CORRELATION OF FLUORIDE LEVELS WITH ANAEMIA AND MATERNAL AND PERINATAL OUTCOME IN PREGNANT WOMEN

By

DR. SNEHA SINGH, MBBS

DISSERTATION SUBMITTED TO SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH, KOLAR, KARNATAKA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR

MASTER OF SURGERY

IN

OBSTETRICS AND GYNAECOLOGY

Under the Guidance of

DR. MUNIKRISHNA. M. MD, DGO

& Co-Guidance of

DR. SHASHIDHAR.K. N, MD, PROFESSOR OF BIOCHEMISTRY

DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY

SRI DEVARAJ URS MEDICAL COLLEGE

TAMAKA, KOLAR-563101

APRIL/MAY 2020

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH, CENTRE, TAMAKA, KOLAR, KARNATAKA

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"CORRELATION OF FLUORIDE LEVELS WITH ANAEMIA AND MATERNAL AND PERINATAL OUTCOME IN PREGNANT WOMEN"** is a bonafide and genuine research work carried out under the guidance**., DR. MUNIKRISHNA. M.** Professor, Department of OBSTETRICS, Sri Devaraj UrsMedical College, Tamaka, and Kolar.

Date:

Place: Kolar

Signature of the candidate

Dr. SNEHA SINGH

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCHCENTRE, TAMAKA, KOLAR, KARNATAKA

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **"CORRELATION OF FLUORIDE LEVELS WITH ANAEMIA AND MATERNAL AND PERINATAL OUTCOME IN PREGNANT WOMEN"** is a bonafide research work done by **Dr.Sneha Singh** in partial fulfilment of the requirement for the Degree of MASTER OF SURGERY in OBSTETRICS AND GYNAECOLOGY

Date:

Place: Kolar

Signature of the Guide

DR.Munikrishna M,Professor,

Department of OBG

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCHCENTRE, TAMAKA, KOLAR, KARNATAKA

CERTIFICATE BY THE CO- GUIDE

This is to certify that the dissertation entitled **"CORRELATION OF FLUORIDE LEVELS WITH ANAEMIA AND MATERNAL AND PERINATAL OUTCOME IN PREGNANT WOMEN"** is a bonafide research work done by **Dr.Sneha Singh** in partial fulfilment of the requirement for the Degree of MASTER OF SURGERY in OBSTETRICS AND GYNAECOLOGY

Date:

Place: Kolar

Signature of the CO-Guide

DR.Shashidhar.K.N, MD

Vice principal

Professor of Biochemestry

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCHCENTER, TAMAKA, KOLAR, KARNATAKA

ENDORSEMENT BY THE HOD,PRINCIPAL / HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled **"CORRELATION OF FLUORIDE LEVELS WITH ANAEMIA AND MATERNAL AND PERINATAL OUTCOME IN PREGNANT WOMEN"** is a bonafide research work done by **Dr. Sneha Singh** under the guidance of **Dr** .Munikrishna M**..,** Professor, Department Of obstetrics and gynecology.

Dr.Sheela S.R

Professor & HOD
Department of Orthopaedics,
Sri Devaraj Urs Medical College,
Tamaka, Kolar

Date:
Place: Kolar

Dr. SREERAMULU P.N.

Principal and Dean
Sri Devaraj Urs Medical College,
Tamaka, Kolar

Date:
Place: Kolar

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCHCENTER, TAMAKA, KOLAR, KARNATAKA

ETHICAL COMMITTEE CERTIFICATE

This is to certify that the Ethical committee of Sri Devaraj Urs Medical College &Research Center, Tamaka, Kolar has unanimously approved

Dr. Sneha Singh

Post-Graduate student in the subject of Obstetrics and gynecology at Sri Devaraj Urs Medical College, Kolar to take up the dissertation work entitled

CORRELATION OF FLUORIDE LEVELS WITH ANAEMIA AND MATERNAL AND PERINATAL OUTCOME IN PREGNANT WOMEN

To be submitted to

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION ANDRESEARCHCENTER, TAMAKA, KOLAR, KARNATAKA,

Date:

Place: Kolar

Member Secretary

Sri Devaraj Urs Medical College,

Tamaka, Kolar-563101

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION ANDRESEARCHCENTER, TAMAKA, KOLAR, KARNATAKA

COPYRIGHT

DECLARATION BY THE CANDIDATE

I hereby declare that the Sri Devaraj Urs Academy of Higher Education and Research Center, Kolar, Karnataka shall have the rights to preserve, use and disseminate this dissertation/thesis in print or electronic format for academic /research purpose.

Date:

Place: Kolar

Signature of the candidate

Dr. Sneha Singh

SDUAHER

Sri Devaraj Urs Academy of Higher Education and Research

Certificate of Plagiarism Check for Thesis/Dissertation

Author Name	Dr. Sneha singh
Course of Study	M.S (OBG)
Name of Supervisor	DR. Muni Krishna. M
Department	Department of OBG
Acceptable Maximum Limit	10%
Submitted By	librarian@sduu.ac.in
Paper Title	Correlation of fluoride levels with anemia and maternal and perinatal outcome in pregnant women
Similarity	10 %
Paper ID	191128090014
Submission Date	2019-11-28 09:00:14

* This report has been generated by DrillBit Anti-Plagiarism Software

Sneha Singh	M.P. Muni Krishna
Signature of Student	Signature of Supervisor
	Professor & H.O.D. 2/12/19. Dept. of Obstetrics & Gynecology SDUMC, Tamaka, KOLAR.
	Head of the Department
Senior Librarian 2/12/19 Library and Information Centre Sri Devaraj Urs Medical College Tamaka, KOLAR-563 101.	2/12/19 Director P.G. STUDIES Sri Devaraj Urs Medical College Tamaka, KOLAR-563 101
University Librarian	Director Of Post Graduate Studies

ACKNOWLEDGEMENT

This dissertation has been one of the most significant academic challenges I have ever had to face. Without the support, patience and guidance of the following people, this study would not have been impossible. It is to them I owe my deepest and most sincere gratitude. Firstly, I would like to thank my Guide **Dr. MUNIKRISHNA. M**, Professor, Dept of OBG, SDUMC Kolar, for his utmost patience, continuous support, guidance and contribution. I would also like to thank him for his constant encouragement and guidance with respect to every aspect of my professional life.

I would also like to thank **Dr. SHASHIDHAR.K.N**, Professor, Department of Biochemestry, Sri Devaraj Urs Medical College for his wholehearted support, constant encouragement and guidance.

I am sincerely thankful to **Dr. SHEELA S.R,** Professor & HOD, Dept. of OBG, SDUMC, Kolar. Without her constant encouragement this study would not have been possible. Her precious advice on both the dissertation as well as the path of my career has been priceless.

I acknowledge **Dr. GOMATHY.E**, professor in the department of OBG, SDUMC Kolar, for her valuable teachings of perseverance and guidance.

I express my deep sense of gratitude and humble thanks to **Dr.Smitha**, **Dr.Jyothi, Dr.Vimarshita, Dr.Nandini** for their timely advice and constant support throughout the study.

I sincerely thank all the other assistant professors & senior residents, Department of OBG, SDUMC, Kolar, for their constant guidance and encouragement.

I sincerely thank to **Dr. Saideepika** for her constant guidance and encouragement.

I extend my gratitude towards all the patients who agreed to participate in this study, without their precious support it would not be possible to conduct this research.

I thank my fellow post graduates and my friends **Dr. Rashmi** and **Dr. Nikita, Dr.kritika and Dr.Supriya** for their unflinching support. Special thanks to all labour room staff for their help and support throughout my study. Heartfelt thanks to my lovely seniors and juniors. I thank all the staff nurses who are our pillars of support.

Words cannot express how grateful I am to my parents, in-laws, husband and my daughter for all of the sacrifices made on my behalf. Their prayer for me and faith in me and my abilities is what has sustained me this far.

DR. SNEHA SINGH

LIST OF ABBREVATIONS USED

Hb-Hemoglobin

IDA- Iron deficiency anaemia

MCHC-Mean Corpuscular Hemoglobin Concentration

MCV- Mean Corpuscular volume

PCV-Packed Cell volumn

RBC-Red Blood Corpuscle

SF-Serum Ferritin

TIBC-Total Iron Binding Capacity

TS-Transferrin Saturation

UNICEF-United Nations Children's Fund

WHO-World Health Organisation

APH-Ante Partum Hemorrhage

PPH-Post Partum Hemorrhage

BMI-Body Mass Index

SES- Socioeconomic Status

CaF2-Calcium Fluorite

CDC-Centre For Disease Control

ICMR-Indian Council for Medical Research

PPM-Parts Per Million

APGAR-Appearance,Pulse,Grimace,Activity And Respiration

LSCS- Low Segment Caesarean Section

LBW-Low Birth Weight

MAS- Meconium Aspiration Syndrome

PROM-Premature Rupture of Membranes

ABSTRACT

CORRELATION OF FLUORIDE LEVELS WITH ANAEMIA AND MATERNAL AND PERINATAL OUTCOME IN PREGNANT WOMEN

INTRODUCTION:

The fluoride ion is a toxic natural element found at varying concentrations in drinking water. It is known that when fluoride is ingested, it is accumulated on the erythrocyte cell membrane, leading to increase permeability, loss of calcium and reduced lifespan of RBCs. This change causes formation of echinocytes and their rapid destruction leads to anemia. Nearly half of the global total number of anaemic women live in Indian sub-continent and, in India alone, the prevalence of anaemia during pregnancy may be as high as 88%.

The cause of anaemias in India during pregnancy is multi-factorial and includes nutritional deficiencies (ex: iron, folate, and vitamin B12). Iron Deficiency anaemia manifests once the iron stores (Ferritin) gets depleted.

Deficiency of Folate or Vitamin B12 inhibits purine and thymidylate syntheses, impairs DNA synthesis, and causes erythroblast apoptosis, resulting in anaemia from ineffective erythropoiesis.

It leaks minerals in the earth's crust and contaminates underground aquifers. Calcium fluorite (CaF2), one of the minerals, has 157 ppm fluoride. Excess ingestion of fluoride is a major cause for anaemia during pregnancy and in low birth-weight babies due to non-absorption of nutrients including iron supplementation.

More so Kolar district has a high prevalence of fluorosis and it is known to have high levels of Fluoride as underground water of kolar is under 1,500ft and the fluoride content in ground water is 0.5ppm.Hence the high fluoride content may be an important cause for anaemia and

unfavourable foetal outcomes in pregnancy which has not been adequately studied and documented in southern India.

OBJECTIVE:

- To estimate the serum and urine fluoride levels in pregnancy.
- To correlate serum and urine fluoride levels with haemoglobin levels in women during pregnancy.
- To document the maternal and perinatal outcome of women having increased fluoride levels in pregnancy.

MATERIALS AND METHODS:

A comparative observational study was done over 18 months period from Dec 2017 to Jun 2019. According to WHO guidelines, Hb<11gm/dl were considered anaemia. 110 pregnant women were included with 55 in each group, namely;

Case group: 55 cases consisting of pregnant women with anaemia

Control group: 55 controls consisting of pregnant women with non- anaemia

Under aseptic precaution 5 millilitre of venous blood samples were collected in clot activator tube and was centrifuged to obtain clear serum, which was stored at -20 degree C and later analysed for serum ferritin, Vitamin B12, folate and fluoride levels.

A comparison was done between the two groups (anaemic and non-anaemic) and correlation was done between fluoride levels and anaemia in pregnancy.

The maternal and perinatal outcome of pregnancy in patients found to have increase fluoride level was documented and compared with outcome of another group.

RESULTS

Mean age of patients in cases and control groups was 23.73 ± 3.36 years and 24.13 ± 3.52, respectively. In cases group, there was significantly higher number of patients of lower-class status. Majority of the patients of anaemic group (40.00%) were gravida 2. There was no significant difference between cases and control groups patients in terms of parity (P=0.081). In present study, most of the patients (74.55%) were having gestational age ≥ 37 weeks followed by 23.64% patients having 32+1-36+6 weeks gestational age. As compared to control group, cases had significantly lower mean Serum ferritin, significantly lower mean serum folate, and significantly lower Serum vitamin B12. In comparison with control group, patients in cases group had significantly higher Serum fluoride(mg/l) (0.48 ± 0.36 vs 0.19 ± 0.14, P <0.0001) and significantly higher Urine fluoride(mg/l) levels (1.68 ± 0.68 vs 0.94 ± 0.48, P <0.0001). Compared to patients with serum fluoride levels <0.205 mg/L, patients with serum fluoride levels >=0.205 mg/L had comparable mode of delivery and significantly more maternal complications.Compared to patients with serum fluoride levels <0.205 mg/L, patients with serum fluoride levels >=0.205 mg/L had significantly higher pre-term births, lower birth weight and higher NICU admissions. The APGAR (1 minute) and perinatal mortality was comparable among the two groups.

CONCLUSION

The study concluded that in pregnant women with anaemia, there were significantly higher levels of Serum fluoride as well as significantly higher urine fluoride as compared to those without anaemia. The serum and urinary fluoride levels had a significantly negative correlation with the haemoglobin level in cases. There was more serum ferritin, serum vitamin b_{12} and folate deficiency in anaemic group.

There was no significant association of Mode of delivery with serum fluoride and urine fluoride levels in both the groups, however maternal complications were significant among anaemia group with high fluoride levels. In addition, there was higher number of preterm deliveries with low birth weight infants requiring more NICU admissions in the anaemia group with high fluoride levels.

Keywords- Pregnancy, Anaemia, Fluoride

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURE

INTRODUCTION

INTRODUCTION

Fluoride (F) is a toxic natural element found at varying concentrations in drinking water.[1] Humans are exposed to fluoride through different sources such as potable water, food, tea and beverages, pesticide residue on food, pharmaceutical drugs, toothpastes, etc.[2] Though fluoride concentration can increase by various sources, a major source is drinking water.[3]

It is known that when fluoride is ingested, it is accumulated on the erythrocyte cell membrane, leading to increase permeability, loss of calcium and reduced lifespan of RBCs. This change causes formation of echinocytes and their rapid destruction leads to anemia.[4]

Anaemia is the commonest medical disorder in pregnancy and has a varied prevalence, aetiology and degree of severity in different populations. The overall prevalence of anaemia is estimated to be about 40% of world's population. The prevalence is 35% for non-pregnant women and 51% for pregnant women globally and tends to be 3-4 times higher in non-industrialized than in industrialized countries (56% vs 18%). The prevalence is very high in South East Asian countries. [5]

Nearly half of the global total number of anaemic women live in Indian sub-continent and, in India alone, the prevalence of anaemia during pregnancy may be as high as 88%.[5] The relative prevalence of mild, moderate and severe anaemia are 13%, 57% and 12% respectively.[6]

In India, it is frequently severe and contributes to maternal mortality and reproductive health morbidity. It deserves more attention than its currently receiving. Recently lot of programmes have been focussed on safe motherhood but maternal anaemia remains a problem of great concern. [7]

The cause of anaemias in India during pregnancy is multi-factorial and includes nutritional deficiencies of iron, folate, and vitamin B_{12}. Iron Deficiency anaemia is the commonest of all nutritional anaemias in pregnancy. About 1000 mg of iron per day is required during pregnancy. [7]

Iron Deficiency anaemia manifests once the iron stores (Ferritin) gets depleted.

Deficiency of Folate or Vitamin B_{12} inhibits purine and thymidylate syntheses, impairs DNA synthesis, and causes erythroblast apoptosis, resulting in anaemia from ineffective erythropoiesis. [7]

One of the causes of anaemia is contamination of drinking water with fluoride which has been recently being focus of research, it leaks minerals in the earth's crust and contaminates underground aquifers.[2] Calcium fluorite (CaF2), one of the minerals, having 157 ppm fluoride. Excess ingestion of fluoride is a major cause for anaemia during pregnancy and in low birth-weight babies due to non-absorption of nutrients including iron supplementation. [2]

There is high prevalence of anaemia in our country especially in rural areas which adversely affect both mother and baby. Since the diet is poor in iron and vitamins, the government of India in 1970 took a decision to supplement iron and folic acid to all pregnant women in the country.[3] Supplementation was made mandatory during all antenatal check-up. However, the problem of anaemia persists in our country. India runs highest in the percentage of low birth weight babies (43%) according to UNICEF report in 2008.[3]

Even though deficiency of Iron, Folate and Vitamin B_{12} remain the important causes for anaemia in pregnancy, a substantial number of patients are found to be anaemic

despite the levels of these found to be normal. In such patients excessive Fluoride intake could be an important reason for the anaemia and unfavourable foetal outcome.

More so, Kolar district has a high prevalence of fluorosis and it is known to have high levels of Fluoride as underground water of kolar is under 1,500ft and the fluoride content in ground water is 0.5-4.1ppm.[8] Hence the high fluoride content may be an important cause for anaemia and unfavourable foetal outcomes in pregnancy which has not been adequately studied and documented in southern India.

The proposed study aims to correlate the serum and urine fluoride levels in pregnancy with anaemia and foetal outcome.

AIMS & OBJECTIVES

OBJECTIVES

1. To estimate the serum and urine fluoride levels in pregnancy.
2. To correlate serum and urine fluoride levels with haemoglobin levels in women during pregnancy.
3. To document the maternal and perinatal outcome of women having increased fluoride levels in pregnancy.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Anaemia is common in India predisposing to adverse maternal and foetal outcome. World Health Organization (WHO) has set the upper limit of fluoride concentration in drinking water at 1.5 mg/L 2000), and the Bureau of Indian Standards, has laid down as 1.0 mg/L as the maximum permissible limit of fluoride.

The normal upper limit of fluoride in urine is 0.1mg/L and normal upper limit for fluoride in serum is 0.02 mg/L [3]

It is known that when fluoride is ingested, it is accumulated on the erythrocyte cell membrane, leading to increase permeability, loss of calcium and reduced lifespan of RBCs. This change causes formation of echinocytes and their rapid destruction leads to anemia.[4]

Studies have also indicated that high fluoride levels in pregnant women leads to decreased absorption of iron due to destruction of microvilli in intestine. [4]

According to World Health Organization (WHO), Haemoglobin less than 11gm/dl and 7 gm/dl in pregnancy is considered as mild and severe anaemia respectively. The Centre for Disease Control and Prevention in 1990 has defined anaemia as < 11gm/dl in the first and third trimester and < 10.5gm/dl in second trimester.[6,7]

Iron Deficiency anaemia is the most common of all nutritional anaemias in pregnancy. The other important and common causes for anaemia in pregnant females are folate and Vitamin B12 deficiency.[8] Iron Deficiency anaemia manifests once the iron stores (Ferritin) gets depleted.

Deficiency of Folate or Vitamin B12 inhibits purine and thymidylate syntheses, impairs DNA synthesis, and causes erythroblast apoptosis, resulting in anaemia from ineffective erythropoiesis. [9]

Interventional study in India have shown that removal of Fluoride diet during pregnancy significantly improve haemoglobin level.[3]

Studies have also documented an inverse association of haemoglobin levels with fluoride levels in serum and urine. [4.] There is direct correlation between gestational age, birth weight and APGAR score. There is documented evidence that serum fluoride levels more than 1 ppm, significantly increase the risk of low birth weight, preterm delivery and low APGAR score. [10]

The risk of LBW can be up to 10 times higher if fluoride content is significantly high.[11] A few studies particularly from middle east has suggested that low birth weight is not associated with higher fluoride content.[9] However, in cattle also fluoride toxicity is known to produce anaemia and the role of vitamin B_{12} in haemoglobin biosynthesis is found to be inhibited by higher fluoride content.[12]

World Health Organisation has highlighted Anaemia to be the leading global health issue which is centred in the developing countries. The literature of Pasricha claims that, the statistics around pregnant women with anaemia is 42 percent.[13] Due to lack of resources for clinical observations and extensive research, women with anaemia often belong to countries like South and South East Asia and Sub-Saharan Africa.[13] While Iron deficiency has been understood to be the factor behind this huge crisis, many literatures suggest that deficiencies of folate and vitamin B_{12}, may also be important depending on the geographic setting and the population.[13]

Pasricha highlights that recent research within the population of South East Asian countries have found that iron deficiency is a small factor in the cause of anaemia

among pregnant women.[13] A study of 399 pregnant women in their first trimester in central Vietnam, as Pasricha explains, found " an unexpectedly low prevalence of anaemia (19.3%), iron deficiency (20.1%) and iron-deficiency anaemia (6%) most of the burden of anaemia could not be explained by iron deficiency."[13] The study speculates different causes in the form of haematinic micronutrient deficiencies. Other weaknesses in the literature can be addressed by finding the relation of fluoride level in pregnant and anaemia.[13]

Susheela et al. in their literature identify the measurement of fluoride intake to identify the cause of anaemia in pregnant women.[3] Their interventional study in India have shown that removal of Fluoride diet during pregnancy significantly improve haemoglobin level.[3] The literature suggest that though iron deficiency is the leading cause of anaemia, fluoride is at cause in large number. It draws conclusion from their study of 2055 pregnant women that, when ingested with fluoride, mainly through potable water, it threatens to cause thyroid hormone production in children which can be traced to intrauterine growth period.[3]

The study took extensive measures to remove all contact of fluoride from the pregnant women. Black rock salt, junk food, sugar, toothpaste with high fluoride and salty snacks were eliminated in addition to potable drinking water. This resulted in a significant rise in haemoglobin level in one sample from 8.2 ± 0.9 to 10.8 ± 2.0 between 1st and 2nd trimester and reduction in urinary fluoride level from 2.082 ± 1.058 to 1.628 ± 1.63.[1322] The increase in body mass index (BMI) and reduction in pre-term deliveries was an additional result.[3(p1324)]

Ho et. al in their literature state anaemia as one of the most prevailing complications during pregnancy.[14] Anaemia concerns are categorised into kinds; physiological and nutritional. Physiological anaemia is trigged by increase in haemodilution and blood volume where haemoglobin is less than 110 g/1.[14] Nutritional anaemia is characterised by competitive levels of nutrients absorption by foetus leading to insufficient maternal nutrition such as iron deficiency anaemia, cobalamin deficiency anaemia and folate deficiency anaemia.

The study examined the blood samples of 221 Chinese women for determining cobalamin, haemoglobin, folate and serum ferritin concentrations at the beginning of labour to compare and analyse their interrelationships.[15] The study criterion of full-term pregnancy with healthy liver and renal functions, normal level of haemoglobin in first trimester and no antepartum haemorrhage and hematonic were met.

As a result, it was observed that 23/221 women showed evidences of clinical anaemia HB<110<g/1) with haemoglobin, cobalamin, serum ferritin and folate levels at 101.8 g/1, 204.6 pmol/1, 13.03 μg/1 and 12.60 nmol/1 respectively.[15] These levels were a clear indication of insufficiency of nutrients leading to correlation between anaemia and iron depletion. As haemoglobin levels in physiological anaemia is rarely less than 110 g/, the likelihood nutritional anaemia occurring during pregnancy rapidly increases, thus calling for unquestionably necessary treatment.

