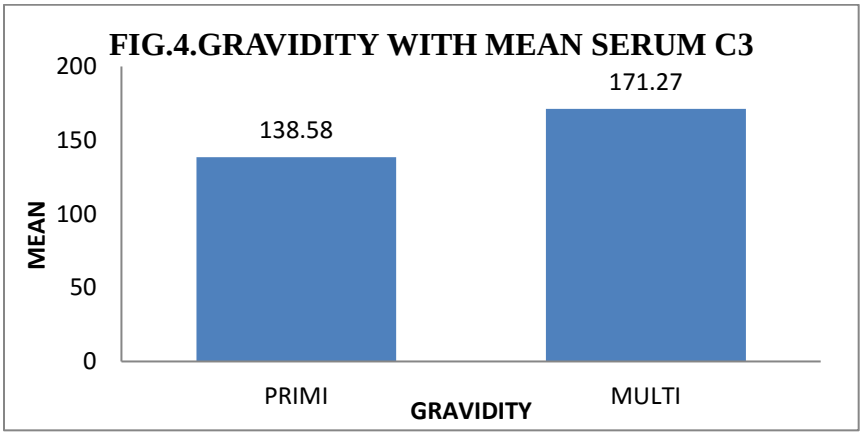




In this study, majority of patients found in the age group 25-30. In test population majority of population in age group 25-30 and in control 20-25.In correlation of age with serum Ig G and C3 , Ig G levels decreased and C3 level increased than normal, in pregnancy. Deepa K, Prithvi Naveen et.al [1] and many more studies suggest that Maternal age is an essential risk factor ,as increases the risk of GDM.

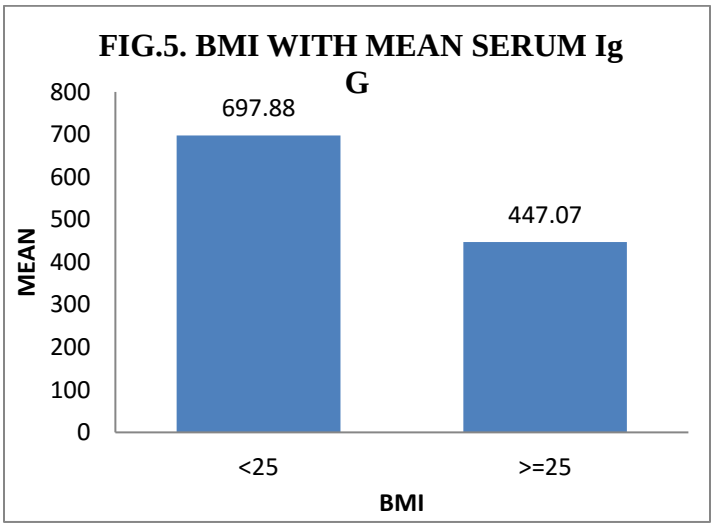
❖ **GRAVIDITY WITH SERUM Ig G AND C3**

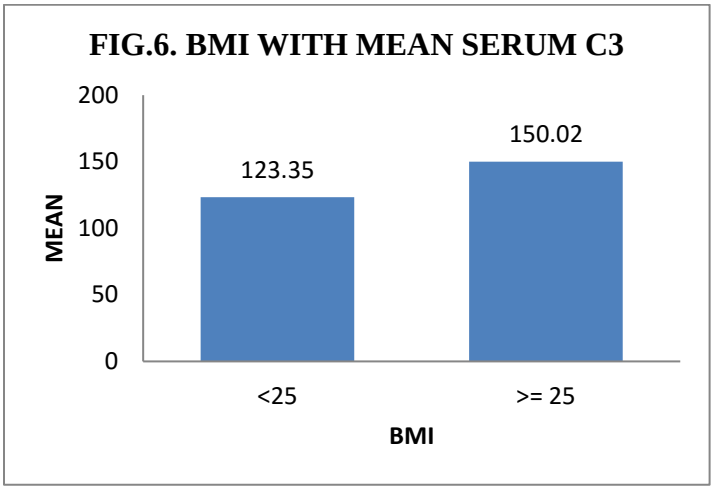




The majority of patients were multigravida (58.33%) and followed by primigravida (41.66%).The level of Ig G was decreased and levels of C3 increased in multigravida. The mean serum Ig G was 723.76 in muitigravida and 598.04 in primigravida.The mean serum C3 was 138.58 in primigravida and 171.27 in mutigravida.

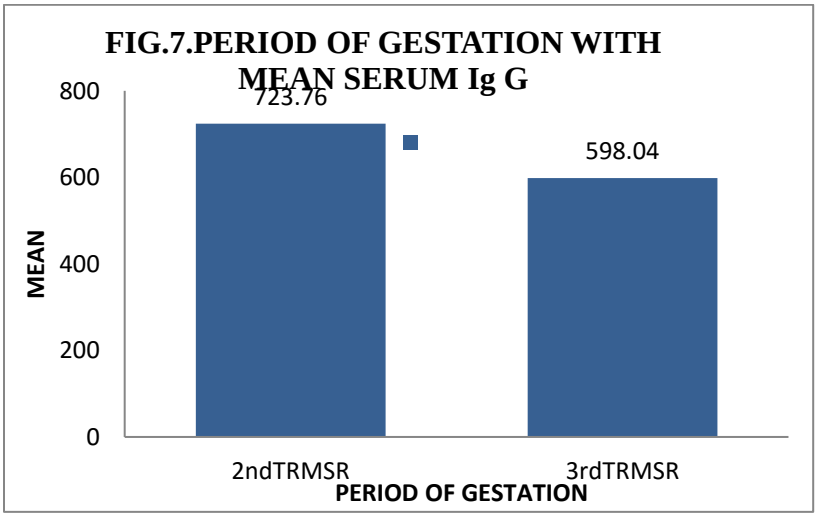
- **BMI WITH SERUM Ig G AND C3**

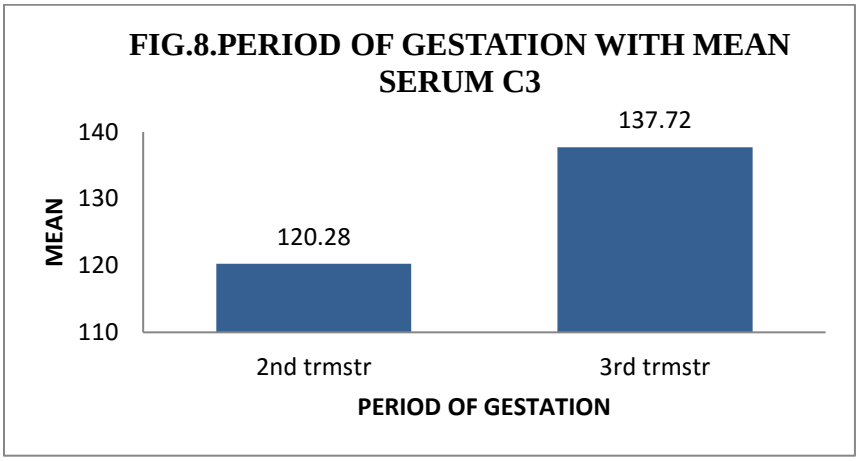




In body mass index, it was found that majority of patients were healthy (73.3%).The C3levels increases and Ig G level decreases with body mass index.The correlation of mean serum Ig G and C3 with BMI was given in above figures. Studies shows that BMI is an important risk factor for GDM.

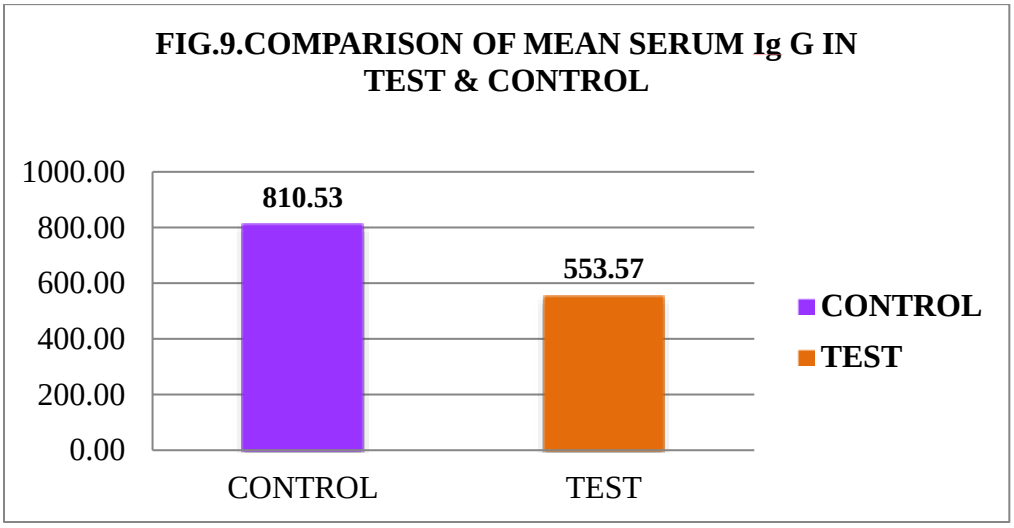
❖ **PERIOD OF GESTATION WITH SERUM Ig G AND C3**