Fluoride may naturally occur at some places which is a concern for residents in that area.[15] Apart from causing dental mottling and skeletal manifestations, Ersoy et. al identifies fluoride to cross the cell membrane and to enter soft tissues when consumed

water with high fluoride level.[15] Such water source was used as the centre of the study conducted by Ersoy et. al in Isparta, South Turkey. The fluoride level in the waters of Isparta was observed to be 2.74 $\pm$ 0.64 in the endemic region.[15] In the study conducted, Ersoy concluded that the toxic effect of fluoride in water reflected on anaemia. Ersoy et. al accuses fluoride to have an effect on bone marrow.

The literature of Diouf addresses the challenges of developing nation like Senegal's limited resources and failure to prevent increase fluoride in drinking water.[16] This has resulted in dental issues among pregnant women who clearly show sign of this problem. The study attempts to find parallels between mother's with dental problem, due to excess exposure to fluoride and the weight of their newborns which is 2500g at standard, according to WHO.[16] The study conducted among 108 mothers when compares the controlled and uncontrolled subjects finds, 6.9% low weight compared to 25.9% of uncontrolled population.[16] Therefore, the literature finds direct relation between high level of fluoride and low birth weight among children in the endemic region.

A study was conducted on endemic fluorosis area in domestic ruminants to assess the toxic effect of fluoride in ruminants in fluoride polluted locality in India. In this study there is significant reduction in haematological parameters such as haemoglobin concentration, packed cell volume, total erythrocyte count and leukocyte count indicative of anaemia due to fluoride stress. It was observed that fluoride level in drinking water in these areas included in study was about 5.0-6.2 ppm.[17]

The literature by Szymaczek and Lewicka raises concern over negative impact of fluoride exposure on foetal development.[18] The study conducted in Poznan, Poland attempts to establish that the standard practice of providing fluoride supplement in pre-natal care may not be the right decision going forward. In their study, they observed the fasting urine samples of pregnant women aged 22-34. This was observed between 28th week which was found to be 0.838 mg/L and 33rd week which was 1.300 mg/L.[18] The Fluoride level increased among these women significantly. Therefore, suggesting the water consumed should be examined in the local area before prescribing Fluoride to pregnant women as it may case rise in Fluoride level into foetal hard tissue.[18]

Valdez et al. in their literature discuss the negative effects of fluoride on cognitive functions in infants from in utero exposure through women residing in high endemic hydrofluorosis area. The Ministry of health, Mexico conducted a research where pregnant women, no more than 12 weeks of gestation, minimum 5 years exposure to study area and no history of thyroid or diabetes were considered.[19] Over the three trimesters, water and urine samples were collected and examined. It was observed that, 33.8% babies were born premature with weight lower than 2.5 kg.[18] This leads to a negative impact on memory, attention and sensory commands during development periods in children. This calls for preventive measures in regulating the concentration of Fluoride in tap water to ensure prevention of development delays or disabilities.

History of Anemia

The word “anaemia” in Greek means “without blood. “In a 2002 report, WHO lists iron deficiency, a major cause of anaemia, as one of the top 10 risk

factors in developing countries, for “lost years of healthy life”. The work of Hedin and Wintrobe in assessing the volume of packed red cells by various types of hematocrit and the work of Keith, Rewntree and Garathy in estimating blood volume led to the accurate laboratory definition of the presence or absence of anaemia.

Pierre Blaud in 1832, discovered that ferrous sulphate tablets were effective therapy for iron deficiency anaemia.

In 1919 Sir William Osler classified anaemia as occurring during pregnancy, following post-partum haemorrhage, associated with post-partum sepsis or other postpartum anaemia. In 1922 Price quantitated the variation in red cell size seen in various types of anaemia.

DEFINITION OF ANAEMIA IN PREGNANCY

Anaemia is the generic name given to a group of disorders characterized by a quantitative or qualitative deficiency of the circulating erythrocytes.[20] Anaemia is a condition of low oxygen carrying capacity of blood in which Hb concentration is two standard deviation below the median for healthy population of same age , sex, and stage of pregnancy. [21]

This however, is a statistical definition and is not easily understandable and practical.

The WHO definition for diagnosis of anaemia in pregnancy is a Hb concentration of less than 11g/dl and a haematocrit of less than 33%, although CDC (Centres for Disease Control, USA) proposes a cut-off of 10.5g/dl during the second trimester.[22]

PREVALANCE OF ANEMIA- [23]

The overall prevalence of anemia is estimated to be 40% of the world's population. Prevalence of anemia is 35% for non-pregnant women and 51% for pregnant women

globally. The prevalence of anemia in Indian pregnant population is about 88%. In India, anemia antedates pregnancy, “Too early, Too frequently, Too many” is the rule. Therefore anaemia is the most common medical disorder in pregnancy in the developing country.

About 20-40%of maternal deaths in India is due to anemia. The national Family Survey conducted by International Institute of Population Sciences in 2015-2016 reported that 50.3% pregnant woman in India were anemic(urban 45.7%, rural 52.1%).

CLASSIFICATION OF ANEMIA

WHO classification[23]

Severity	Hb g/dL
Mild anaemia	9.1-11g/dl
Moderate anaemia	7.1-9g/dl
Severe anaemia	<7g/dl

ICMR classification [23]:

Category	Anaemia	Hb level
1	Mild	10-10.9g/dl
2	Moderate	7-10g/dl
3	Severe	<7
4	Very severe (decompensated)	<4

CAUSES OF ANAEMIA DURING PREGNANCY[23]

Acquired

- Iron deficiency anaemia (60%)

- Macrocytic anaemia (10%) due to deficiency of folic acid and/or vitaminB12
- Dimorphic anaemia (30%) both due to deficiency of iron and folic acid and/orB12
- Protein deficiency due to protein deficiency in extreme malnutrition
- Megaloblastic anaemia
- Anaemia caused by acute blood loss
- Anaemia of inflammation or malignancy
- Acquired haemolytic anaemia
- Aplastic or hypoplastic anaemia

Hereditary

- Thalassaemias
- Sickle cell haemoglobinopathies
- Other haemoglobinopathies
- Hereditary haemolytic anaemias

Bone marrow insufficiency- hypoplasia or aplasia due to radiation, drugs (Aspirin, indomethacin)

Haemorrhagic (due to acute blood loss / chronic blood loss -hook worm, bleeding piles)

Chronic disease (renal) or neoplasia

PHYSIOLOGICAL ANAEMIA OF PREGNANCY

In pregnancy, there is an increase in plasma volume [30–40%] which is greater than the rise in RBC volume [10 – 15%] so that there is a fall in hemoglobin. This fall is physiological because24

a).It begins in the first trimester when iron needs are fully met.

b) It occurs in well-nourished women also

c) It is not eliminated by the administration of Iron.

The decrease in blood viscosity results in a reduced load on the heart during pregnancy and may also facilitate blood flow through the placenta. The increase blood volume also offers protective buffer against blood loss in third stage of labour.

Criteria of Physiological Anemia – The lower limit of physiological anemia during second half of pregnancy should fulfill the following hematological values-

a) Hb – 10gm%

b) RBC- 3.2 Million/mm3

c) PCV- 32% and

d) Peripheral smear showing normal morphology of the RBC with central pallor

IRON DEFICIENCY ANAEMIA

Iron deficiency anemia is the commonest type and depending upon the socio-economic status. It may be affecting approximately 20% of the world's population[25.] In pregnant women, the prevalence of iron deficiency anaemia exceeds 80%. It is directly or indirectly responsible for 20% of maternal deaths and also a significant contributor of fetal wastage, premature births and low birth weight.[26]

Reasons for high prevalence of IDA in India[27,28]

Apart from dietary insufficiency, inadequate absorption of iron ia an importantly cause of iron deficiency. Multiparty, previous menorrhagia and malnutrition contributes to this condition. Intestinal chronic diarrhea interferes with iron absorption. Worm infestation is a common predisposing and aggravating factor that

may or may not be documented. In India one common problem leading to anemia is Poverty compounded by population explosion, illiteracy and food faddism where pregnant women are not allowed to eat many types of foods because of customs and rituals

Clinical features[29,30,31]

Symptoms –Storage iron is depleted before a fall in Hb and as iron is an essential element in all cells, symptoms of iron deficiency may occur even without anaemia. These include fatigue, weakness, exhaustion, lassitude, indigestion, loss of appetite, irritability, poor concentration and hair loss.

Clinical symptoms and signs of iron deficiency anaemia in pregnancy are usually non-specific, unless the anaemia is severe. Fatigue is the most common symptom. Patients may complain of pallor, weakness, headache, giddiness, palpitations, dizziness, dyspnoea and irritability. Rarely pica develops where there is a craving for non-food items such as ice and dirt. Iron deficiency anaemia may also impair temperature regulation and cause pregnant women to feel colder than normal

Signs of iron deficiency anemia[27]:

- Pallor, edema, koilonychias (spoon shaped nails), nails become brittle and lose luster, bald and magenta colour tongue, angular stomatitis etc.
- Soft systolic murmur can be heard in the mitral area due to hyperdynamic circulation. There can be fine crepitations at bases of lungs due to congestion.

EFFECT OF ANEMIA ON PREGNANCY-[27]

Maternal Effect-

- Preeclampsia

- Preterm labor
- Cardiac failure
- Postpartum hemorrhage
- Hemorrhagic shock
- Sub involution of uterus
- Pulmonary embolism
- Maternal death
- Puerperal sepsis
- Failure of lactation

High incidence of toxemia, hydramnios, APH, preterm labor, PROM, PPH, increased maternal morbidity and mortality are seen more in anaemic mothers[31,32].

Foetal Effects -

Intrauterine growth restriction, Preterm birth ,low birth weight, still births and increased perinatal mortality and morbidity are seen more in babies born to anaemic mothers[33,34]

IRON METABOLISM IN PREGNANCY :

The total amount of iron stores in the adult non pregnant woman is approximately 2.2 gm which increases to 3.2 gm in a pregnant woman at term[35]. In the non-pregnant woman, haemoglobin accounts for approximately 70% of iron stores and ferritin accounts for approximately 25%.

The iron requirement during pregnancy is about 900 mg[35].

Expansion of erythrocyte mass	- 500 – 600 mg
Fetus and placenta	- 300 mg

Iron requirements in pregnancy[36]

Upto 20th wk	-	0.7 mg/day
20 – 32 wk	-	5 mg / day
> 32 wks	-	8 mg/ day

Diet contains 7 mg of iron per 1000 kcal of food. While 20-30% of haem iron is absorbed, only 5% or less of non haem iron is absorbed. Iron is absorbed in the ferrous state from the proximal part of duodenum which is taken up by apotransferrin in the interstitium and circulate in the plasma as transferrin bound iron. This iron is taken up by the developing erythroid cells in the marrow.

Iron which is released from the transferrin receptor complex is incorporated into protoporphyrin to form haem. Any excess iron in the plasma is stored as ferritin.

On the basis of a 10% iron absorption rate, the recommended dietary allowance in a non-pregnant woman's diet is 15mg of elemental iron / day which is increased to 30 mg a day during pregnancy[35].

Enhancers of iron absorption [35]

- Haem iron
- Meat, poultry and fish
- Ascorbic acid
- Low gastric p^H

Inhibitors of iron absorption

- Phytates
- Bran
- Calcium rich antacids
- Milk, Tea or coffee
- Egg

Iron bioavailability:[36,37]

1. Low bio-availability diet

Cereals, roots and tubers such as maize, rice, beans, whole wheat flour and ragi with negligible amounts of meat, fish and ascorbic acid.

2. Intermediate bio-availability diet

Cereals, roots and tubers, but include some animal foods like meat, fish and ascorbic acid.

3. High bio-availability diet

Meat, fish, poultry and foods with generous amount of ascorbic acid.

Iron rich foods:

Non haem iron - Pulses, cereals, jaggery, beet root, green leafy vegetables, legumes, dry beans, iron rich white breads etc.

Haem iron - haemoglobin and myoglobin from red meat, viscera, fish and poultry.

Food should be cooked in iron utensils and too much cooking should be avoided.

Pathophysiology of iron deficiency anaemia[35]

- If iron stores are depleted there is impaired erythropoiesis resulting in reduced erythrocyte indices
- Three commonly recognized states of iron deficiency exist.

First Stage: Reduction of iron stores.

Second Stage: Iron deficiency starts when iron stores are depleted but anaemia has not yet resulted. This leads to a state of iron deficient erythropoiesis.

Third Stage: Severe degree of iron deficiency is manifested as overt microcytic anaemia.

DIAGNOSIS OF IDA IN PREGNANCY [37]

1) **Hemoglobin concentration**: It is the simplest, noninvasive practical test that is widely acceptable.

• It is first preceded by depletion of iron stores and reduction in S. iron

2) **Red cell indices:** These values are not so useful in pregnancy because these values change slowly and the changes occur too late in pregnancy to be diagnostically useful[19].

MCV, MCH, MCHC are all reduced.

3) **Peripheral smear:** Anisocytosis, ovalocytosis, frank microcytosis, and hypochromia, occurrence of target cells and elongated pencil cells are commonly seen.

4) Assessment of iron stores

- Done by indirect, noninvasive methods

Iron profile –

a) S. Iron
b) Total Iron binding capacity (TIBC)
c) Transferrin saturation (TS)
d) Serum ferritin (SF)

a) S. Iron

Normal – 60 – 175 μg / dl –

In IDA < 60 μg / dl –

b) TIBC and transferrin

These are the measurements of transport iron

- Average value of TIBC is 340 g/dl and increases in IDA
- Normal range of transferrin is 20 – 50% and decreases to less than 15% in IDA

In anaemia of chronic disease, TS is often normal with low serum iron levels.

c) Serum ferritin [38]

This is the best noninvasive test for evaluating iron stores[38].

- Normal range – 12 – 300 μ g / lit (50-155 ηg / ml)
- Values < 20 μ g / lit are indicative of IDA

S. ferritin loses its diagnostic value in the presence of infection, inflammation, ineffective erythropoiesis, malignancy and liver disease in all of which is elevated.

5) Bone marrow aspiration

This is indicated only in cases where there is no response to iron therapy after 4 wks (or) for diagnosis of kala azar (or) in suspected aplastic anaemia [38].

Characteristic blue granules of stainable iron are seen in the erythroblasts by staining with potassium ferrocyanate.

6) Reticulocyte count

Provides a reliable measure of red cell production

Normal range – 1 – 2%

7) Molecular genetics of Iron deficiency[39]

Human transferrin gene is known to show many types of polymorphisms and it has been reported that human transferrin G277S mutation is a risk factor of iron deficiency.

8) Other tests

- Stool examination (to R/o hook worm infestation)
- Urine examination for microscopy
- Rule out malaria in peripheral smear
- Serum proteins in hypoproteinaemia
- Renal function tests in suspected renal disease.

PREVENTION OF IRON DEFICIENCY ANAEMIA IN PREGNANCY

Propylactic Iron Therapy[40]

- National nutritional anemia prophylaxis programme recommends 100 tablets of ferrous sulphate 200mg with 60 mg of elemental iron with folic acid 0.5 mg to all pregnant women in third trimester of pregnancy and lactating women.

Also, iron prophylaxis was recommended for family planning acceptor and children of age 1-11 yrs.

- WHO recommends universal oral iron supplementation with 60 mg of elemental iron daily for 6 months in pregnancy in areas where the prevalence of iron deficiency is less than 40%. In areas where prevalence is greater than 40%, recommendation is to continue 3 months postpartum
- Government of India recommendations– 100 mg elemental iron +500mcg of folic acid in 2nd half of pregnancy for 100 days
- Some recent studies have shown that weekly or twice weekly iron supplements also give equally good results. Intramuscular iron dextran 250mg 2-3 doses 4 weeks apart was found to be effective prophylaxis. Iron sucrose also can be used in place of iron dextran.

Suggestions for better prophylaxis [40]

- Dietary modifications to add iron rich foods: jaggary, liver, meat, kidney, eggs, dates and green vegetables are rich in iron
- Community based distribution of tablets
- Fortification of common food stuffs (eg.wheat and salt)
- Educating adolescent girls about diet, personal hygiene – Girls in India are deprived of good diet from their childhood as compared to their brothers and thus enter adulthood with malnutrition, anaemia or low iron stores. So, most women start their pregnancy with anaemia or low iron stores. As a public health approach, prolonged oral supplementation beginning before the women become pregnant may be a better strategy to benefit majority of the

population. Hence, women of child bearing age in non-industrialised countries should receive a 2-4 months' course of 60mg of iron daily.

- Hookworm infestation should be treated- Single dose of Albendazole 400 mg or Mebendazole 100mg twice daily for three days should be given to all anaemic patients in 2^{nd}& 3^{rd} trimester. Change in defecation habits & avoid bare foot walking.
- Chemoprophylaxis for malaria in endemic and high-risk areas

Treatment of Iron Deficiency Anaemia[40]

Goal of Iron Therapy

Is to correct Hb deficit & replenish iron stores in tissues.

Choice of therapy depends on 3 factors

1.Severity of anaemia

2.Gestational age

3.Tolerance of therapy chosen

- Mild anaemia–treat with oral iron irrespective of gestational age
- Moderate anaemia- <36weeks-oral iron
  >36weeks-parenteral iron
- Severe anaemia – Local guidelines for blood transfusion in chronic anaemia are broadly divided into 3 groups-

1.Duration of pregnancy is < 36weeks gestation

- Hb ≤ 5g%

- Hb 5-7g% and in the presence of established or incipient cardiac failure or clinical evidence of hypoxia, pneumonia or any other serious bacterial infection & malaria

2.Duration of pregnancy ≥ 36 weeks gestation

- Hb ≤6g% and >6g% in the presence of established or incipient cardiac failure or clinical evidence of hypoxia, pneumonia or any other serious bacterial infection & malaria

3. When elective caesarean section is planned & there is history of antepartum haemorrhage, post-partum haemorrhage, previous caesarean section with Hb 8-10g%, then 1 unit of blood should be cross matched and if Hb is <8g% then 2 units of blood should be cross matched.

Severe anaemia with >5g/dl before 36 weeks and >6g/dl after 36 weeks considers parenteral iron therapy in the absence of established or incipient cardiac failure or clinical evidence of hypoxia, pneumonia or any other serious bacterial infection & malaria

ORAL IRON THERAPY

This is generally the preferred method of treatment.

1) Ferrous salts

- Since iron is absorbed in ferrous form, only ferrous salts should be used.
- These are the least expensive preparations.

Percentage and amount of iron in common iron preparations[25]

Preparation	Molecular iron (mg/tab)	Percentage of iron	Elemental iron (mg / tab)
* Ferrous sulphate	300	20%	60
* Ferrous fumarate	200	33%	66
* Ferrous gluconate	300	12%	36

Iron polysaccharide complex[42]

- This is ferric polysaccharide polymer
- It causes fewer side effects
- It is 8 – 10 times more expensive than the conventional iron salts
- It has 100 mg of elemental iron per tablet and avoids frequent dosing.

Carbonyl iron[42]

- It is a metallic iron powder with particle size less than 5 mm.
- Bio-availability is 70% to that equivalent of ferrous sulphate.
- It has slow release iron and is not absorbed until it is converted to ionic form.

Sodium feredetate[43]

- This is iron chelated by sodium EDTA.
- Absorption of iron from NaFe EDTA is 2.1 to 2.9 times more than the absorption of iron from $FeSo_4$[14].

Dosage of iron for oral therapy

- The usual therapeutic dosage is 60 – 120 mg of elemental iron a day in three divided doses.
- This should be decreased to 30 mg a day after anaemia is corrected.

PARENTERAL IRON THERAPY

It has no advantage over oral iron if the latter is well tolerated.

Indications

a) Poor compliance to oral therapy

b) Intolerance to oral iron

c) Malabsorption syndromes

d) Severe iron deficiency anaemia presenting late in pregnancy

The main advantage is the certainty of administration to correct haemoglobin deficit and the response is predictable

Calculation of dosage[44]

Elemental iron needed (mg) = (Normal Hb% - Pt's Hb%) x Wt (in kg) x 2.21 + 1000

(or)

0.23 x wt (in kg) x (150 – pt's Hb in g / dl) + 500 [to replenish iron stores]

Routes of administration: **IM/IV**

It is mandatory to give a test dose prior to parenteral iron as the risk of anaphylaxis is high.

Preparations:

Intramuscular

a. Iron-dextran complex (Imferon)
b. Iron-sorbitol-citrate complex (Jecofer)
c. Iron sorbital citrate +folic acid+ Vit B12(Jectofer plus)

Usage:100 mg of elemental iron given on alternate days after test dose deep IM by Z technique

Side effects: fever, myalgia, arthralgia, injection site discolouration and abscess.

Intravenous[44]

Newer iron preparations

a. Iron sucrose (Orofer-s)
b. Iron carbymaltose (Ferri)
c. Iron gluconate (Globac)
d. Fractionated iron dextran

Ferric carboxymaltose (Ferrinject)[44]

Ferric Carboxymaltose Injection contains iron in a stable ferric state as a complex tightly bound within a carbohydrate polymer. It allows for controlled delivery of iron within the cells of the reticuloendothelial system (primarily

bone marrow) and subsequent delivery to the iron binding proteins ferritin and transferrin.

It is administered intravenously, as a single dose of 1000mg over 15 minutes (maximum 15mg/kg by injection or 20 mg/kg by infusion).

Hb(g/dl)	Body Wt 35 Kg to<70Kg	Body Wt ≥ 70 Kg
≥ 10	1000mg	1500mg
<10	1500mg	2000mg

BLOOD TRANSFUSION

Indications

1) Severe anaemia beyond 36 wks to withstand the strain of labor and to tolerate blood loss at delivery.
2) Patients not responding to oral or parenteral iron therapy.
3) To correct anaemia due to antepartum and postpartum haemorrhage.

Advantages

1) Increases oxygen carrying capacity
2) Improvement is expected in 2-3 days
3) Stimulates erythropoiesis

Disadvantages

1) Blood transfusion reactions
2) Precipitated preterm labor
3) Overload may lead to CCF and pulmonary edema which is rare.

Blood transfusions should be preferably done with packed cells.

Partial exchange in severely anaemic pregnant patients increase the haemoglobin rapidly as against slow packed cell transfusion in cases needing immediate pregnancy intervention[45]

MEGALOBLASTIC ANAEMIA IN PREGNANCY [45]

In megaloblastic anaemia, DNA replication is affected. There is derangement of red cell maturation with production of abnormal precursors known as megaloblasts.

It is caused by deficiency of folic acid and / or vitamin B12.

Megaloblastic anemia in pregnancy is nearly always secondary to folate deficiency which complicate upto one third of all pregnancies in non-industrialised countries and is more common in multiple pregnancies.

Pathophysiology

Folate and vit B_{12} deficiency results in impaired growth and maturation in erythrocytic, granulocytic and megakaryotic cell lines, causing significant anaemia because of ineffective erythropoiesis and neutropenia with hypersegmented nuclei and thrombocytopenia[46].

Folic Acid Deficiency Anaemia:

The prevalence of folic acid deficiency may complicate upto one-third of all pregnancies in non industrialized countries and is more common in multiple pregnancies[45.]

Daily Requirement

Non pregnant women-50-100µg/day

Pregnant women-800µg/day

Lactation-600µg/day

Sources: green vegetables, spinach, broccoli, fruits, liver and kidney.

Goat's milk is poor in folic acid.

Etiology

1. Dietary deficiency (strict vegetarians) – very rare.
2. Pernicious anaemia caused by lack of intrinsic factor – rare.
3. Malabsorption syndrome.

Pathogenesis: [45]

Deficiency of folate and vitamin B12 reduces tetrahydrofolate responsible for thymid ylate production. This thymidylate deficiency impairs DNA synthesis. RNA synthesis proceeds normally leading to to larger proportion of cytoplasm compared to the nucle us resulting in megaloblasts.

Effects during pregnancy[45]

1. Women with folate deficiency were believed to exhibit an increased incidence of obstetric complications including spontaneous abortions, abruption, third trimester bleeding but other studies failed to confirm this.
2. Embryopathies such as neural tube defects are associated with folate deficiency and supplementation appears to result in diminution of such embryopathies

IUGR – attributed to folate deficiency but probably related to decreased maternal blood volume and failure of adequate placental growth

Investigations:[46]

1. Blood film:

Oval macrocytes, anisocytosis, poikilocytosis

Hypersegmented neutrophils (>5 lobes).

2. Red cell folate level is low measured by radioimmunoassay (<150ng/ml). I n combination with low serum folate level (<3ng/ml) is diagnostic of folic a cid deficiency.
3. Bone marrow: abnormal red cell precursors (megaloblasts).

For the diagnosis atleast 2 of the following features must be present :

>4 % neutrophils must have > 5 lobes

Presence of orthochromic macrocytes (diameter >12 mcg)

Nucleated RBC's are found

Howell Jolly bodies

Macropolycytes

Folic Acid Prophylaxis:[46]

- Diet- rich in folic acid like fortified grains, dried beans, green leafy vegetables liver.
- All women planning for pregnancy are advised to take 500µg /day of folate 3 months preconceptionally to prevent neural tube defects. Those who have had a previous fetus with neural tube defects, on anticonvulsants or carrying a mult iple pregnancy and in malaria endemic areas are advised to take 5mg/day of folic acid.
- WHO recommends folic acid intake of 400µg daily for 6 months during pregn ancy and continuing for 3 months postpartum in low risk and 5mg/day for high risk
- Vitamin C increases folate absorption by converting it into folinic acid.

DIMORPHIC ANAEMIA

It is a deficiency of both iron and folate with findings of both anaemias but dominance of one.

- Blood film may show macrocytic or normocytic, normochromic or hypochromic pictures.
- Treatment is both prescription of iron and folic acid in therapeutic doses.

Treatment of a coexistent iron deficiency may lead to correction of microcytic hypocromasia and make megaloblastic anaemia more easily diagnosed.

FIRST STAGE

1. Sedation and pain relief.
2. Oxygen is given if patient has dyspnoea.
3. In cases of preterm labor, β – mimetics and steroids should be given with caution to avoid the risk of pulmonary edema.
4. Digitalization may be required in cardiac failure due to severe anaemia.

SECOND STAGE

Can be curtailed with forceps.

THIRD STAGE

Active management to be done except in severe anaemia for fear of cardiac failure.

Any PPH should be treated energetically as the patients tolerate bleeding very poorly[47].

PUERPERIUM:

The mother should have adequate rest, iron and FA therapy for 3 months. Any infection is energetically treated. Puerperal sepsis, failing lactation, subinvolution of uterus 8,23 and thrombolism are common problem of the puerperium. The anemic patient should be given contraceptive advice and asked not to conceive for at least 2 years. [48]

Antenatal Care in Next Pregnancy:

The WHO recommends estimation of haemoglobin at first visit, 16 weeks and 36 weeks of gestation in all patients. Prognosis is good if anemia is detected and treated in time.[48]

PREVENTION OF NUTRITIONAL ANAEMIAS

1. Health education
2. Food fortification
3. Improvement of dietary habits
4. Iron supplementation during pregnancy

SUPPLEMENTATION –INTERVENTIONS IMPLEMENTED BY MINISTRY OF HEALTH AND FAMILY WELFARE[49]

THE NATIONAL IRON+ INITIATIVE-

It is an attempt to prevent IDA comprehensively across all life stages. There are age specific interventions with iron and folic acid supplementation and deworming for improving the hemoglobin levels for all age group.

- Adolescents 11-19 years receive weekly dose of 100 mg elemental iron and 500 mg of folic acid with bimanual de-worming in school.

- Women at reproductive age group (20-49) years: ASHA workers provide IFA supplement with 100 mg elemental iron and 500mcg folic acid throughout the calendar years with bimanual de-worming.
- Pregnant and lactating women are provided with 100mg of elemental iron and 500 mcg of folic acid daily for 100 days during routine antenatal visits.
- Long lasting insecticide nets are also provided to pregnant woman.

PRADHAN MANTRI SURAKSHIT MATRITVA ABHIYAN-

This program aids to provide assured comprehensive and qualitative antenatal care, free of cost, universally to all pregnant women on the 9th of every month, that will also help in reducing prevalence of anemia in pregnant mothers.

NATIONAL NUTRITION MISSION

This was launched under the oversight of the ministry of woman and child development in March 2018 with the aim to reduce anemia among young children and women of reproductive age group.

FLUORIDE

Fluorosis is an important public health problem many parts of the world including India. Of the 85 million tons of fluoride deposits on the earth's crust, 12 million are found in India (Teotia, 1984).

Fluorosis was first published in the year 2000, although the disease was detected and reported in India in 1937. Fluoride endemicity has been reported in 230 districts of 19 states of the country. National programme for prevention and control of fluorosis was is being implanted in Karnataka state from 2009-2010. During 2009-10 the programme was started in Mysore and Bellary District of the state.[50]

Hence it is natural that fluoride contamination is widespread, intensive and alarming in India. It has been estimated that the total population consuming containing elevated levels of fluoride is over 66 million.[51]

Endemic fluorosis resulting from high fluoride concentration in groundwater is a public health problem in India (Kotecha et al., 2012).

Chemical composition of Fluorine:

Fluorine is the 13th most abundant element in the earth's crust and is the most electronegative in nature. As a result, it has high reactivity and strong affinity to combine with other elements to produce compound know as Fluoride.[50]

The recommended level of fluoride in drinking water in India is 0.5 to 0.8 mg/L (Park, 2011). The available data suggest that 15 States in India are endemic for fluorosis (fluoride level in drinking water >1.5mg/L), and about bout 62 million people in India suffer from dental, skeletal and non- skeletal fluorosis.[51]

Recommendations from American organizations concerning the minimum of adequate intake of F mention 0,1-0,5mg / day for infants up to six months and 1,5-4,0 mg / day (0,7-2,0mg / liter in drinking water) to older ages and adults. Taking into consideration the number of sources from where someone can be exposed daily, the upper limit for children and adults is defined to 1mg/day. Later surveys refer to a minimum concentration of F in drinking water 0.7-1mg / liter. Conclusively, the minimum dose of adequate ingested intake is determined to 0.05 mg / kg / day for infants and children over 6 months and 3mg/day for women, including during pregnancy and 4mg/day for men. Adult 's upper limit is 10mg/day. [53]

India is one of the worst fluorosis affected countries, with large number of people suffering as many Indians depend on groundwater for drinking purposes and water at many places is rich in fluoride.

Molecular Mechanisms of Fluoride Toxicity[55]

"Fluoride can interact with a wide range of cellular processes such as gene expression, cell cycle, proliferation and migration, respiration, metabolism, ion transport, secretion, endocytosis, apoptosis/necrosis, and oxidative stress, and that these mechanisms are involved in a wide variety of signaling pathways.

Fluoride Inhibits the Enzyme Enolase[55]

Fluoride is a well-known inhibitor of enzymes of the glycolytic pathway, first of all enolase. Exposure of red blood cells (erythrocytes) to fluoride produces a variety of metabolic alterations via its effect on enolase in human red blood cells, fluoride inhibits active sodium transport, aerobic glucose utilization, and lactate formation.

The human gut is the natural habitat for a large and dynamic bacterial community. Major functions of the gut microflora include metabolic activities that result in salvage of energy and absorbable nutrients. Colonic microorganisms also play a part in vitamin synthesis and in absorption of calcium, magnesium, and iron. Lactobacillus acidophilus belongs to a group of bacteria that live in the human small intestine. These beneficial microorganisms aid digestion, help maintain a healthy intestinal tract, and prevent harmful bacteria from congregating there.

When fluoride meets L. acidophilus, it inhibits this beneficial bacterium that aids in the absorption of iron.

ASSOCIATION OF FLUORIDE WITH ANEMIA AND LOW BIRTH WEIGHT[55]

About half of all pregnant women don't have enough iron in their body. Pregnant women need about twice as much iron as usual, therefore they have a higher risk of iron-deficiency anemia, which can increase the risk of preterm delivery and low birthweight. As with most mineral nutrients, iron from digested food is absorbed in the intestinal lining by epithelial cells whose microvilli provide the huge surface area needed to efficiently absorb nutrients.

Mucus production by the goblet cells is considerably reduced and microvilli of the mucosa fall off which are the most damaging effects of fluoride consumption. The function of the microvilli is to absorb nutrients from the diet, including orally administered iron and folic acid provided to pregnant women. If there is scanty mucus production, the individual would be constipated. Secondly, fluoride ingestion destroys the probiotics, (the good bacteria) in the intestine which produce vitamin B12; an essential ingredient of Hemoglobin.

It is known that when fluoride is ingested; it will also accumulate on the erythrocyte membrane, which in turn loses calcium content. This change causes formation of echinocytes. The life span of this echinocytes is less than the normal life span of RBC hence early destruction of RBCs in form of echinocytes causes anemia. The membrane, which is deficient in calcium content, is pliable and is thrown into folds. The RBCs attain the shape of an amoeba with pseudopodia like folds projecting in different directions. Such RBCs are termed as Echinocytes.[4]

The Echinocytes will be found in circulation in large numbers, depending upon the extent of fluoride poisoning and duration of exposure to fluoride. RBCs, in human, although have a life span of 120-130 days, the echinocytes undergo phagocytosis (eaten-up by macrophages) and are eliminated from circulation. This would mean that RBCs in individuals exposed to fluoride poisoning, shall not live the entire life span, but are likely to be eliminated as echinocytes. This would lead to low hemoglobin levels in patients chronically ill due to fluoride toxicity.[4]

Patsouri K (2015) also examined the intake of Fluoride during pregnancy and found that Fluoride placenta from maternal to fetal blood. The significance of placenta barrier as a minor obstacle in the free passage of F from mother to fetus has been disputed by researchers. Although they agree with the difference in concentration of various substances, like F, in mother and fetus, this difference may be also attributed to the rapid maternal kidneys 'action and to the absorption from her bones. It is found that when the quantity of F in drinking water is increased then the concentration in maternal plasma, in fetus plasma and in fetal enamel of deciduous teeth is also increased. In general, concentration in fetus plasma is usually detected 25% less from maternal. [53]

Fluoride ingestion can also derange the structure and function of the thyroid gland, leading to reduction in thyroid hormone production, resulting in inadequate stimuli on erythropoietic tissues to produce erythrocytes. A high percentage of erythrocytes produced in an environment high in fluoride, are abnormal with crenations known as echinocytes. The echinocytes do not survive the normal RBC life span of 120-l30

days but get phagocytosed and eliminated from blood stream. Thus, less number of erythrocytes result in low hemoglobin. [53]

The increased severity of anemia causes the further effects of pre-term babies and low birth weight babies. Even in the infants and growing children, it has been shown recently that increased fluoride levels are associated with Vitamin D resistant rickets. Thus, a strict dietary modification is advised.[53]

In brief fluoride and anemia are associated due to following [55]

1. Decrease production of erythrocytes by the bone marrow and other hemopoietic tissues and increases erythrocytes abnormalities resulting in premature death of RBCs. Owing to fluoride induced thyroid hormone deficiency an adequate stimulus was also lacking for erythrocyte production.
2. Reduces blood folic acid activity.
3. Diminishes beneficial microbial growth in the gut and inhibits production of vitally needed vitamin B12
4. It causes loss of microvilli in the intestinal lining, resulting in poor absorption of nutrients critical for the biosynthesis of hemoglobin.

   Fluorosis can also manifest in the form of [52]

   1. Dental fluorosis (DF): This affects children during development of teeth when the mother has consumed or inhaled fluoride through food, drinking water, dental products and/or industrial emission. It usually starts from intra-uterine life when tooth germ erupts. It affects both temporary and permanent teeth and later in life the discoloration and cosmeses may become permanent.

2. Skeletal fluorosis (SkF): Skeletal fluorosis is not easily recognisable until the disease has developed to an advanced stage. Excessive quantities of fluoride, when deposited in the skeleton, tend to get distributed more in cancellous compared to cortical bone. This affects the bones and major joints of young and old, men and women without discrimination. In advanced stages of skeletal fluorosis, it is not reversible. Severe pain in joints and rigidity or stiffness in joints would incapacitate an individual. Patients of skeletal fluorosis may also get paralysis later in life. [52]

Xray: Xray would reveal increased girth, thickening and density of bones. In certain patients, due to calcium deficiency, Osteomalacia type changes would assure revealing weak bone with the trabeculla pattern instead of dense bone, Calcified membranes or ligaments may be observed.

3.Non-skeletal fluorosis: This is the earliest manifestation of fluorosis. Fluorosis may not be suspected based on the history and the symptoms. The manifestations that may overlap with other diseases are quite non-specific. Yet another possibility is that the health complaints are considered as non-specific and ignored. The elicitation of proper history and conduct of diagnostic tests for confirmation of the disease are of prime importance. [52] These are the general symptoms that may erupt in life.

It has been suggested that it could provide a benefit on the prevention of caries as a)it inhibits the demineralization and reduces the enamel solubility when incorporated into the mineral structure(b) enhances remineralization by F released from dissolved

enamel (although a greater effect of remineralization occurs from F acquired topically by saliva and dental plaque) (c) reduces oral bacteria causing a reduction of the rate of acid production of oral bacteria causing carious lesions, (d) inducts the re-precipitation of fluoridated hydroxyapatite phases within enamel, it reduces the net rate of transport of matter out of the enamel surface.

Despite the benefits of F incorporation, an excess in dosage causes a greater incidence of adverse health effects. Plenty of side effects are reported in literature. These occur when the levels F in humans exceed 3.5-4 ppm in drinking water. Important parameters in the occurrence of side effects are the timing of F intake and the way in which it is received, because the last one determines the amount of intake, dose, duration of administration and the concentration of other minerals or vitamins. [53]

Prevention[52]

The 3 major reasons for low hemoglobin production can be reversed to normal by mere withdrawal of fluoride i.e. diet editing and simultaneous diet counselling for promotion of consumption of nutrients essential for hemoglobin production.

All pregnant women must be counselled to get their blood and drinking water tested for fluoride to establish the risk and take precautionary measures. If the drinking water is contaminated with F-, they have to shift to an existing safe source of drinking water in the neighbourhood. This is considered as the best option particularly for pregnant women.

The intake of iron and folic acid in pregnancy to prevent anemia is effective if the fluoride levels are not high in the drinking water. The management of Fluorosis

patients and the complete recovery from adverse health effects of fluoride can be achieved in shorter span of time, if nutritional intervention focussing on adequate intake of calcium, Vitamin C and E and other antioxidents is also practised simultaneously with drinking safe water.

It may be necessary to point out that the drugs or tablets containing the above nutrients should not be prescribed as the patient basically requires a nutritive diet and due to deficient and inadequate dietary intake, he/ she has become a victim of fluorosis.[64]

Susheela AK et al (2018) [54] in a latest review article addressed the genesis of the disease, diagnostic protocols developed, mitigation and recovery through nutritional interventions. The authors found that the structural and functional damages caused to skeletal muscle and erythrocytes, leads to clinical manifestations due to fluorosis. Hormonal derangements resulting in serious abnormalities in the health of children were also seen. Fluoride toxicity destroys the probiotics in the gut, resulting in vitamin B12 depletion, an essential ingredient in haemoglobin (Hb) biosynthesis.

Thus keeping all the factors and the literature in mind the proposed study was done with an aim to correlate the serum and urine fluoride levels in pregnancy with anaemia and foetal outcome.

MATERIALS & METHODS

MATERIALS AND METHODS:

Study Design: It is a Comparative observational study

Source of Data: The main source of data for the study were antenatal cases attending the department of Obstetrics and Gynaecology, R.L Jalappa Hospital and research center, attached to Sri Devaraj Urs Medical College, Tamaka, Kolar

Study population: All antenatal cases who met inclusion criteria enrolling to OPD and admitted in Obstetrics and Gynaecology Department consenting for study.

Duration of Study: 18 months. Dec 2017 to Jun 2019

Sample size:

Sample size as per previous study was calculated to be 110, 55 in each group.

Sample size is calculated based on the correlation coefficient of 0.2 between serum fluoride and haemoglobin as reported in study by Prashant Sinha [4] where serum and urine fluoride levels were correlated with anaemia with confidence interval of 95% with 80% power fixing an alpha error at 5%. The calculated study sample size per group around 55 anaemic and 55 non-anaemics. Total study sample 110. n MASTER used for calculation.

$$n = \frac{[\, Z_{1-\alpha/2} + Z_{1-\beta}\,]}{[FZ(\rho 1) - FZ(\rho 0)]^2} + 3$$

where

ρ0 –Population correlation coefficient

ρ1-sample correlation coefficient

$Z_{1-\alpha/2}$ –Desired confidence level

β – Power

Inclusion Criteria

All Antenatal cases with singleton pregnancy attending OPD or admitted in OBG department.

Exclusion Criteria

Antenatal cases with

1. Previous history of Iron deficiency anaemia due to Bleeding disorder,
2. Gastritis
3. Thyroid disorders
4. Systemic Lupus Erythrosis (anti- phospholipid syndrome)
5. Sickle cells disease
6. Antenatal cases with other comorbidities like preeclampsia, Gestational Diabetes, chronic liver disease and chronic renal disease.

Methodology

A total of 110 pregnant women were included in this comparative observational study. This study was approved by institutional ethics committee (IEC) and obtained informed consent signed by subjects after explaining the study in detail to the study subjects. They were screened for anaemia by estimating CBC and performing peripheral smear.

According to WHO guidelines, Hb<11gm/dl were considered anaemia.[6]

110 study subjects were divided into 2 groups

Case group: 55 cases consisting of pregnant women with anaemia

Control group: 55 controls consisting of pregnant women with non- anaemia

SAMPLE COLLECTION-

Under aseptic precaution 5 millilitre of venous blood samples were collected from ante cubital vein from study subjects in vacutainer.

For biochemical investigations blood samples was collected in clot activated vacutainers, for haematological parameters. The samples were allowed to retract at room temperature for 10 minutes and samples were centrifuged at 3000 rpm for 10 minutes to obtain serum. Clear serum was stored at -20 degree C and later analysed for serum ferritin, Vitamin B12, folate and fluoride levels.

15 ml spot urine samples were collected in plastic screwed capped bottles for fluoride estimation. Serum and urine fluoride were estimation by using Orion Ion selective fluoride electrode (ISE)(ORION-3822090) at pH 5.0 adjusted with TISAB (Total Ionic Strength Adjustment Buffer) II buffer. The instrument was calibrated and standardized using four solutions having F concentrations of 0.01 ppm, 0.1 ppm, 1 ppm and 10 ppm. The standards were run before analysis of each sample and the electrode was calibrated periodically.

Serum ferritin was done by Vitros 5.1 FS fusion chemistry analyser based on principle reflectance photometry. Vitamin B12 and folic acid was done by electrochelumence(e CLIA).

A comparison was done between the two groups (anaemic and non-anaemic) and correlation was done between fluoride levels and anaemia in pregnancy.

The maternal and perinatal outcome of pregnancy in patients found to have increase fluoride level was documented and compared with outcome of another group.

STATISTICAL ANALYSIS

Categorical variables were presented in number and percentage (%) and continuous variables were presented as mean ± SD and median. Normality of data was tested by Kolmogorov-Smirnov test. If the normality was rejected then non parametric test was used.

Statistical tests were applied as follows-

1. Quantitative variables were compared using Independent t test/Mann-Whitney Test (when the data sets were not normally distributed) between the two groups and ANOVA/Kruskal Wallis test (when the data sets were not normally distributed) between the three groups.

2. Qualitative variables were correlated using Chi-Square test/Fisher's Exact test.

A p value of <0.05 was considered statistically significant.

Correlation was done between the groups using spearman's rank correlation for non-parametric data.

The data was entered in MS EXCEL spreadsheet and analysis was done using Statistical Package for Social Sciences (SPSS) version 21.0.

Biochemical parameters

Serial no	PARAMETERS	REFERENCE RANGE
1.	Serum fluoride	Yet to be established
2.	Urine fluoride	Yet to be established
3.	Serum ferritin (Vitros 5.1)	12-200 ng/ml
4.	Serum folate (EIC)	2-20 ng/ml
5.	Serum vitamin B_{12} (EIC)	170- 900 pg/ml

RESULTS

RESULTS

This is a comparative cross sectional observational study conducted at the Department of Obstetrics and Gynaecology, R.L Jalappa Hospital and research center, attached to Sri Devaraj Urs Medical College, Kolar, Karnataka from Dec 2017 to Jun 2019. After taking informed consent, antenatal cases enrolled and admitted to Obstetrics and Gynaecology Department were included in the study. Relevant history was taken, and antenatal check-up was performed. Patients were screened for anaemia. Two groups were formed, one group consist of anaemic case group and the other group containing control group of nonanemic study subjects. Serum ferritin, vitamin B12, folate was evaluated. Serum and urine sample were processed for fluoride levels estimation. Following are the results pertaining to the study.

Table 1: - Study of age distribution between cases and control groups.

Age distribution (years)	Group		Total	P value
	Case(n=55)	Control(n=55)		
18-20	13 (23.64%)	11 (20.00%)	24 (21.82%)	0.641*
21-25	29 (52.73%)	28 (50.91%)	57 (51.82%)	
26-30	12 (21.82%)	15 (27.27%)	27 (24.55%)	
30-35	1 (1.82%)	0 (0.00%)	1 (0.91%)	
>35	0 (0.00%)	1 (1.82%)	1 (0.91%)	
Total	55 (100.00%)	55 (100.00%)	110 (100.00%)	
Mean ± Stdev	23.73 ± 3.36	24.13 ± 3.52	23.93 ± 3.43	0.471#
Median (IQR)	24(21 - 25)	24(22 - 26)	24(21 - 26)	

*-Chi square test

#-Mann Whitney test

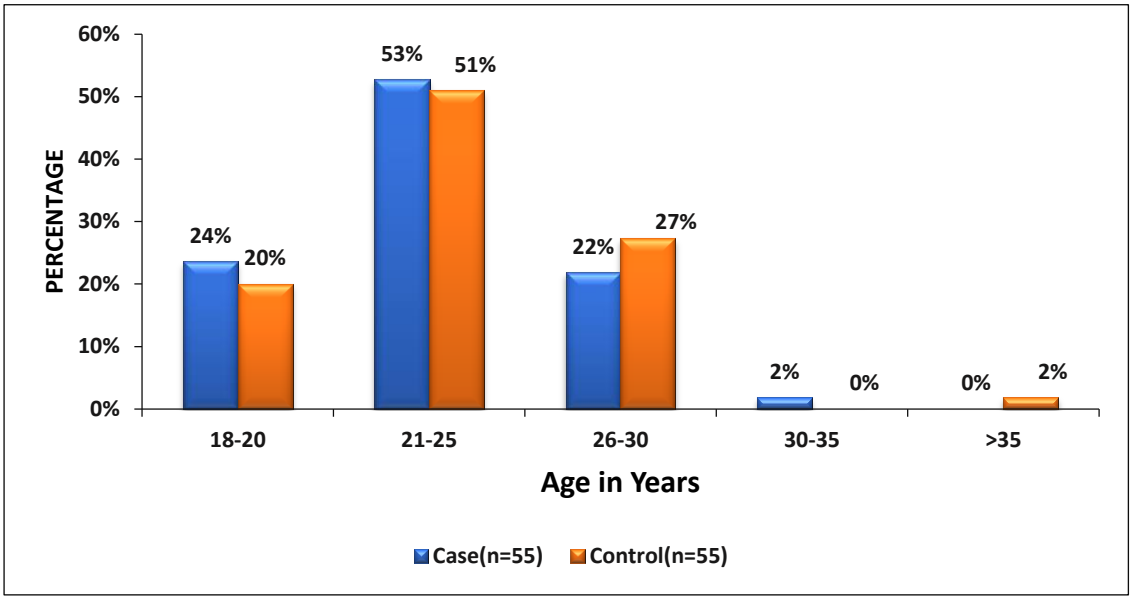

Figure 1: - Study of age distribution between cases and controls.