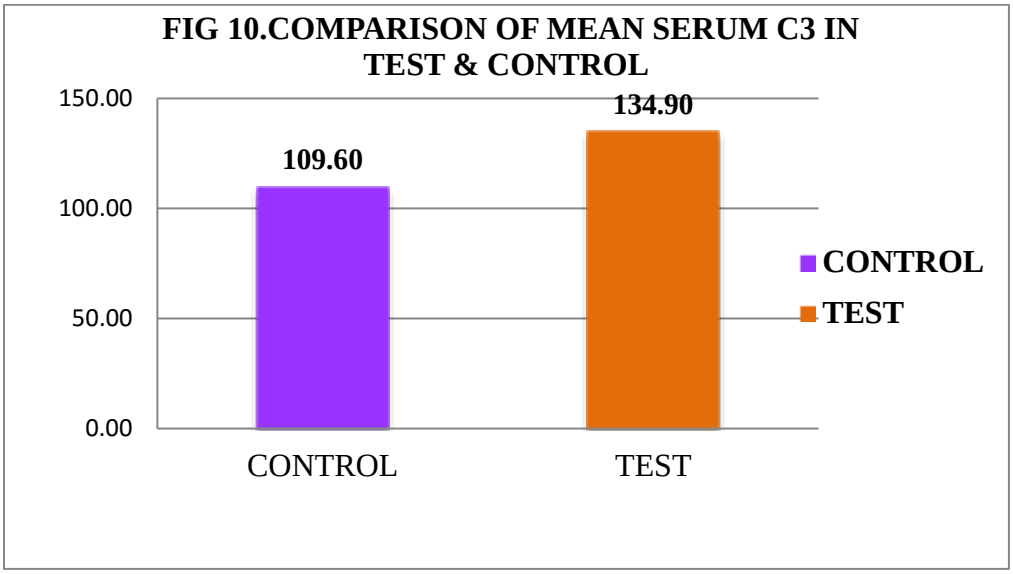




Period of gestation, majority of patients in the third trimester (28 weeks- up to birth).the Ig G levels decreased in trimester wise. The mean of Ig G in second trimester was 723.76 and in third trimester 508.94.But the level of C3 increases with trimester. The mean C3 in second trimester was 120.28 and in third trimester 137.72.

COMPARISON OF SERUM Ig G AND C3 IN TEST AND CONTROL GROUP



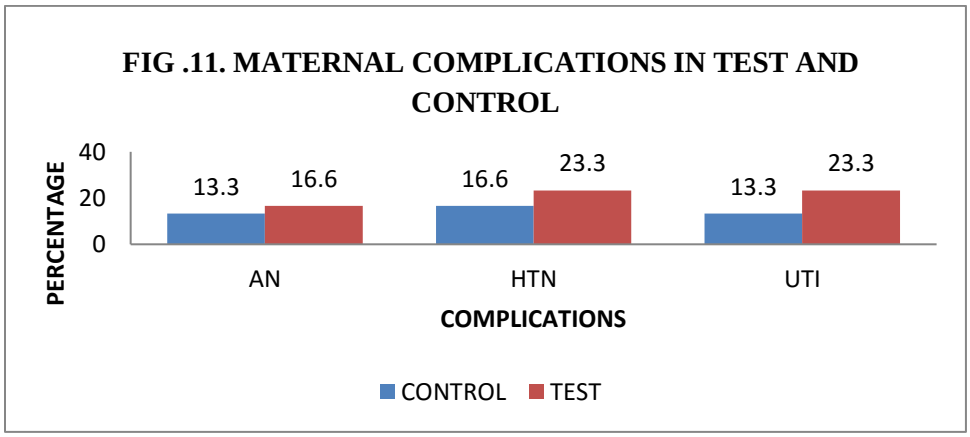


In this study the serum levels of Immunoglobulin G was significantly low in pregnant women with gestational diabetics (test) when compared with normal healthy pregnant women (control) and the levels of complement C3 was significantly high in test group when compared with control group.