Table 1 and figure 1 indicates mean age of the patients was 23.93 ± 3.43 years. Mean age of patients in cases and control groups was 23.73 ± 3.36 years and 24.13 ± 3.52, respectively. Majority of the patients (51.82%) belonged to the age group 21-25 years followed by 24.55% patients in 26-30 years age group and 21.82% patients <=20 years of age. Only 1 patient each was of 31-35 years and >35 years of age. Majority of the patients of cases and control groups also belonged to the age group 21-25 years. Mean age was comparable between cases and control group (P value > 0.05).

Table 2: - Study of booking status between cases and control groups

Booking Status	Group		Total	P value
	Case(n=55)	Control(n=55)		
Booked	20 (36.36%)	34 (61%)	25 (22.73%)	0.0006*
Unbooked	35 (63.64%)	21 (38.18)	85 (77.27%)	
Total	55 (100.00%)	55 (100.00%)	110 (100.00%)	

*-Chi square test

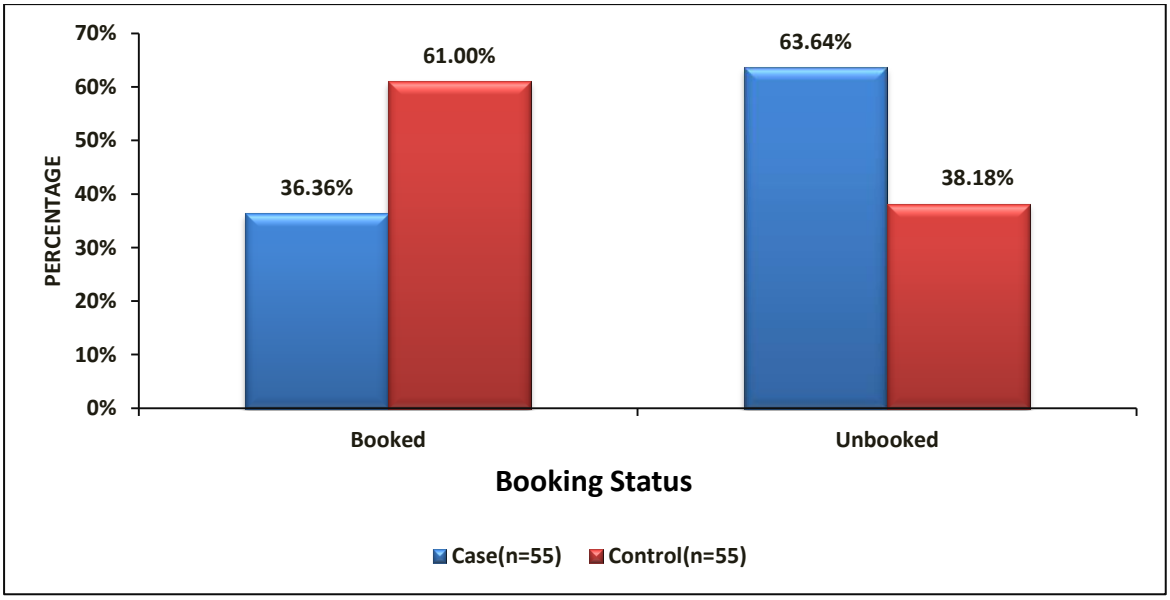

Figure 2: - Study of booking status between cases and control groups

Table 2 and fig3 explains booking status, majority of cases (77.27%) were unbooked. As compared to control group, patients of cases group had significantly lower number of booked cases (36.36% vs 61%, P < 0.001) and significantly higher number of unbooked cases (63.64% vs 38.18%, P < 0.001).

Table 3: - Study of parity

Parity	Group		Total	P value
	Case(n=55)	Control(n=55)		
Primigravida	16 (29.09%)	28 (50.91%)	44 (40.00%)	0.081*
Gravida -2	22 (40.00%)	19 (34.55%)	41 (37.27%)	
Gravida -3	13 (23.64%)	6 (10.91%)	19 (17.27%)	
Gravida -4	4 (7.27%)	2 (3.64%)	6 (5.45%)	
Total	55 (100.00%)	55 (100.00%)	110 (100.00%)	

*-Chi square test

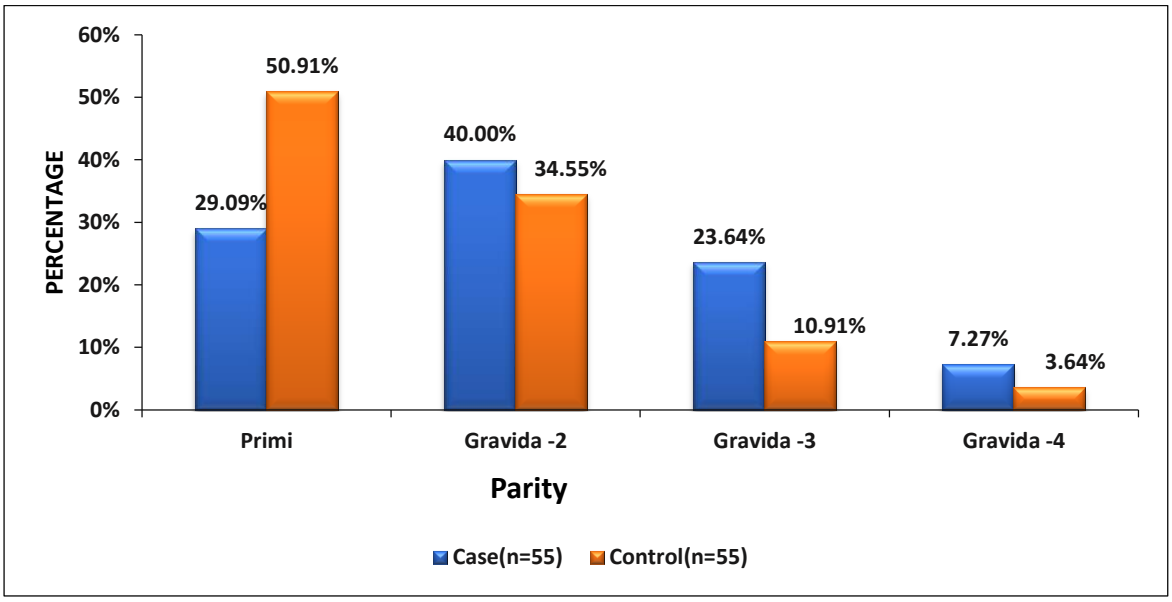

Figure 3: - Study of parity

Table 3 and Figure 3 indicate that most of the patients (40.00%) were primigravida followed by gravida 2 (37.27%). Majority of the patients of cases group (40.00%) were gravida 2, while that of control group (50.91%) were primigravida. However, there was no significant difference between cases and control groups patients in terms of parity (P> 0.05).

Table 4: - Study of gestational age

Gestational age (weeks)	Group		Total	P value
	Case(n=55)	Control(n=55)		
28--31+6	1 (1.82%)	1 (1.82%)	2 (1.82%)	0.026*
32+1--36+6	19 (34.55%)	7 (12.73%)	26 (23.64%)	
37+1 and above	35 (63.64%)	47 (85.45%)	82 (74.55%)	
Total	55 (100.00%)	55 (100.00%)	110 (100.00%)	

*-Chi square test

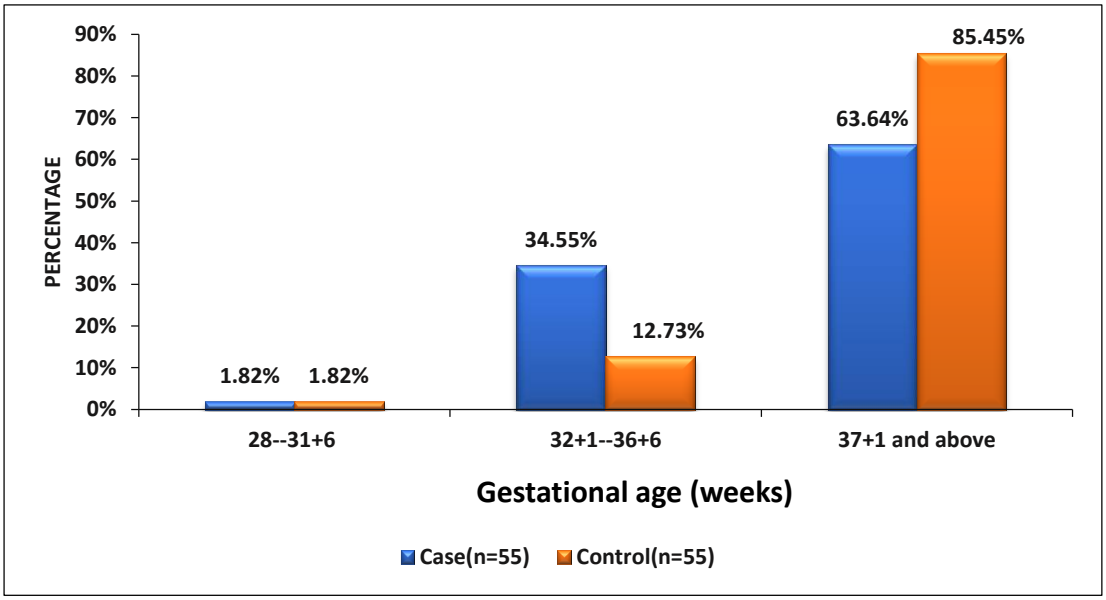

Figure 4: - Study of gestational age

Majority of the study subjects (74.55%) were in gestational age ≥ 37 weeks followed by 23.64% patients were in 32+1-36+6 weeks gestational age. As compared to control group, in cases group there were significantly higher number of patients with gestational age 32+1-36+6 weeks (34.55% vs 12.73%, P= 0.026) and significantly lower number of patients with gestational age > 37 weeks (63.64% vs 85.45%, P< 0.05).Tabulated in Table 4 and Figure 4.

Table 5: - Comparison of social economic status between cases and controls groups

Social economic status	Group		Total	P value
	Case(n=55)	Control(n=55)		
Upper	6 (10.91%)	15 (27.27%)	21 (19.09%)	0.027*
Middle	29 (52.73%)	30 (54.55%)	59 (53.64%)	
Lower	20 (36.36%)	10 (18.18%)	30 (27.27%)	
Total	55 (100.00%)	55 (100.00%)	110 (100.00%)	

*-Chi square test

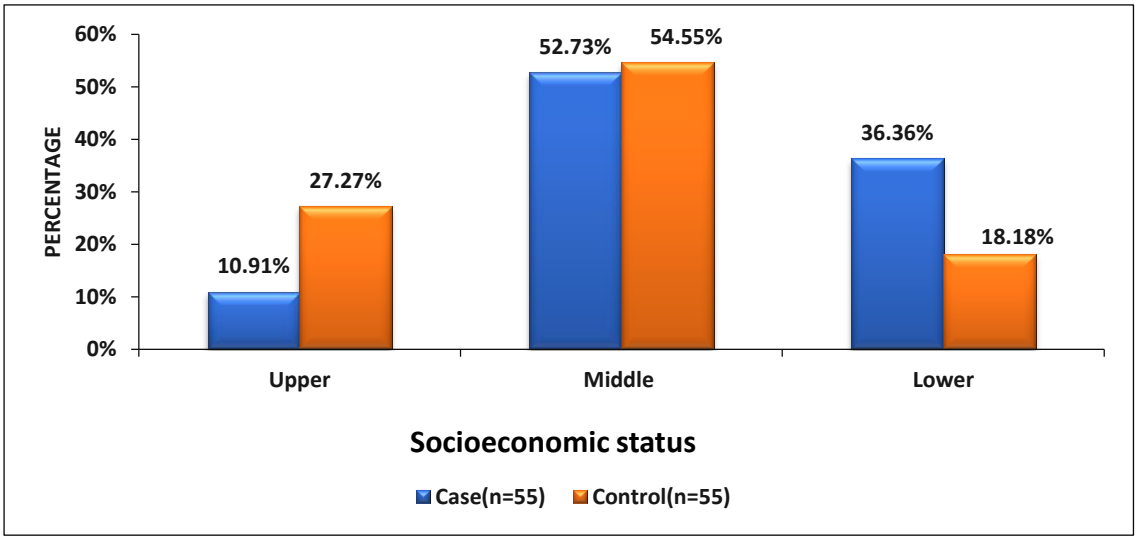

Figure 5: - Comparison of social economic status between cases and controls group

Compared to control group, there were significantly lower number of patients of upper class (10.91% vs 27.27%, p- value <0.05); significantly lower number of patients of middle class (52.73% vs 54.55%, p- value <0.05); and significantly higher number of patients of lower class (36.36% vs 18.18%, p- value <0.05) in case group observed from table5 and fig5.This is shown in Table 5 and Figure 5.

Table 6: - Study of severity of anaemia in cases

Haemoglobin	Number	Percentage
Mild anaemia (10-10.9)	13	23.64%
Moderate anaemia (7-9.9)	28	50.91%
Severe anaemia (<7)	14	25.45%
Total	55	100.00%

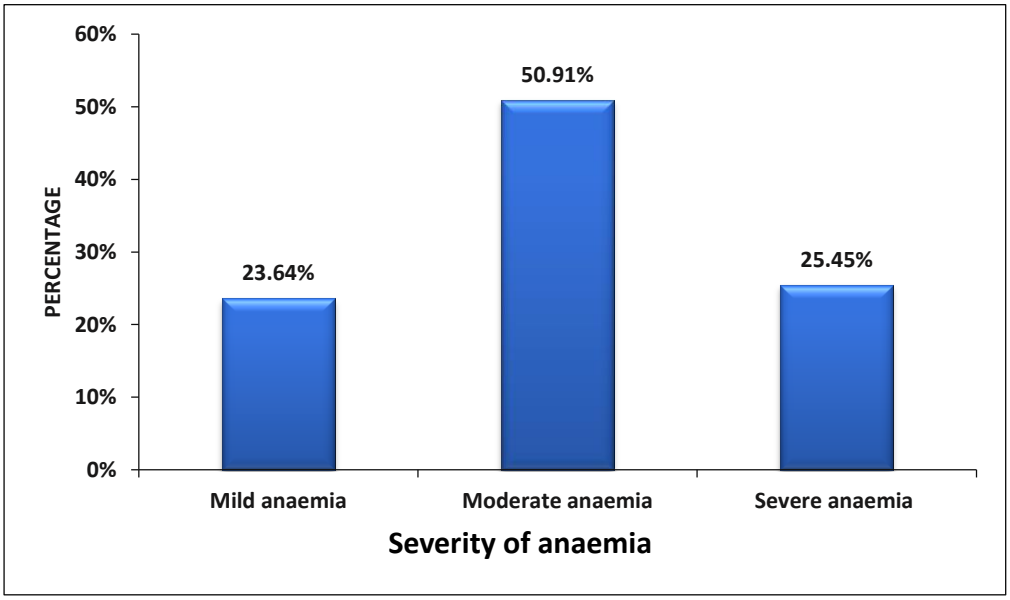

Figure 6: - Study of severity of anaemia in cases.

According to table 6 and figure 6, majority (50.91%) of the patients were moderate anaemic followed by severe anaemia (25.45%) and mild anaemia (23.64%).

Table 7: -Comparison of serum ferritin levels between cases and controls groups.

Serum ferritin levels (ng/ml)	Group		Total	P value
	Case(n=55)	Control(n=55)		
Deficient	18 (32.73%)	10 (18.18%)	28 (25.45%)	0.08*
Normal	37 (67.27%)	45 (81.82%)	82 (74.55%)	
Mean ± Stdev	16.38 ± 8.03	22.59 ± 14.79	19.49 ± 12.25	0.048#
Median (IQR)	14(10.550 - 18.550)	15.05(12.500 - 31.475)	14.8(11.900 - 24)	

*-Chi square test

#-Mann Whitney test

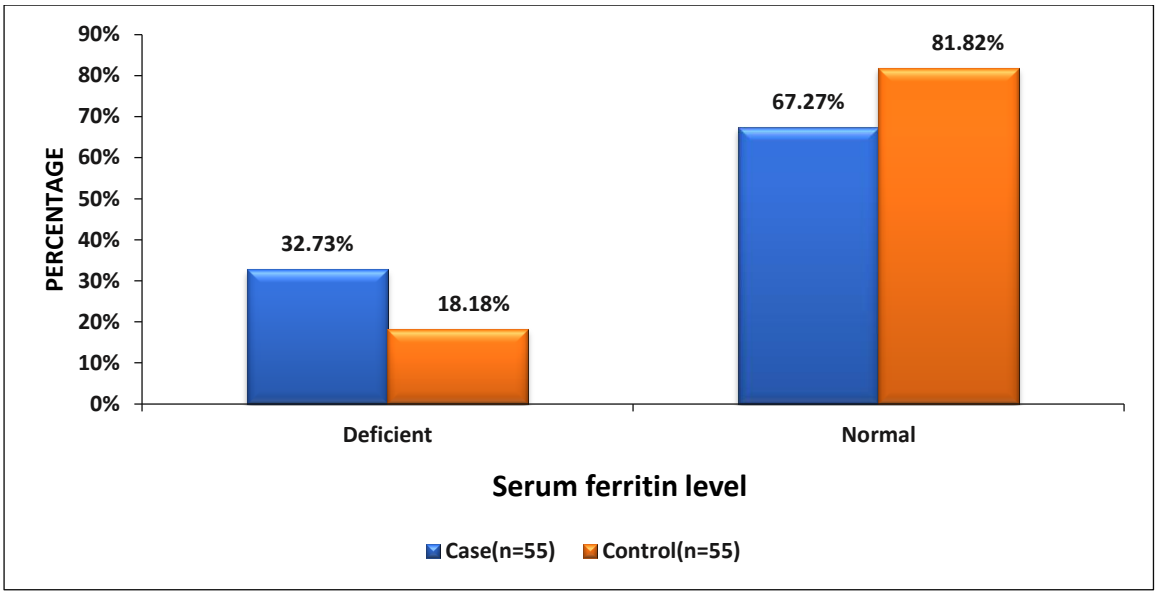

Figure 7.1: - Comparison of serum ferritin levels between cases and controls group

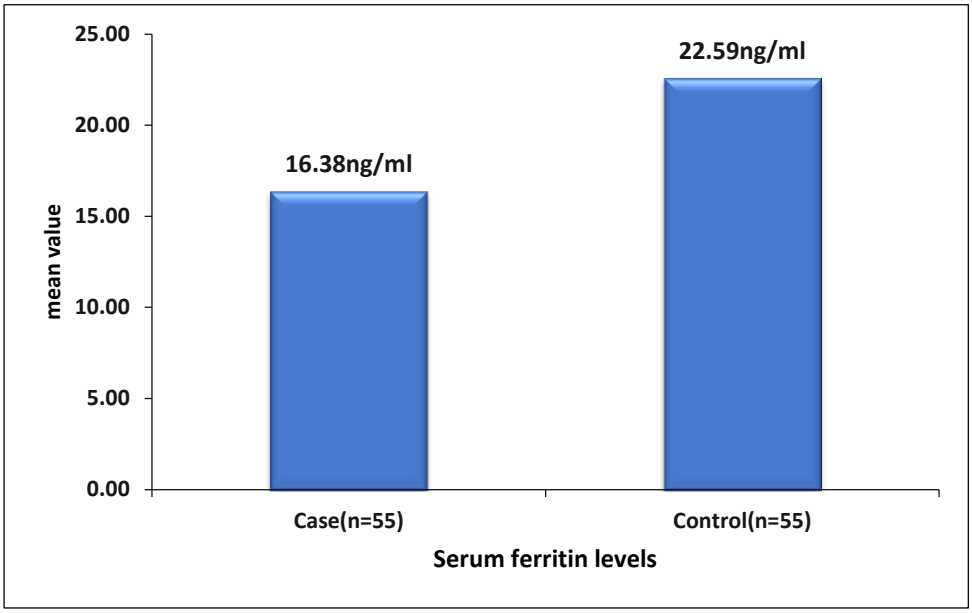

Figure 7.2: - Comparison of serum ferritin levels between cases and controls group

As compared to control group, patients in cases group had significantly lower mean Serum ferritin (ng/ml) **(**16.38 ± 8.03 vs 22.59 ± 14.79, P=0.048). The number of patients with deficient Serum ferritin were comparable between cases and control group (32.73% vs 18.18%, P = 0.080). This is shown in Table 7 and Figure 7.1 and 7.2.

Table 8: - Comparison of serum folate levels between cases and controls group

Serum folate levels (ng/ml)	Group		Total	P value
	Case(n=55)	Control(n=55)		
Deficient	28 (50.91%)	8 (14.55%)	36 (32.73%)	<.0001*
Normal	27 (49.09%)	47 (85.45%)	74 (67.27%)	
Mean ± Stdev	3.39 ± 2.36	6.51 ± 3.81	4.95 ± 3.52	<.0001#
Median (IQR)	2.82(1.532 - 4.297)	5.05(3.600 - 8.645)	3.7(2.340 - 6.860)	

*-Chi square test

#-Mann Whitney test

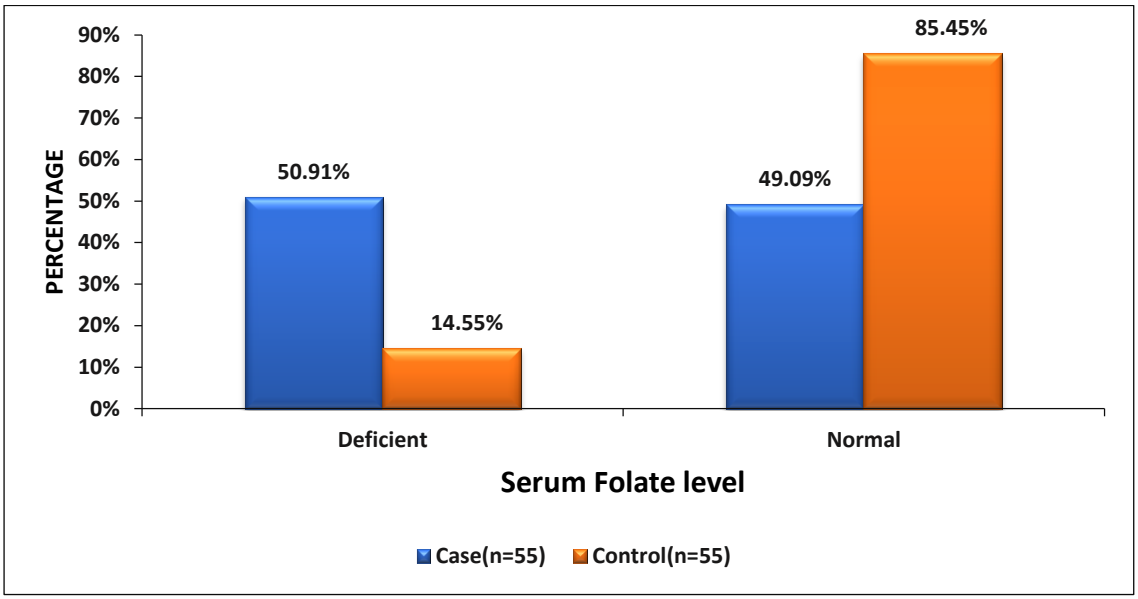

Figure 8.1: - Comparison of serum folate between cases and controls group

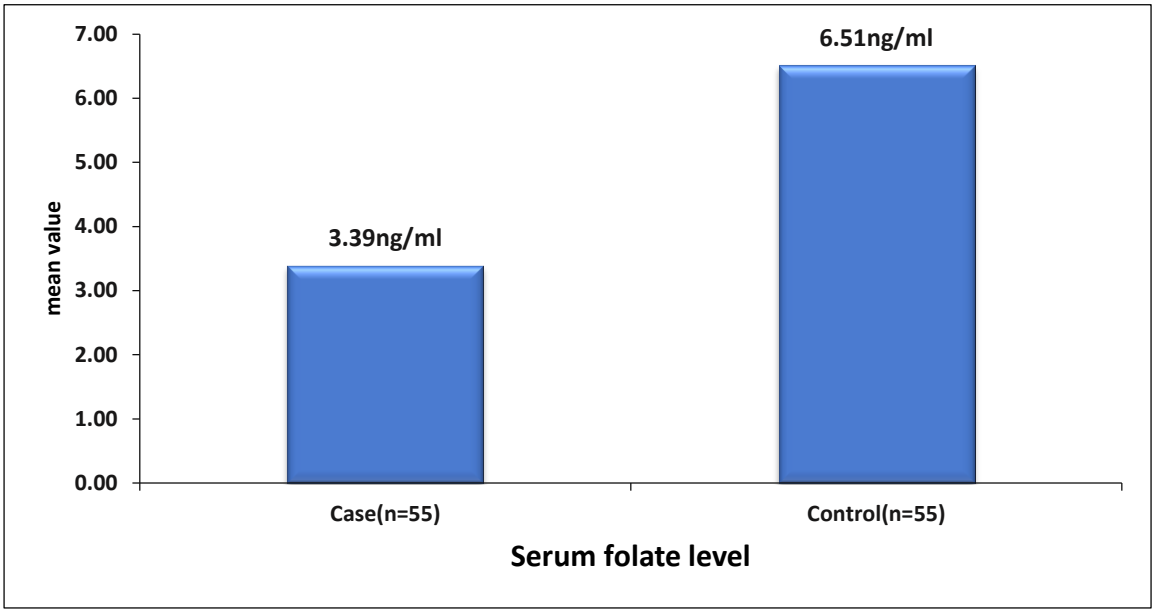

Figure 8.2: - Comparison of serum folate between cases and controls group

As compared to control group, patients in cases group had significantly lower mean serum folate (ng/ml) (3.39 ± 2.36 vs 6.51 ± 3.81, P<.0001). As compared to control group, patients in cases group had significantly higher number of patients with deficient serum folate (ng/ml) (50.91% vs 14.55%, P<.0001). This is shown in Table 8 and Figure 8.1 and 8.2.

Table 9: - Comparison of serum vitamin B12 level between cases and controls group

Serum vitamin B12(pg/ml)	Group		Total	P value
	Case(n=55)	Control(n=55)		
Deficient	31 (56.36%)	11 (20.00%)	42 (38.18%)	<.0001*
Normal	24 (43.64%)	44 (80.00%)	68 (61.82%)	
Mean ± Stdev	178.91 ± 51.08	237.4 ± 90.32	208.16 ± 78.72	<.0001#
Median (IQR)	160(159 - 200.750)	202(170 - 264.250)	178.5(159 - 230)	

*-Chi square test

#-Mann Whitney test

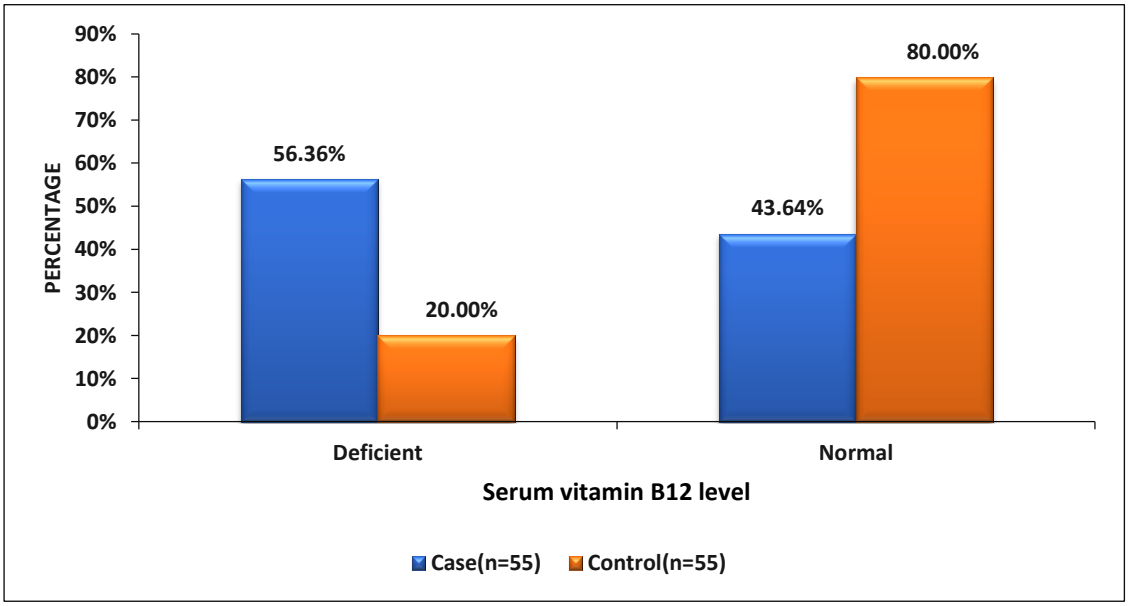

Figure 9.1: - Comparison of serum vitamin B12 between cases and controls group

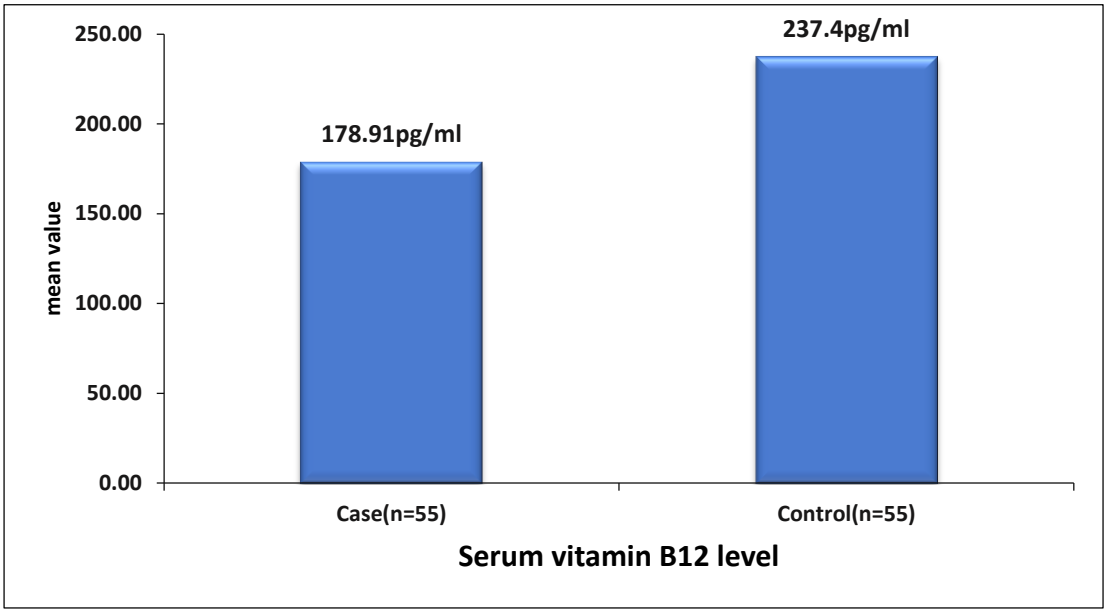

Figure 9.2: - Comparison of serum vitamin B12 between cases and controls group

As compared to control group, patients in cases group had significantly lower Serum vitamin B12 (pg/ml) (178.91 ± 51.08 vs 237.4 ± 90.32, P <0.0001) and also the number of patients with deficient Serum vitamin B12 were higher among cases than control group (56.36% vs 20.00%, P<0.0001). This is shown in Table 9 and Figure 9.1 and 9.2.

Table 10: - Comparison of serum and urine fluoride levels between cases and controls group

Serum and Urine fluoride levels(mg/l)	Group		Total	P value
	Case(n=55)	Control(n=55)		
Serum fluoride(mg/l)				
Mean ± Stdev	0.48 ± 0.36	0.19 ± 0.14	0.34 ± 0.31	<.0001 *
Median (IQR)	0.38(0.120 - 0.880)	0.1(0.0600 - 0.300)	0.2(0.100 - 0.480)	
Urine fluoride(mg/l)				
Mean ± Stdev	1.68 ± 0.68	0.94 ± 0.48	1.31 ± 0.69	<.0001 *
Median (IQR)	1.76(1.200 - 2.208)	0.85(0.640 - 1.072)	1.17(0.720 - 1.890)	

*-Mann Whitney test

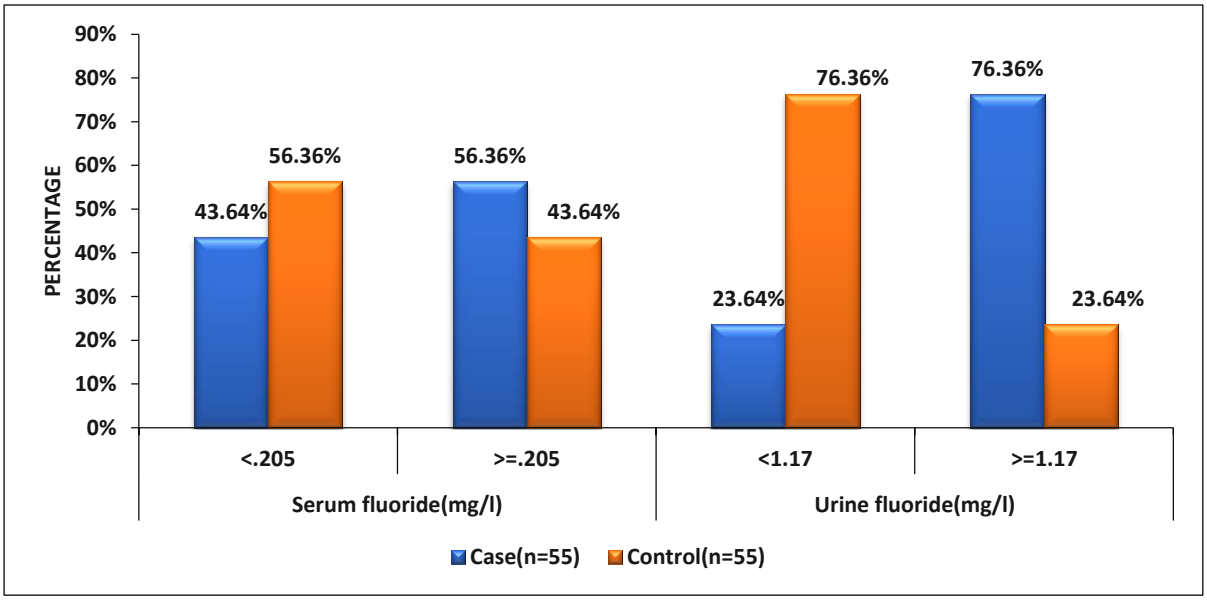

Figure 10: - Comparison of serum and urine fluoride levels between cases and controls groups.

In comparison with control group, patients in cases group had significantly higher Serum fluoride(mg/l) (0.48 ± 0.36 vs 0.19 ± 0.14, P <0.0001) and significantly higher Urine fluoride(mg/l) levels (1.68 ± 0.68 vs 0.94 ± 0.48, P <0.0001). This is shown in Table 10 and Figure 10.

Table 11: - Correlation of haemoglobin with serum and urine fluoride.

Correlation matrix		Haemoglobin
Serum fluoride (mg/l)	Correlation Coefficient	-0.365
	P value	0.0001
	n	110
Urine fluoride(mg/l)	Correlation Coefficient	-0.444
	P value	<0.0001
	n	110

Speearman rank correlation coefficient

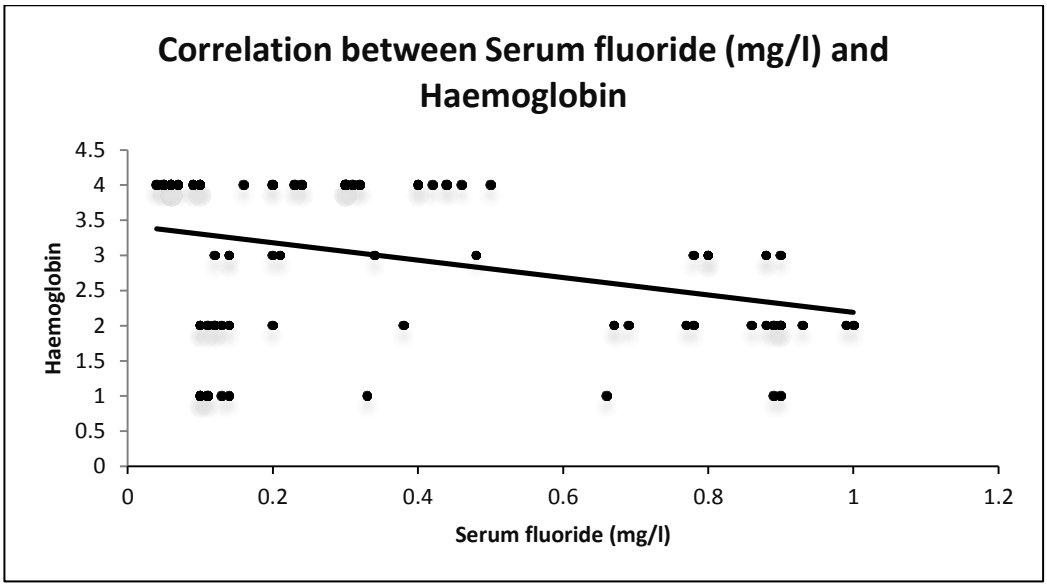

Figure 11.1: Correlation between Serum fluoride (mg/l) and Haemoglobin

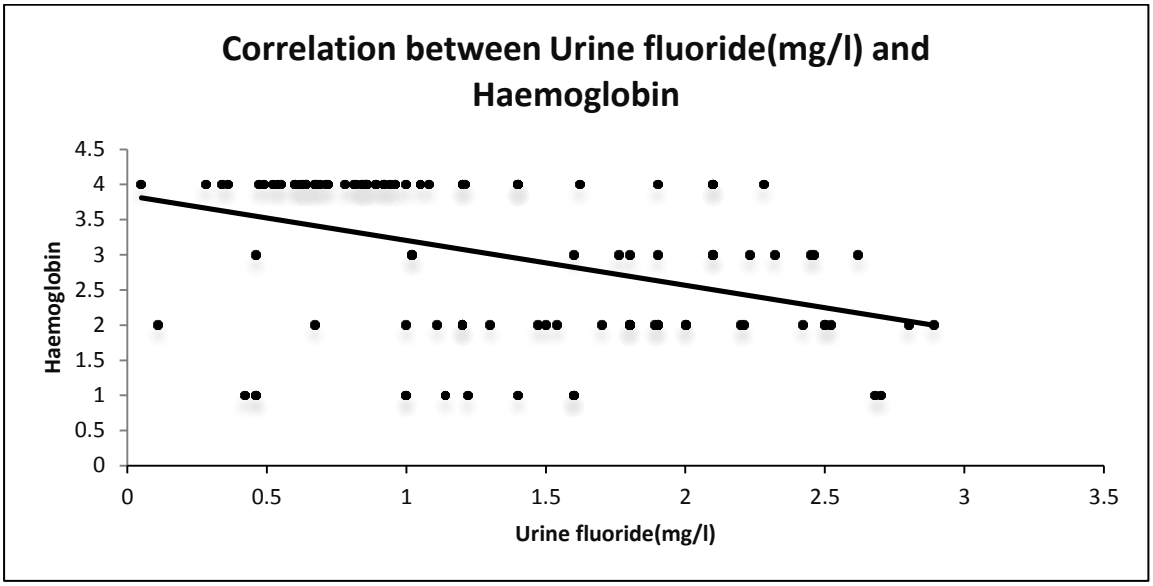

Figure 11.2: - Correlation of haemoglobin with urine fluoride.

We found a significant negative correlation of Haemoglobin with Serum fluoride (r=-0.365, P=0.0001) and urine fluoride levels (r=-0.444, P<0.0001). The haemoglobin levels decreased significantly with increase in the serum and urine fluoride levels. It is shown in Table 11 and Fig. 11.1 and 11.2

Table 12: - Study of type of anaemia in cases

Peripheral smear	Number	Percentage
Normocytic normochromic anaemia	22	40.00%
Microcytic hypochromic anaemia	21	38.18%
Dimorphic anaemia	8	14.55%
Megaloblastic anaemia	4	7.27%
Total	55	100.00%

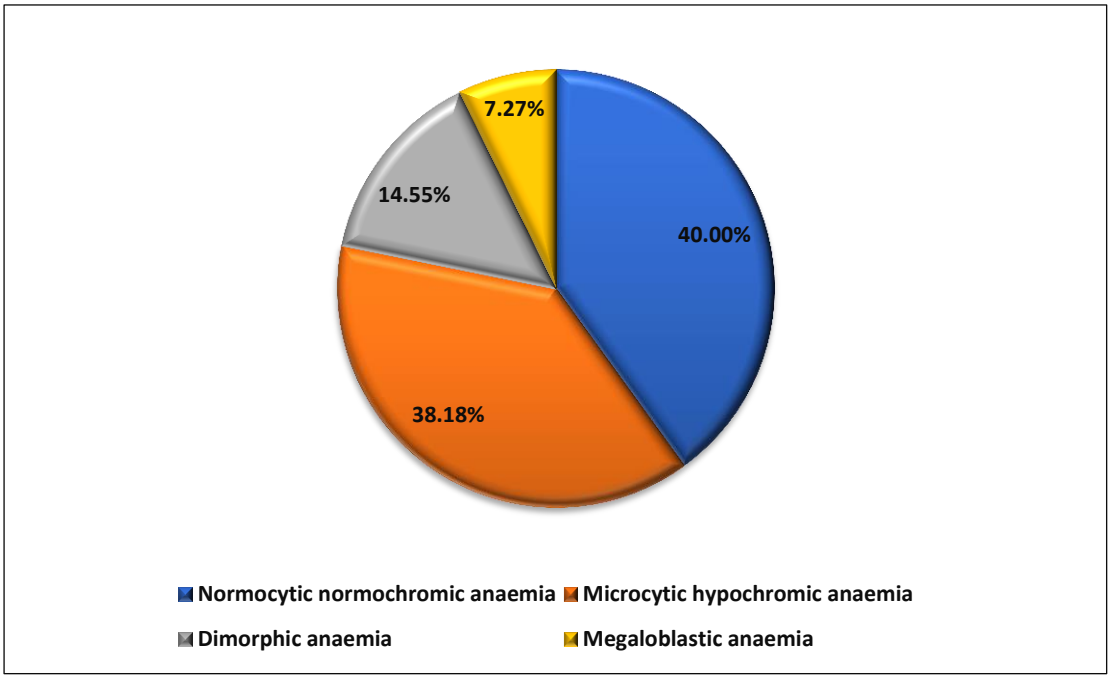

Figure 12: - Study of type of anaemia in cases

As tabulated in table 12, peripheral smear shows, normocytic normochromic anaemia was noted in 40.00% (majority), microcytic hypochromic anaemia in 38.18%, megaloblastic anaemia in 7.27% and dimorphic anaemia in 21.82% patients. This is shown in Table 12 and Figure 12.

Table 13: - Comparison of symptoms between cases and controls groups

Symptoms	Group		Total	P value
	Case(n=55)	Control(n=55)		
Fatiguability	15 (27.27%)	0 (0.00%)	15 (13.64%)	<.0001*
Dyspnoea	6 (10.91%)	0 (0.00%)	6 (5.45%)	
Pedal edema	16 (29.09%)	0 (0.00%)	16 (14.55%)	
Asymptomatic	18 (32.73%)	55 (100.00%)	68 (61.82%)	
Total	55 (100.00%)	55 (100.00%)	110 (100.00%)	

*-Chi square test

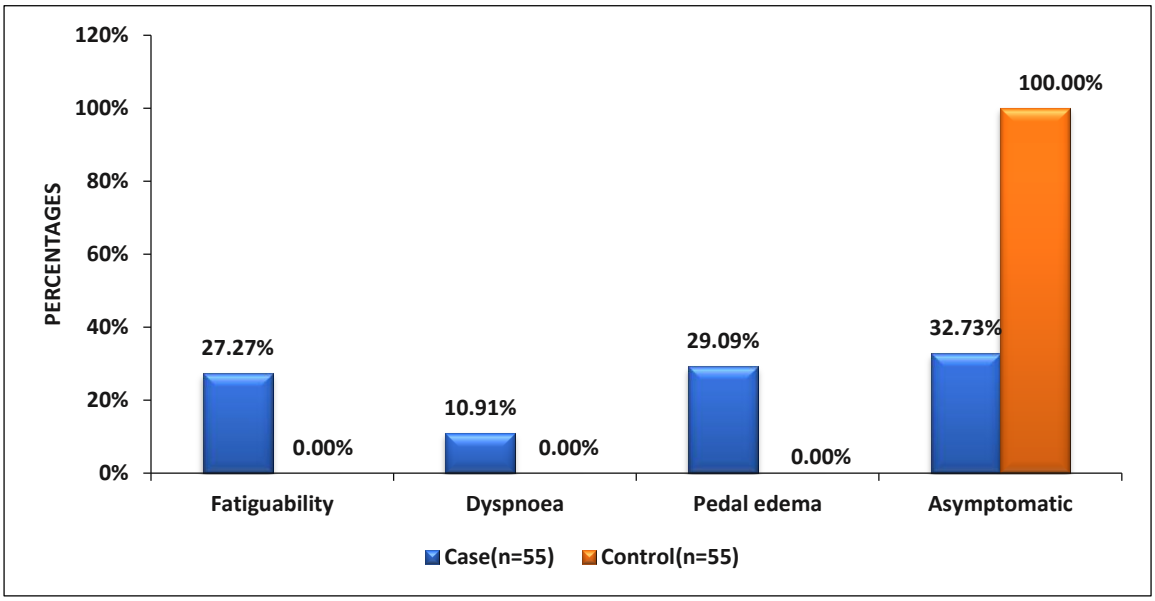

Figure 13: - Comparison of symptoms between cases and controls group

All controls were asymptomatic, as compared to only 32.73% of patients in cases. In comparison with control group, patients in cases group had significantly higher number of patients with Fatiguability (27.27% vs 0.00%, P<.0001), Dyspnoea (10.91% vs 0.00%, P<.0001), and Pedal edema (29.09% vs 0.00%, P<.0001).