- The mean value of Ig G in test group was 553.57 (182.11) and control group was 810.53 (265.95) (P<0.01)
- The mean value of C3 in test was 134.90(22.93) and control was 109.60 (23.33) (P<0.01)

This study shows significantly increase in complement C3 levels and significantly decrease in immunoglobulin G levels in Gestational Diabetes, when compared with normal pregnancy. Which is in agreement with the study done by Deepa K, Prithvi Naveen et.al.

MATERNAL COMPLICATIONS



The maternal complications were uncommon in study populations. Some noticeable complications were acanthosis nigricans, hypertension, and infections like urinary tract infection. The complications were seen in both test and control group.

- AN was more seen in test group (16.6%) than in control (13.3%).
- Hypertension was more common .but its chance was more in test(23.3%) than control(16.6%).
- The chance of Urinary tract infection was more in test (23.3%) than control (13.3%)

The other complications were not seen in this study population. These complications were seen in both pregnant women with GDM and normal healthy pregnant women. But the test group, pregnant women with gestational diabetics was more chance to develop these complications when compared with control, normal healthy pregnant women.

MANAGEMENT OF GDM

The GDM is can be prevented, or the risk for developing it at least minimized, but to adopt more healthy habits before and during the pregnancy is better for mother and child.

First to identify or determining the risk factors like hereditary ,being overweight before your pregnancy, being 25 years of age or older, If developed GDM in a previous pregnancy, If previously had large baby (9 pounds or more) or a stillbirth , If had abnormal blood sugar tests before, including glycosuria (sugar in your urine), history of polycystic ovarian syndrome.

Meet with a doctor early on in pregnancy, and frequently throughout, in order to ensure the health of mother and baby. It is recommended that those at average risk for GDM have a screening performed during their second trimester -- between 24 and 28 weeks. If at high risk, the doctor may elect to test for diabetes during the first prenatal visit. The GDM can be identified mainly through oral glucose tolerance test.

Lowering the risk through diet-

- Eat more fiber
- Increase protein intake
- Take fresh fruit in moderation
- Avoid “white” foods

Lowering Risk Through Exercise -Exercising before and during pregnancy can help protect women from developing gestational diabetes. Research has shown that woman who were physically active for 4 hours a week or 30 minutes a day, before and during pregnancy can reduce their risk of GDM by about 70%.Always ask doctor about how much and what type of physical activity is right for you. Safe exercises to participate in during pregnancy typically include low impact exercises, such as walking and swimming.

If Medical Nutrition Therapy [MNT] and lifestyle changes are not sufficient to maintain the blood glucose at the recommended targets within 1-2 weeks, the ADA and AACE generally recommend the initiation of insulin. For basal insulin requirements, both insulin NPH and detemir can be used in pregnancy. For prandial and correction insulin requirements, regular human insulin, as well as the analogs lispro and aspart can be used. Typically, 50% of the total daily dose is used for basal requirements and 50% for prandial requirements (divided into three doses), with frequent adjustment to dosing until glycemic targets are reached.Mainly insulin is used in this population as pharmacological part.

In this study population by using these above methods GDM can be controlled in pregnant women. The blood sugar level was reached to normal level then the serum Ig G and C3 levels were also reached to normal level.

CONCLUSION

This prospective comparative study shows the serum levels of Ig G were decreased and serum levels of C3 were increased in gestational diabetics when compared with normal healthy pregnant women. In test group 76.33% were decreased levels of Ig G and 70% were increased levels of C3 than normal value when compared with normal healthy pregnant women. The major risk factors were age, gravidity, BMI, family history of diabetes mellitus and history of GDM in previous pregnancy. The Ig G levels and C3 levels were varying with age, gravidity ,BMI, period of gestation. The levels of Ig G decreased with period of gestation, the level was decreased in third trimester than in second trimester. Also the value of C3 is increased with POG, the value is greater in third trimester than second trimester. The Ig G levels were increased and C3 levels were decreased with increasing age. The patients with BMI < 25 had decreased Ig G levels and increased C3 level. The maternal complications; Acanthosis nigricans, hypertension and pre-eclampsia, infections like UTI were seen in this study population. The women with gestational diabetes mellitus were more chance to develop these complications than normal healthy pregnant women. The patients with this complications had decreased levels of Ig G and increased level of C3. The GDM can be controlled through proper medical screening, heaithy diet ,exercise and also some patients insulin was used. The patients

who controlled their blood sugar level to normal also their Ig G and C3 levels were reached to normal.

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