This is shown in Table 13 and Figure 13.

Table 14: - Comparison of significant past obstetric history between cases and controls group

Significant past obstetric history	Group		Total	P value
	Case(n=55)	Control(n=55)		
Not significant	36 (65.45%)	55 (100.00%)	91 (82.73%)	<.0001*
H/o blood transfusion	9 (16.36%)	0 (0.00%)	9 (8.18%)	
H/o iron sucrose infusion	10 (18.18%)	0 (0.00%)	10 (9.09%)	
Total	55 (100.00%)	55 (100.00%)	110 (100.00%)	

*-Chi square test

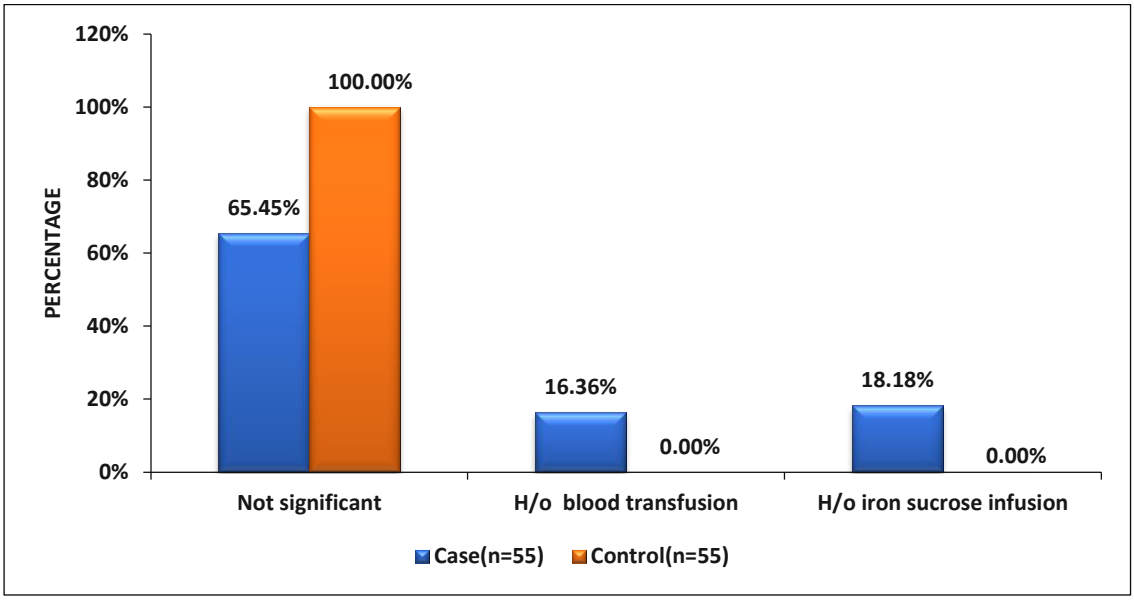

Figure 14: - Comparison of significant past obstetric history between cases and controls group

34.5% cases had significant past obstetric history with no controls having significant past history. (P <0.0001). Among the cases, 9 patients had H/o blood transfusion and 10 patients had H/o iron sucrose infusion. This is shown in Table 14 and Figure 14.

Table 15: - Comparison of blood transfusion requirement between cases and controls group

Blood Transfusion Requirement	Group		Total	P value
	Case(n=55)	Control(n=55)		
Yes	20 (36.36%)	0 (0.00%)	20 (18.18%)	<.0001*
No	35 (63.64%)	55 (100.00%)	90 (81.82%)	
Total	55 (100.00%)	55 (100.00%)	110 (100.00%)	

*-Fisher's Exact test

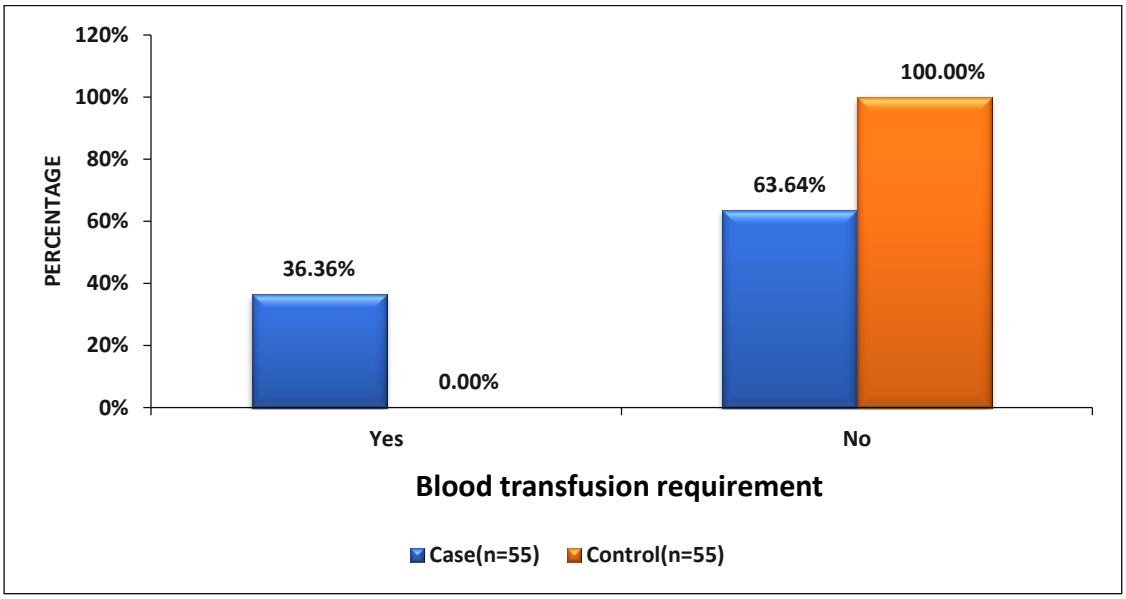

Figure 15: - Comparison of blood transfusion requirement between cases and controls group

Blood transfusion was required in 36.36% patients. In comparison with control group, patients in cases group had significantly higher number of patients who required blood transfusion (36.36% vs 0.00%, P<.0001). This is shown in Table 15 and Figure 15.

Table 16: - Comparison of mode of delivery between cases and controls group

Mode of delivery	Group		Total	P value
	Case(n=55)	Control(n=55)		
Normal delivery	22 (40.00%)	25 (45.45%)	47 (42.73%)	0.643*
Instrumental	8 (14.55%)	5 (9.09%)	13 (11.82%)	
LSCS	25 (45.45%)	25 (45.45%)	50 (45.45%)	
Total	55 (100.00%)	55 (100.00%)	110 (100.00%)	

*-Chi square test

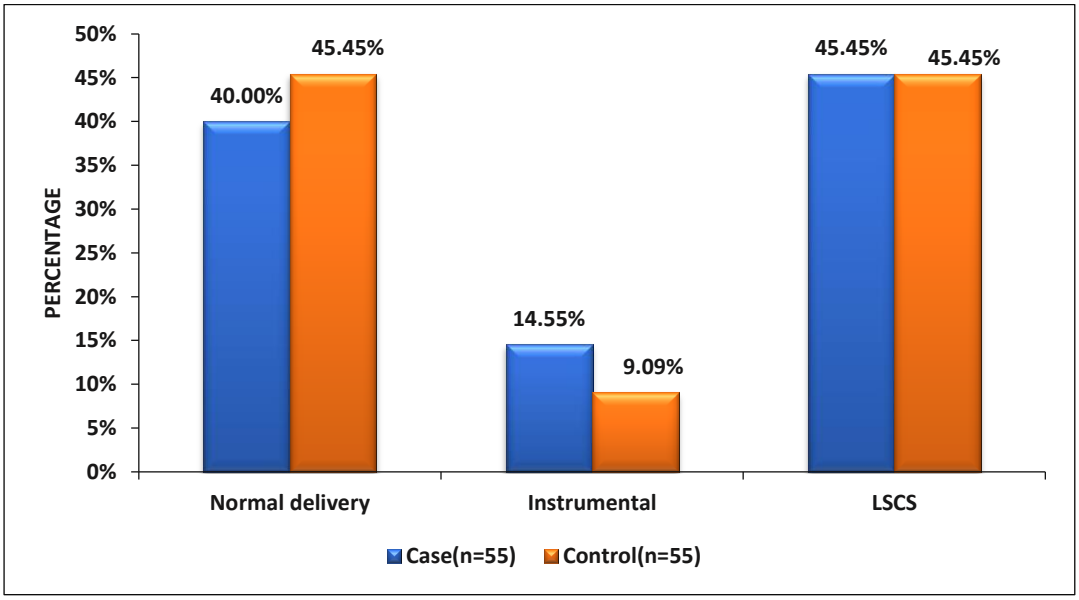

Figure 16: - Comparison of mode of delivery between cases and controls group

In this study, in majority of the patients, mode of delivery was caesarean section. Mode of delivery between cases and controls was comparable (P = 0.643). This is shown in Table 16 and Figure 16.

Table 17: - Comparison of indication of LSCS between cases and controls group

Indication of LSCS	Group		Total	P value
	Case(n=55)	Control(n=55)		
Fetal distress	6 (24.00%)	5 (20.00%)	11 (22.00%)	0.607*
Previous LSCS	9 (36.00%)	10 (40.00%)	19 (38.00%)	
Oligohydramnios	2 (8.00%)	5 (20.00%)	7 (14.00%)	
Breech	5 (20.00%)	2 (8.00%)	7 (14.00%)	
Cephalopelvic disproportion	3 (12.00%)	3 (12.00%)	6 (12.00%)	
Total	25 (100.00%)	25 (100.00%)	50 (100.00%)	

*-Chi square test

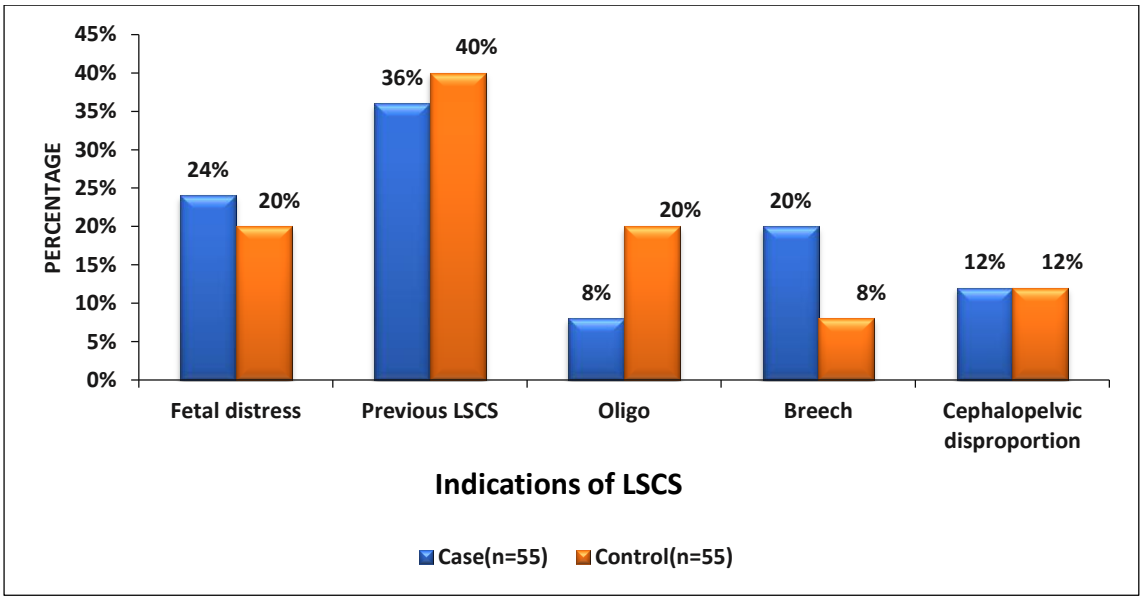

Figure 17: - Comparison of indication of LSCS between cases and controls group

Indication of LSCS was Previous LSCS. Indications of LSCS between cases and controls were compared with p- value p- value> 0.05) tabulated in Table 17 and Figure 17.

Table 18: - Comparison of maternal complications between cases and controls group

Maternal complications	Group		Total	P value
	Case(n=55)	Control(n=55)		
Wound gaping	6 (10.91%)	2 (3.64%)	8 (7.27%)	**0.021***
Postpartum haemorrhage	2 (3.64%)	0 (0.00%)	2 (1.82%)	
Failed lactation	4 (7.27%)	0 (0.00%)	4 (3.64%)	
Sub involution of uterus	2 (3.64%)	0 (0.00%)	2 (1.82%)	
No complication	41 (74.55%)	53 (96.36%)	94 (85.45%)	
Total	55 (100.00%)	55 (100.00%)	110 (100.00%)	

*-Chi square test

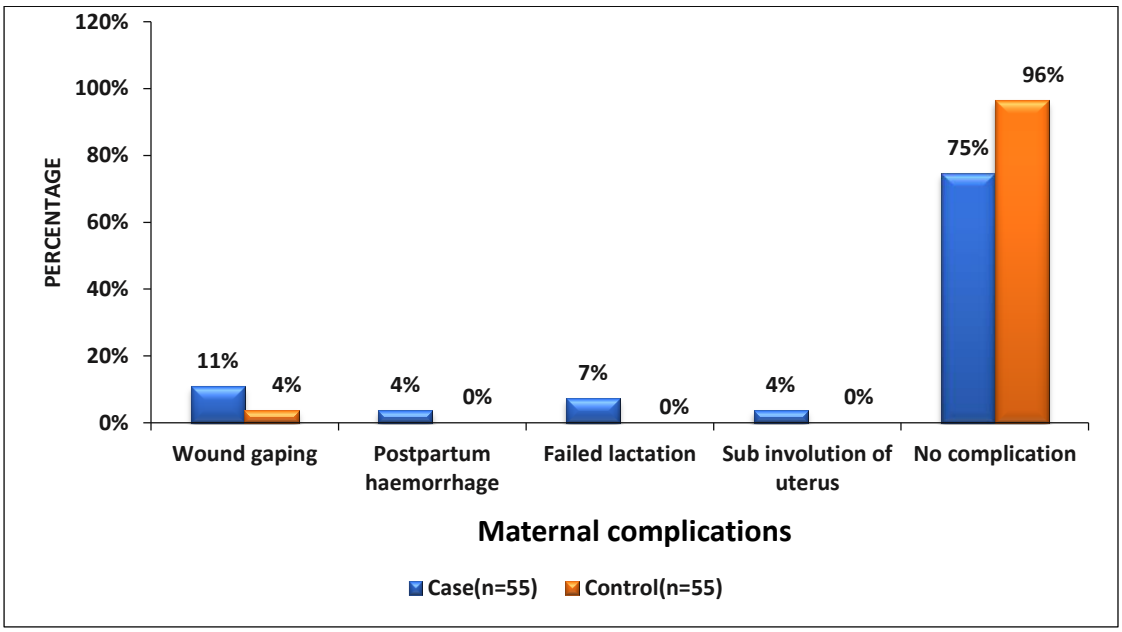

Figure 18: - Comparison of maternal complications between cases and controls group

In comparison of data in table 18, cases had significantly higher Wound gaping (10.91% vs 3.64%), significantly higher postpartum haemorrhage (3.64% vs 0.00%), higher failed lactation (7.27% vs 0.00%), and higher subinvolution of uterus (3.64% vs 0.00%), (P = 0.021). This is shown in Table 18 and Figure 18.

Table 19: - Comparison of fetal outcome between cases and controls group

Fetal outcome	Group		Total	P value
	Case(n=55)	Control(n=55)		
Term infants	37 (67.27%)	46 (83.64%)	83 (75.45%)	0.046*
Preterm infants	18 (32.73%)	9 (16.36%)	27 (24.55%)	
Total	55 (100.00%)	55 (100.00%)	110 (100.00%)	

*-Chi square test

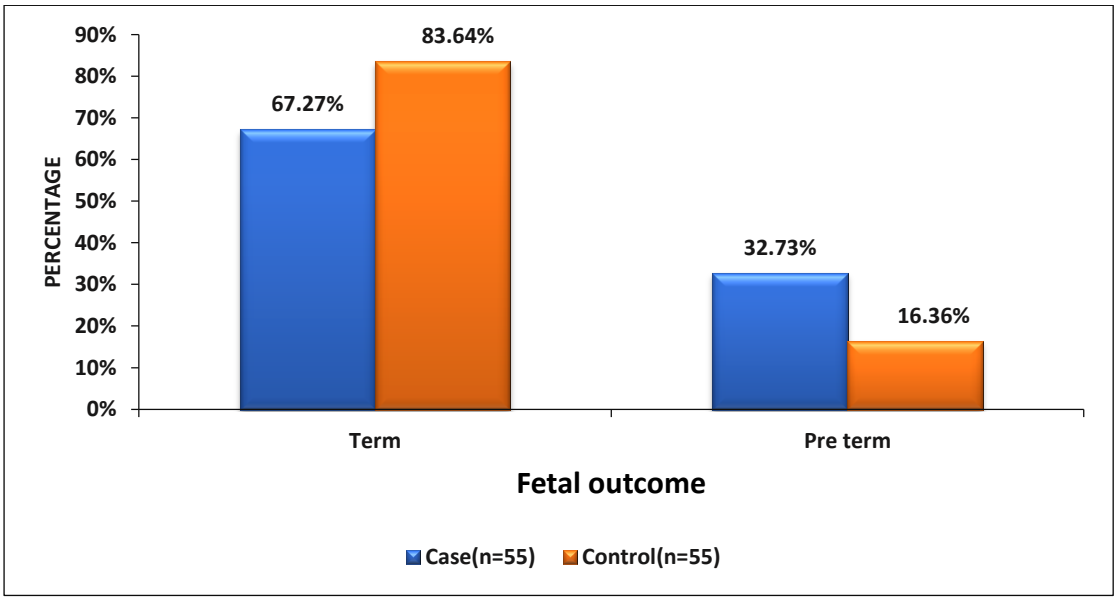

Figure 19: - Comparison of fetal outcome between cases and controls group

In this study, majority (75.45%) were full term infants. In comparison with control group, patients in cases group had significantly lower number of full-term infants (67.27% vs 83.64%, P = 0.046) and significantly higher number of preterm infants (32.73% vs 16.36%, P = 0.046). This is shown in Table 19 and Figure 19.

Table 20 : - Comparison of birth weight between cases and controls group

Birth weight(kg)	Group		Total	P value
	Case(n=55)	Control(n=55)		
1-1.45	4 (7.27%)	3 (5.45%)	7 (6.36%)	0.034*
1.5 -2.49	21 (38.18%)	8 (14.55%)	29 (26.36%)	
2.5-3.49	29 (52.73%)	43 (78.18%)	72 (65.45%)	
>3.5	1 (1.82%)	1 (1.82%)	2 (1.82%)	
Total	55 (100.00%)	55 (100.00%)	110 (100.00%)	

*-Chi square test

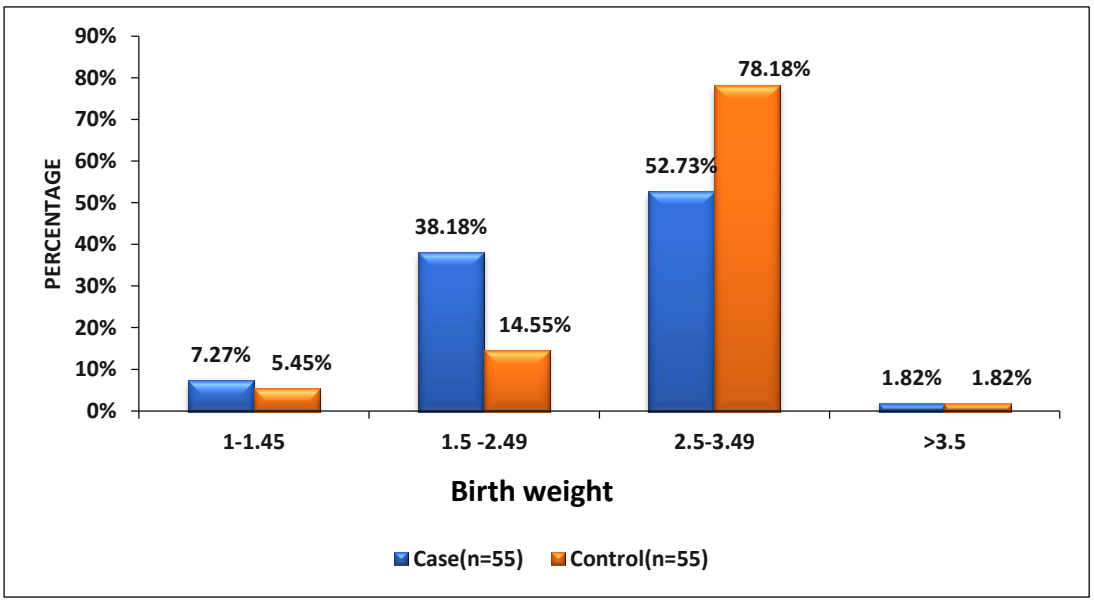

Figure 20: - Comparison of birth weight between cases and controls group

In this study, majority (65.45%) had birthweight 2.5-3.49 kg. In comparison with control group, babies in cases group had significantly higher number of babies with birthweight 1.5 -2.49 kg (38.18% vs 14.55%, P = 0.034), and significantly lower number of babies with birthweight 2.5-3.49 kg (52.73% vs 78.18%, P = 0.046).This is shown in Table 20 and Figure 20.

Table 21: - Comparison of APGAR 1'min between cases and controls group

APGAR 1'min	Group		Total	P value
	Case(n=55)	Control(n=55)		
<7/10	6 (10.91%)	3 (5.45%)	9 (8.18%)	0.489*
>7/10	49 (89.09%)	52 (94.55%)	101 (91.82%)	
Total	55 (100.00%)	55 (100.00%)	110 (100.00%)	

*-Fisher's Exact test

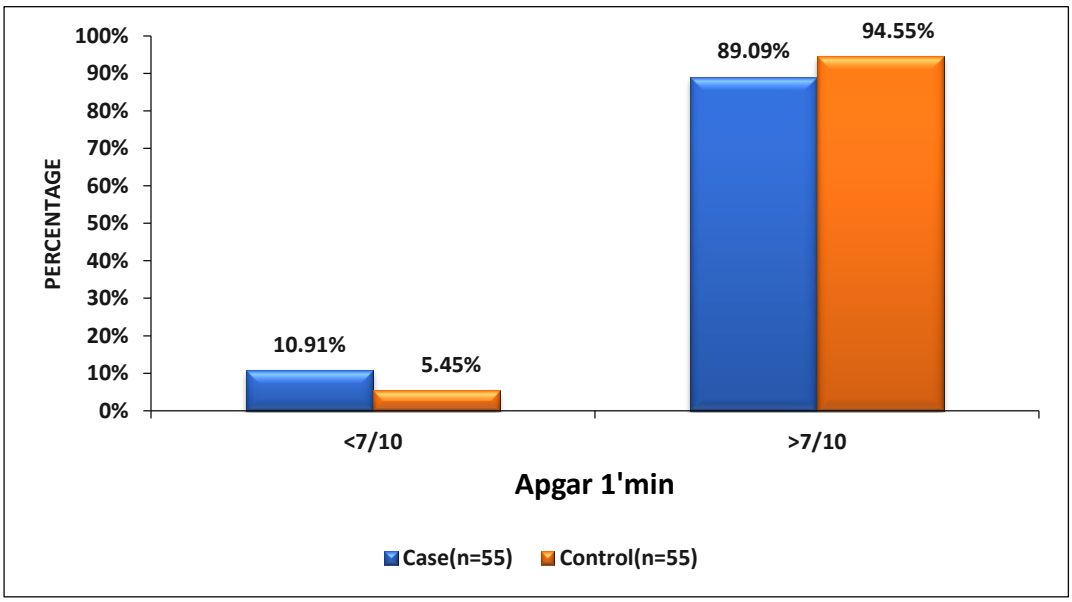

Figure 21: - Comparison of APGAR 1'min between cases and controls groups.

In this study, majority (91.82%) of infants had APGAR score at 1 min >7/10. The APGAR score between cases and control group was comparable, <7/10 - 10.91% vs 5.45% and >7/10- 89.09% vs 94.45%(P=0.489). This is shown in Table 21 and Figure 21.

Table 22: - Comparison of NICU admission between cases and controls group

NICU admission	Group		Total	P value*
	Case(n=55)	Control(n=55)		
Yes	30 (54.55%)	15 (27.27%)	45 (40.91%)	0.004
No	25 (45.45%)	40 (72.73%)	65 (59.09%)	
Reasons of NICU admission	**Case(n=30)**	**Control(n=15)**	**Total**	**P value**
Respiratory distress syndrome	17 (56.67%)	9 (64.29%)	26 (59.09%)	0.68
Jaundice	2 (6.67%)	2 (14.29%)	4 (9.09%)	
Meconium aspiration syndrome	5 (16.67%)	1 (7.14%)	6 (13.64%)	
Preterm care	6 (20.00%)	2 (14.29%)	8 (18.18%)	

*-Chi square test

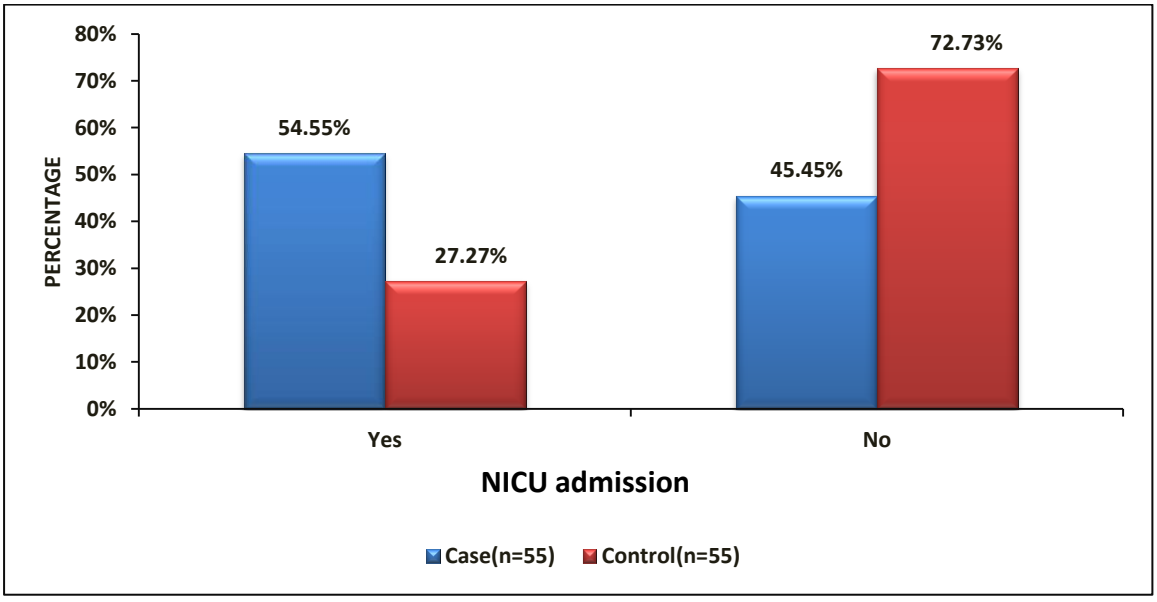

Figure 22.1: - Comparison of NICU admission between cases and controls group

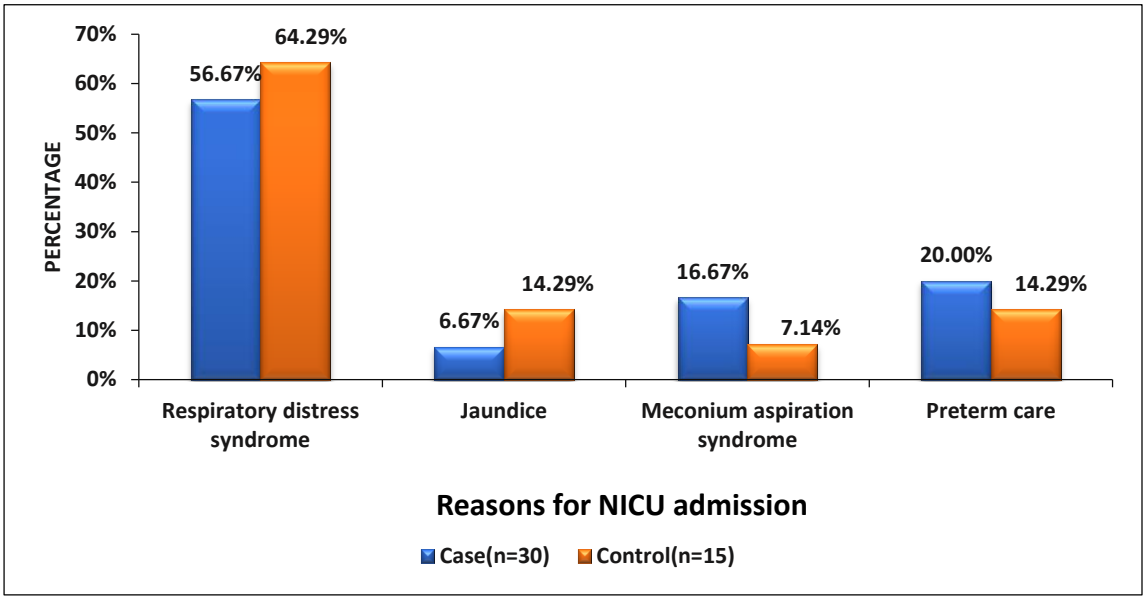

Figure 22.2: - Comparison of cause of NICU admission between cases and controls group

Based on the data collected, 40.91% babies required NICU admission. There were significantly higher number of babies in cases group who required NICU admission (54.55% vs 27.27%, P = 0.004). There was no significant difference between cases and controls in terms of reasons for NICU admission (p- value> 0.05).

This is shown in Table 22 and Figure 22.1 and 22.2.

Table 23: - Comparison of perinatal mortality between cases and controls group

Perinatal mortality	Group		Total	P value
	Case(n=55)	Control(n=55)		
Yes	4 (7.27%)	2 (3.64%)	6 (5.45%)	0.679*
No	51 (92.73%)	53 (96.36%)	104 (94.55%)	
Total	55 (100.00%)	55 (100.00%)	110 (100.00%)	

*-Fisher's exact test

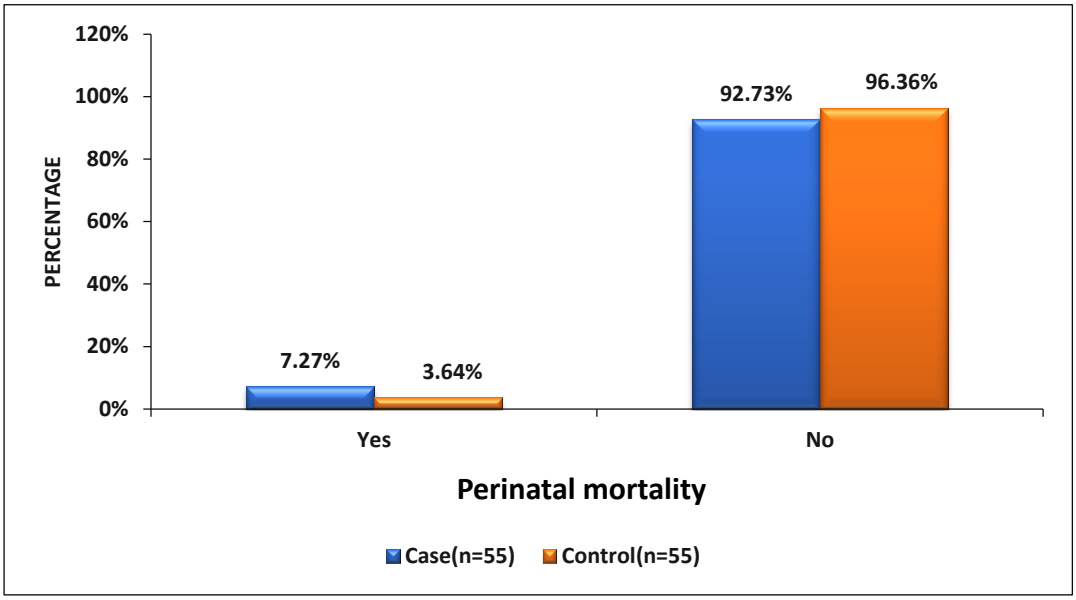

Figure 23: - Comparison of perinatal mortality between cases and controls group

The above data represents, perinatal mortality to be 5.45%%. The perinatal mortality between cases and controls were compared (7.25% vs 3.64% p> 0.05) and represented in Table 23 and Figure 23.

Table 24: - Maternal outcome with serum fluoride levels

Maternal outcome	Serum fluoride(mg/l)		Total	P value*
	<0.205	>=0.205		
Mode of delivery				
Normal delivery	25 (45.45%)	22 (40.00%)	47 (42.73%)	0.643
Instrumental	5 (9.09%)	8 (14.55%)	13 (11.82%)	
LSCS	25 (45.45%)	25 (45.45%)	50 (45.45%)	
Maternal complications				
Wound gaping	2 (3.64%)	6 (10.91%)	8 (7.27%)	**0.038**
Postpartum haemorrhage	2 (3.64%)	0 (0.00%)	2 (1.82%)	
Failed lactation	4 (7.27%)	0 (0.00%)	4 (3.64%)	
Sub involution of uterus	2 (3.64%)	0 (0.00%)	2 (1.82%)	
No complication	45 (81.82%)	49 (89.09%)	94 (85.45%)	

*-Chi square test

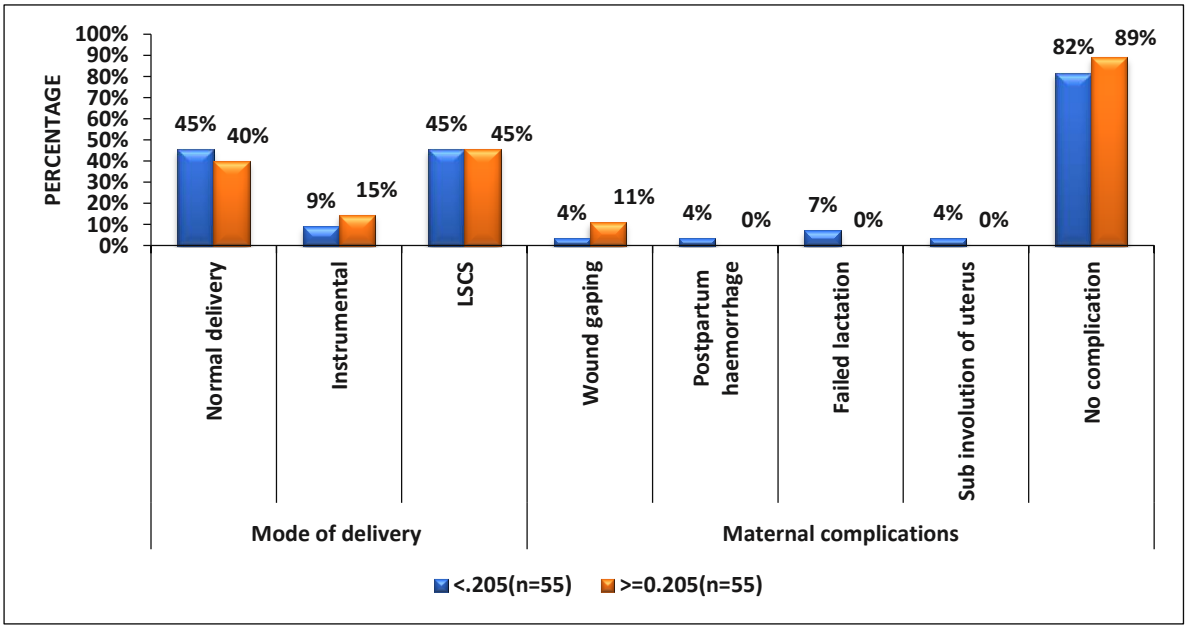

Figure 24: - Maternal outcome with serum fluoride levels

Compared to patients with serum fluoride levels <0.205 mg/L, there was no significant association of Mode of delivery with patients with serum fluoride levels >=0.205 mg/L (P>0.05); with most of the patients in both groups having LSCS.

Among maternal complications, Postpartum haemorrhage, Failed lactation and Sub involution of uterus was significantly higher in patients with serum fluoride levels >=0.205 mg/L patients and there were significantly less complications in patients with serum fluoride <0.205 mg/L (P<0.05) as shown in the Table 24 and Figure 24.

Table 25: - Maternal outcome with urine fluoride levels

Maternal outcome	Urine fluoride(mg/l)		Total	P value*
	<1.17	>=1.17		
Mode of delivery				
Normal delivery	26 (47.27%)	21 (38.18%)	47 (42.73%)	0.286
Instrumental	8 (14.55%)	5 (9.09%)	13 (11.82%)	
LSCS	21 (38.18%)	29 (52.73%)	50 (45.45%)	
Maternal complications				
Wound gaping	1 (1.82%)	7 (12.73%)	8 (7.27%)	0.064
Postpartum haemorrhage	0 (0.00%)	2 (3.64%)	2 (1.82%)	
Failed lactation	2 (3.64%)	2 (3.64%)	4 (3.64%)	
Sub involution of uterus	2 (3.64%)	0 (0.00%)	2 (1.82%)	
No complication	50 (90.91%)	44 (80.00%)	94 (85.45%)	

*-Chi square test

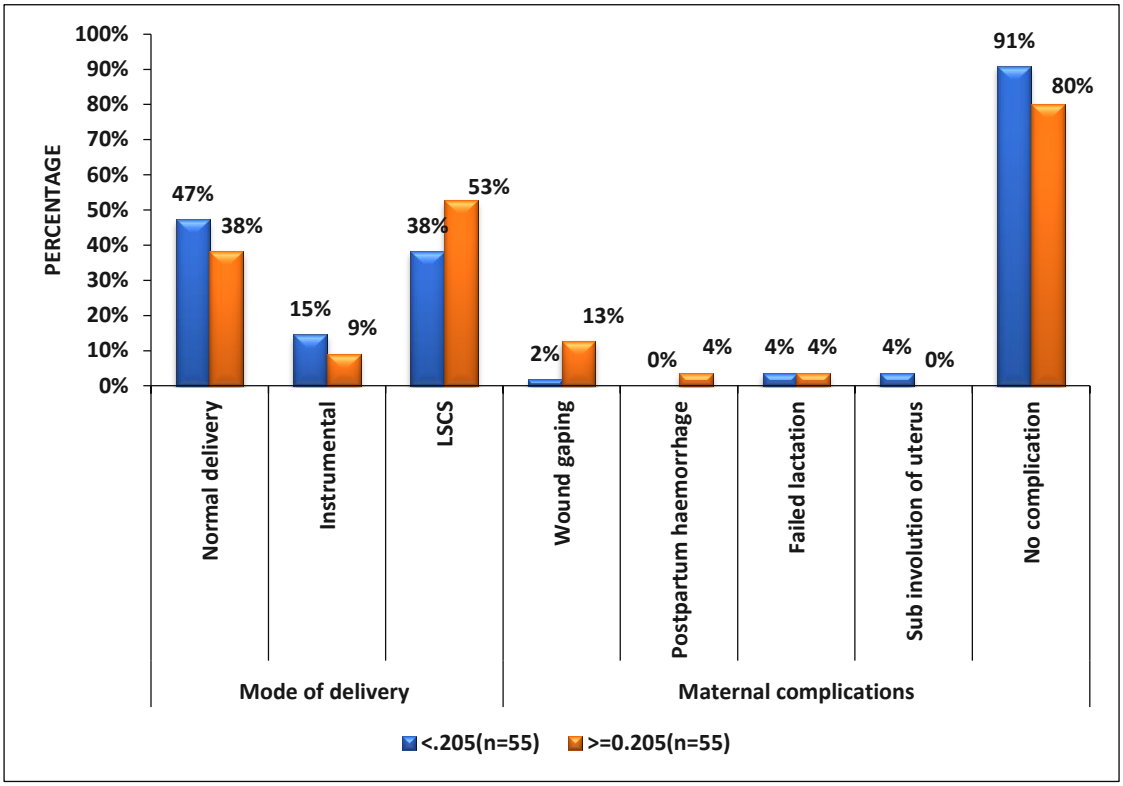

Figure 25: - Maternal outcome with urine fluoride levels

Subjects with urine fluoride levels >=1.17 mg/L had comparable mode of delivery and maternal complications as shown in the Table 25 and Figure 25.

Table 26: - Fetal outcome with serum fluoride levels

Fetal outcome	Serum fluoride (mg/l)		Total	P value
	<0.205	>=0.205		
Term/preterm birth				
Term infants	48 (87.27%)	35 (63.64%)	83 (75.45%)	**0.004***
Preterm infants	7 (12.73%)	20 (36.36%)	27 (24.55%)	
Birth weight(kg)				
1-1.45	3 (5.45%)	4 (7.27%)	7 (6.36%)	**<.0001***
1.5 -2.49	3 (5.45%)	26 (47.27%)	29 (26.36%)	
2.5-3.49	49 (89.09%)	23 (41.82%)	72 (65.45%)	
>3.5	0 (0.00%)	2 (3.64%)	2 (1.82%)	
Apgar 1'min				
<7/10	3 (5.45%)	6 (10.91%)	9 (8.18%)	0.489#
>7/10	52 (94.55%)	49 (89.09%)	101 (91.82%)	
NICU admission				
Yes	17 (30.91%)	28 (50.91%)	45 (40.91%)	**0.033***
No	38 (69.09%)	27 (49.09%)	65 (59.09%)	
Perinatal mortality				
Yes	1 (1.82%)	3 (5.45%)	4 (3.64%)	0.618#
No	54 (98.18%)	52 (94.55%)	106 (96.36%)	

*-Chi square test

#-Fisher's Exact test

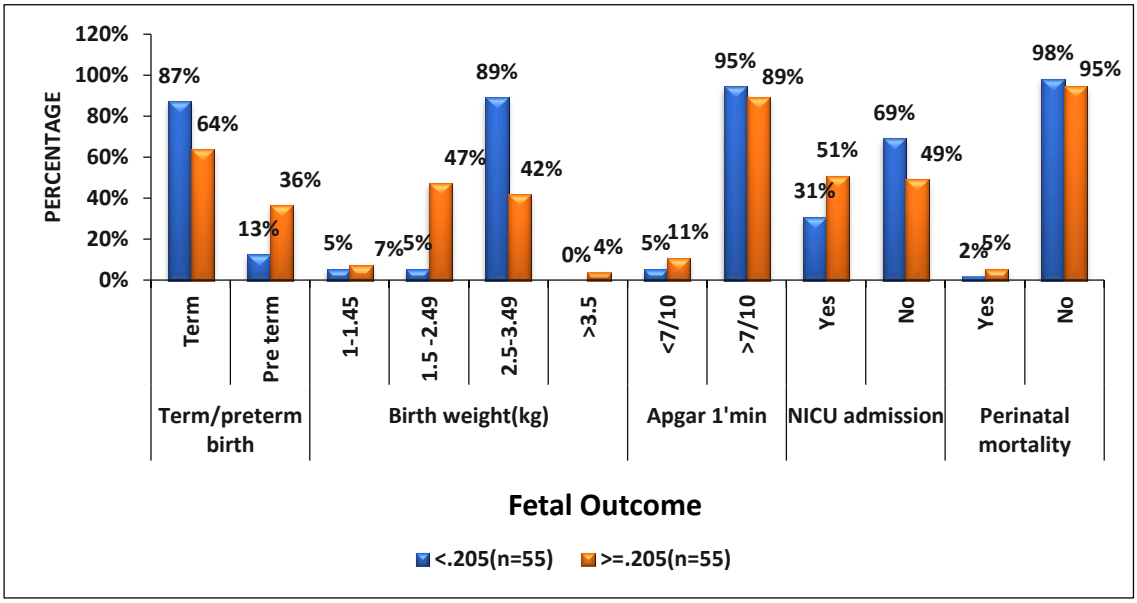

Figure 26: -Fetal outcome with serum fluoride levels

Significant higher pre-term births(12.73%vs36.36%)P value-0.004, lower birth weight(5.45%vs47.27%) P value < 0.0001 and higher NICU admissions (30.91%vs5091%)P value 0.033. The APGAR (1 minute) and perinatal mortality was comparable among the two groups as shown in the Table 26 and Figure 26.

Table 27: - Fetal outcome with urine fluoride levels.

Fetal outcome	Urine fluoride(mg/l)		Total	P value
	<1.17	>=1.17		
Term/preterm birth				
Term	46 (83.64%)	37 (67.27%)	83 (75.45%)	**0.046***
Pre term	9 (16.36%)	18 (32.73%)	27 (24.55%)	
Birth weight(kg)				
1-1.45	3 (5.45%)	4 (7.27%)	7 (6.36%)	**0.0004***
1.5 -2.49	5 (9.09%)	24 (43.64%)	29 (26.36%)	
2.5-3.49	46 (83.64%)	26 (47.27%)	72 (65.45%)	
>3.5	1 (1.82%)	1 (1.82%)	2 (1.82%)	
Apgar 1'min				
<7/10	3 (5.45%)	6 (10.91%)	9 (8.18%)	0.489#
>7/10	52 (94.55%)	49 (89.09%)	101 (91.82%)	
NICU admission				
Yes	15 (27.27%)	30 (54.55%)	45 (40.91%)	**0.004***
No	40 (72.73%)	25 (45.45%)	65 (59.09%)	
Perinatal mortality				
Yes	0 (0.00%)	4 (7.27%)	4 (3.64%)	0.118#
No	55 (100.00%)	51 (92.73%)	106 (96.36%)	

*-Chi square test

#-Fisher's Exact test

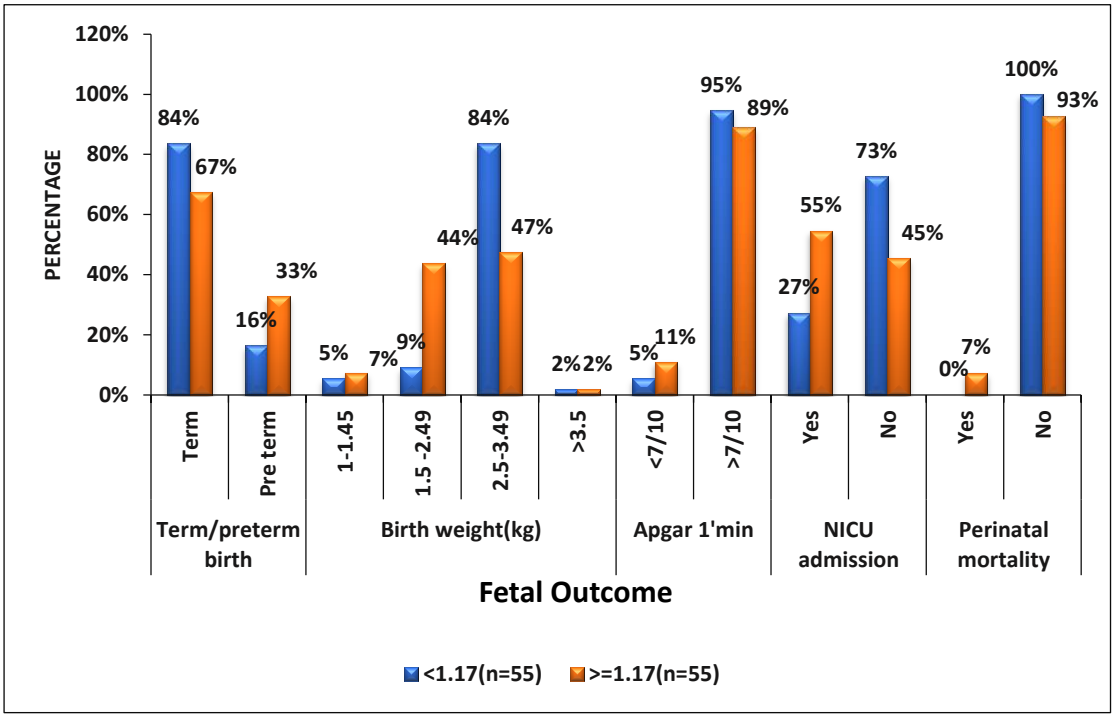

Figure 27: - Fetal outcome with urine fluoride levels

Compared to patients with urine fluoride levels <1.17 mg/L, patients with urine fluoride levels >=1.17 mg/L had significantly higher preterm birth (16.36%vs32.73%)P value-0.046, lower birth weight(9.09%vs47.27%) P value 0.0004 and higher NICU admissions(27.27%vs 54.55%)P value 0.004. The APGAR (1 minute) and perinatal mortality was comparable among the two groups as shown in the Table 27 and Figure 27.

DISCUSSION

DISCUSSION

Anemia is a major health concern in India, which is prevalent in several communities with low hemoglobin (Hb) levels in adults and children. Iron deficiency is commonly seen especially in the case of pregnancy, which affects millions of women globally. In spite of adequate nutritional intake, most women are yet found to be anemic due to other deficiencies of folic acid, vitamin B12.[56]Furthermore, excessive intake of toxic agent such as fluoride (F^-) along with nutritional deficiencies produce a cumulative effect in causing anemia.[57]

In the Kolar district of Karnataka, a high prevalence of fluorosis was also noted. The fluoride content in ground water was noted to be 0.5 ppm.[8] There is paucity of studies in Southern India on effect of excessive fluoride intake in pregnancy on maternal and foetal outcomes. Thus, we did this comparative observational study at the Department of Obstetrics and Gynaecology, R.L Jalappa Hospital and research center, Kolar, Karnataka from December 2017 to June 2019 to correlate the serum and urine fluoride levels in pregnancy with anaemia and foetal outcomes.

Demography and clinical characteristics:

In our study, the mean age of patients in cases and control groups was 23.73 ± 3.36 years and 24.13 ± 3.52, respectively. Most of the patients (40.00%) were primigravida. Mean age and parity were comparable between cases and control group (P >0.05). Similar age and parity distribution were seen in other similar studies as well.

In a study by Sastry et al, mean age of the patients was 26.4 ± 5.2 years; the patients included in their study were gravida 1 to 4; the parity was 0 to 4. Mean gestational

age in patients was 262 days.[10] In study by Apelberg et al, majority of the patients (84%) in their study were in age group 18 to 35 years. Only 41.6% were primigravida. Majority of the patients (87%) were with gestational age ≥ 37 weeks.[58] However in our study, most of the anaemic patients had gestational age <37 weeks.

Anaemia, Pregnancy and Fluoride Levels

India is one among the worst fluorosis-affected countries. Presently, it is a significant health problem in several parts of the world.[4] In the past, there are many studies conducted to study the impact of fluoride on various hematological parameters among experimental animals. But only few studies have evaluated the effects of fluorosis on hematological parameters in pregnant women.[4]

In present study, the mean serum and urine fluoride in the study patients were 0.34 ± 0.31 mg/L and 1.31 ± 0.69 mg/L respectively. In comparison with control group(non-anaemic), patients in cases group (anaemic) had significantly higher Serum fluoride(mg/l) (0.48 ± 0.36 vs 0.19 ± 0.14, P <0.0001) and significantly higher Urine fluoride(mg/l) levels (1.68 ± 0.68 vs 0.94 ± 0.48, P <0.0001). We found a significant negative correlation of Haemoglobin with Serum fluoride (r=-0.365, P=0.0001) and urine fluoride levels (r=-0.444, P<0.0001). The haemoglobin levels decreased significantly with increase in the serum and urine fluoride levels during pregnancy. Other studies have also shown similar relationship between anaemia and fluoride.[3,4,15]

Association of mean Fluoride levels (mg/L) with anemia in pregnancy

Studies	Anemic women	Non-anemic women	P value
Present study Serum fluoride Urine fluoride	 0.48 ± 0.36 1.68 ± 0.68	 0.19 ± 0.14 0.94 ± 0.48	 P<.0001 P<.0001
1. Mahapatra PS et al* (2016) Serum fluoride Urine fluoride	 0.2475 ± 0.0018 1.8778 ± 0.4015	 0.0331 ± 0.0029 0.8019 ± 0.1677	 P= 0.017 P=0.000
2. **Susheela AK et al** (2010) Urine fluoride	1.939 ± 1.122 mg/l	1.364 ± 1.038 mg/l	P<0.05
3.**Opydo-Szymaczek J et al** Serum fluoride (2005)	0.838	1.300	P<0.01

*Sample population included both male and females

Serum Fluoride levels

We found a significant negative correlation of Haemoglobin with Serum fluoride (r=-0.365, P=0.0001) levels. The haemoglobin levels decreased significantly with increase in the serum fluoride levels. Similar to present study, in a study by Mahapatra PS et al, as compared to control group, case group had higher average serum fluoride levels; and the difference was statistically significant.[4] They observed that serum fluoride and urinary fluoride levels were more in cases than controls whereas the hemoglobin level in cases was lower than that of controls.

There are very few studies that have investigated the correlation of serum fluoride level and anaemia during pregnancy. Most of the studies have evaluated this correlation in animals.

In a study by Karadeniz et al., it was demonstrated that there was a significant association between fluoride and reduction in RBC as well as WBC counts, in addition to reduction in hematocrit levels, as well as the hemoglobin levels in rats. The anemia induced by fluorosis noted in that study could be due to inhibition of globulin synthesis; depression of erythropoiesis or a decrease in the level of blood folic acid.[59]

Machalinski et al, reported that sodium fluoride had marked negative effects on hematopoiesis.[60] Choubisa et al, also found that, decreased RBC and hemoglobin were present in an endemic fluorosis zone.[61] Hillman et al, reported that cattle afflicted with fluorosis developed anemia and eosinophilia.[12]

This shows the negative impact of increased serum fluoride levels on the haematopoiesis. The mechanism behind this is the negative effect of Fluoride on the GIT mucosa- decreased mucus production and shedding of the microvilli- causing ill-absorption of the dietary iron as well as the supplemental iron for the pregnant women. In addition, the destruction of the probiotic bacteria by the fluoride also causes B_{12} deficiency in such women leading to increased chances of Dimorphic anaemia.

2.**Urine Fluoride levels**

The mean urine fluoride in the study patients were 1.31 ± 0.69 mg/L. In comparison with control group, patients in cases group had significantly higher Urine fluoride(mg/l) levels (1.68 ± 0.68 vs 0.94 ± 0.48, P <0.0001). We found a significant

negative correlation of Hemoglobin with urine fluoride levels (r=-0.444, P<0.0001). The haemoglobin levels decreased significantly with increase in the urine fluoride levels.

Similar results were obtained in a study by Susheela AK et al, mean urinary fluoride levels in cases were significantly higher as compared to controls (1.939 ± 1.122 mg/l vs 1.364 ± 1.038 mg/l.) The difference between cases and control group in terms of urinary fluoride levels was significant (P<0.05).[3]

Valdez JL et al concluded that mean urinary fluoride levels in 2nd trimester of pregnancy 2.0±1.1mg/l.[19] In a study in Opydo-Szymaczek J et al, mean urine F levels for 31 pregnant women in their 28th and 33rd week of pregnancy were 0.653 and 0.838 mg/L, respectively whereas it was 1.3 mg/L in 30 healthy non-pregnant women in the control group. Decreasing urinary fluorine levels occurred during pregnancy and this was probably related to the mobilization.[18] In a study by Mahapatra PS et al, as compared to control group, case group had higher average urinary fluoride levels and the difference was statistically significant.[4]

We found a significantly higher serum and urine fluoride levels among anemia patients than non-anemic patients and as explained the high fluoride levels could explain various outcomes in anemic pregnant women like low, B_{12}, low ferritin levels.

Fluoride levels and Maternal and Foetal Outcomes

1. **Maternal outcome parameters**

On complete evaluation, considering a mean serum fluoride level of 0.205 mg/L for Serum fluoride and 1.17 mg/L for urine fluoride to categorise the women with high and normal fluoride levels. Compared to patients with serum fluoride levels <0.205 mg/L, there was no significant association of mode of delivery with patients with

serum fluoride levels >=0.205 mg/L (P>0.05); with most of the patients in both groups having caesarean section. However, the maternal complications such as postpartum haemorrhage, failed lactation, and subinvolution of uterus and wound gaping was significantly higher in patients with higher serum fluoride levels. (P<0.05) Compared to patients with urine fluoride levels <1.17 mg/L, patients with urine fluoride levels >=1.17 mg/L had comparable maternal outcomes.

In present study, majority of case group patients and control group patients, mode of delivery was LSCS (P = 0.643). Indication of LSCS between cases and controls were comparable (P=0.607). Among maternal complications, cases group had significantly higher postpartum haemorrhage, failed lactation, and subinvolution of uterus. (P<0.05). Similar findings to present study were reported previously with increased maternal complications associated with increased serum fluoride levels.[10,51,55] However in terms of mode of delivery, there were some differences.

In study by Sastry et al, in 54.62% cases, mode of delivery was LSCS and in 45.38% cases normal vaginal delivery.[10] Whereas, in another study by Apelberg et al, in majority of the patients (77.8%) mode of delivery was normal vaginal delivery.[58]
This can be due to the different set of patients enrolled in the different studies and the mode of delivery is dependent upon various factors other than serum fluoride levels as well.

Foetal outcomes parameters

Compared to patients with serum fluoride levels <0.205 mg/L, patients with serum fluoride levels >=0.205 mg/L had significantly higher pre-term births, lower birth weight and higher NICU admissions. The APGAR (1 minute) and perinatal mortality

was comparable among the two groups. Even compared to patients with urine fluoride levels <1.17 mg/L, patients with urine fluoride levels >=1.17 mg/L had significantly higher pre-term births, lower birth weight infants and higher NICU admissions. The APGAR (1 minute) and perinatal mortality was comparable among the two groups. The perinatal mortality between cases and controls were also comparable.

Similarly, as per a study by Susheela et al, it was observed that among a sample group of mothers who avoided the intake of Fluorine, the number of low-birth weight babies decreased to 22%, while LBW was present in 52% in the control group.[3] The findings corroborate with our study.

In addition, a study conducted by Sastry et al, on the relationship between serum fluoride levels in 17-36 years old pregnant women and adverse foetal outcomes found a significant negative correlation between maternal serum fluoride, birth weight and gestational age. The researcher concluded that there was a tendency towards premature birth and low birth weight with increasing serum fluoride.[10] In addition he documented that the mean gestational age was 262 days, mean birth weight was 2.51 kg, and mean APGAR score was 7.6.[10]

Furthermore, it was observed that there was a statistically positive correlation was found low-birth weight in newborns in the mothers who were living in the endemic areas during the period of pregnancy as per a case control study conducted by Diouf M et al, in an endemic region in Senegal.[16] In contrast a study by Aghaei et al, it was found that there existed mild positive correlation between the weight of babies and the water F in the study regions ($r= 0.196$, $p<0.001$). This implies that an increase of the

Fluoride level in the drinking water, there was as an increase in the birth weight. They also found strong correlation of fluoride levels with height of babies.[62]

In a study conducted by He H et al, conducted to study the effects of Fluoride intake on the human fetus, it was found that there was higher F content present in fetal brain as well as bone tissue in an Fluoride-endemic area as compared to fetal tissues in control group ($p< 0.05$). Reason given by authors for this was because of excess amount of Fluorine taken by the mothers previously. The mechanisms underlying this are no clear; it was also reported in the study that fluoride on entering into the mother's body, crosses the fetus through the placenta. It then enters into the many tissues and organs of the fetus.[63] Findings from the studies suggest that with increased serum fluoride in the pregnant women, there is an increase in preterm delivery, low-birth weight, as well as poor APGAR count.

Along with factors such as parity, period of gestation, mother's adult size, as well as the mother's own birthweight, there is an effect of maternal blood glucose levels on the size at birth. Doull J et al, reported that there was an impairment in glucose tolerance among humans at intake of fluoride at dose of 0.07–0.4 mg/kg/day, which corresponds to concentrations of serum F more than 0.1 mg/L. The primary mechanism that is involved behind this is inhibition of production of insulin. The possible mechanism underlying the increased birth height and increased birth weight with increase in F levels in drinking water can be because of increased F levels that are associated with greater levels of maternal blood glucose.[64]

High levels of fluoride in drinking water can also lead to damage to the small intestinal microvilli that result in impairment in maternal nutrition and reduced fetal growth. Preventing Damage to gastrointestinal tract can be prevented by reducing the intake of fluoride that can result in improved absorption of nutrients and increase in fetal growth.

In many countries, fluoridation of a variety of products is still a common practice, the high percentage of pregnant women exposed to fluoride ingestion and anaemic in developing countries is difficult to overlook. In such a high percentage of pregnant women, a simple procedure for evaluating fluoride levels in urine and Hb in women is adequate to introduce interventions to control anaemia. From the data reported in this it is evident that maternal and child under-nutrition and anaemia are not necessarily due to insufficient intake of food, but due to nutrient absorption derangement due to damage to GI, mucosa due to ingestion of undesirable chemical, such as fluoride through food, water as well as other sources. These aspects have been not been explored so far, and this is the first time such a possibility has been investigated and results have been reported. The results of this approach in pregnancy anaemia provide a new way to reduce the burden of children with disabilities and mental challenges by reducing the percentage of babies with low birth weight.

To conclude, therefore, a new and effective approach to fluorosis management has the potential to reduce anaemia during pregnancy and increase the birth weight of infants. Fluoride toxicity has not been extensively studied as a risk factor in highly endemic regions of India and around the globe for maternal and foetal outcomes. This is the among the few studies that deals with fluoride, pregnancy, anemia, and maternal and

fetal outcomes which shows that there is a significant association between increasing fluoride levels in serum and urine and increasing occurrence of anemia.

STRENGTHS OF OUR STUDY

1. One of the most common medical disorder in pregnancy is anaemia, which is also a common reason for morbidity and mortality in India. Causes of anaemia are manifold and also includes nutritional deficiencies, e.g. iron, folate, and vitamin B12. In addition, contamination of drinking water with fluoride may lead to anaemia. This study will throw some light on this largely unrecognized cause of anaemia which has not been adequately studied and documented in southern India.
2. There is a dearth of studies in India regarding the effect of increased intake of fluoride by pregnant women on maternal and foetal outcomes. Thus, present study can act as a stepping zone for further larger studies to find out high fluoride content being an important cause for anaemia .
3. The results of this study show that, there is an urgent need to promote research and give greater importance in the medical curriculum to know the etiology and adopt preventive measures for the same.

LIMITATIONS OF THE STUDY

1. Being a single- centre hospital- based study; its results cannot be extrapolated to study the effect of fluoride intake in pregnant women in the general population.
2. In our study, we were not able to confirm high levels of fluoride in the long bones of our patients via X-ray imaging due to restrictions of imaging studies permitted to be done on pregnant women.

CONCLUSION

CONCLUSION

The present study concluded that in pregnant women with anaemia(cases), there were significantly higher levels of Serum fluoride as well as significantly higher urine fluoride as compared to those without anaemia (control).

The serum and urinary fluoride levels had a significantly negative correlation with the haemoglobin level in cases. The haemoglobin level decreases significantly with increase in serum and urine fluoride level. There was more serum ferritin, vitamin b_{12} and folate deficiency in cases as compared to control.

There was no significant association of Mode of delivery with serum fluoride and urine fluoride levels. Maternal complications such as postpartum haemorrhage, failed lactation and uterine subinvolution were significantly higher in pregnant women with high fluoride levels. In addition, there was higher number of preterm deliveries, low birth weight infants requiring more NICU admissions with high serum and urine fluoride levels compare to low fluoride levels.

So, it can be interpreted from findings of our study that fluorosis can affect haematological system resulting in anaemia. Therefore, creating general awareness of the use of fluoride-free drinking water is imperative to ameliorate fluorosis and the accompanying anaemia, however, further multi- centered large cohort studies in comparison with fluoride exposed and non- exposed subjects need to be conducted to assess and determine the maternal and foetal effects of fluorosis during pregnancy.

SUMMARY

SUMMARY

In the study conducted at Department of Obstetrics and Gynaecology, R.L Jalappa Hospital, Kolar, Karnataka from Dec 2017 to Jun 2019, subjects attending OBG OPD and admitted in Obstetrics and gynaecology Department were included in the study were recruited after required informed consent. The study subjects were grouped as anaemic case group and Second group consisted of nonanemic control group. Biochemical parameters were analysed for study subjects which include Serum ferritin, vitamin B12, folate was estimated. Serum and urine samples were evaluated for fluoride estimation. Following are the results pertaining to the study.

- Mean age of patients in cases and control groups was 23.73 ± 3.36 years and 24.13 ± 3.52, respectively. Majority (77.27%) were unbooked. In cases group, there was significantly higher number of patients of lower-class status.
- Majority of the patients of anaemic group (40.00%) were gravida 2. There was no significant difference between cases and control groups patients in terms of parity (P=0.081).
- In present study, most of the patients (74.55%) were having gestational age ≥ 37 weeks followed by 23.64% patients having 32+1-36+6 weeks gestational age. As compared to non-anaemic group, anaemic group had significantly a greater number of patients with preterm gestational age <37 weeks.
- Anaemic group had significantly lower mean Serum ferritin, significantly lower mean serum folate, and significantly lower Serum vitamin B12.
- In comparison with non-anaemic group, patients in anaemic group had significantly higher Serum fluoride(mg/l) (0.48 ± 0.36 vs 0.19 ± 0.14, P <0.0001)

and significantly higher Urine fluoride(mg/l) levels (1.68 ± 0.68 vs 0.94 ± 0.48, P <0.0001).

- Normocytic normochromic anaemia was noted in 40.00% (majority)
- We found a significant negative correlation of Haemoglobin with Serum fluoride (r=-0.365, P=0.0001) and urine fluoride levels (r=-0.444, P<0.0001). The haemoglobin levels decreased significantly with increase in the serum and urine fluoride levels.
- Anaemic group had significantly more patients with Fatiguability, dyspnoea, and Pedal edema.
- Cases group had significantly higher number of patients who required blood transfusion.
- There was no significant association of Mode of delivery with serum fluoride levels (P>0.05); with most of the patients in both cases and controls having caesarean section.
- Among maternal complications, Postpartum haemorrhage, Failed lactation and Sub involution of uterus was significantly higher among cases group (P<0.05).
- There were significantly higher number of infants in cases group who required NICU admission. Cause of NICU admission and perinatal mortality was comparable.
- Compared to patients with serum fluoride levels <0.205 mg/L, patients with serum fluoride levels >=0.205 mg/L had comparable mode of delivery and significantly more maternal complications.
- Compared to patients with serum fluoride levels <0.205 mg/L, patients with serum fluoride levels >=0.205 mg/L had significantly higher pre-term births, lower birth weight and higher NICU admissions. Preterm births and higher NICU

admission was significantly increased in higher (>1.17mg/l) urine fluoride levels also.

- The APGAR (1 minute) and perinatal mortality was comparable among the two groups.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Jiménez LV, Fregozo CS, Beltrán M, Coronado OG, Vega MP. Effects of the fluoride on the central nervous system. Neurología. 2011;26:297-300.
2. Edzwald JK. Water quality & treatment: a handbook on drinking water. 6th ed. New York: McGraw-Hill;2011.
3. Susheela AK, Gupta R, Ganesh K, Brahmankar S, Bhasin S, Gupta G. Effective interventional approach to control anaemia in pregnant women. Current Science. 2010;98:1320-30.
4. Mahapatra PS, Chatterjee S, Sarkar P. Fluorosis Induced Anemia in Fluoride Endemic Zone of Bankura District, West Bengal, India. International Journal of Current Research 2016;8:37700-3.
5. MacArthur JD. Fluoride, Premature birth and impaired neurodevelopment. Townsend Letter.2013.
6. World Health Organisation. The prevalence of anemia in pregnancy, WHO Technical reports (1992-1993).
7. Centers for disease Control, Criteria for anemia in children and childbearing aged women. MMWR. 1989;38:400-04.
8. Sruthi M N, Santhuram A N, Arun H S ,Kishore Kumar B N.A comparative study of skeletal fluorosis among adults in two study areas of bangarpet taluk,Kolar.Indian J Public Health 2016;60:203-9
9. Milman N, Bergholt T, Byg KE, Erikson L, Gradual N. Iron status and balance during pregnancy A critical reappraisal of iron supplementation. Acta Obstetris Gynaecol Scand. 1999;78:749-57.

10. Sastry G M, Mohanty S, Bhongir AV, Mishra AK, Rao P. Association of Higher Maternal Serum Fluoride with Adverse Fetal Outcomes. Int. J. Med. Public health. 2011;1.

11. Derakhshani AR, Raoof M, Dehghani M, Hossein A. Effect of fluoride in drinking water on birth height and weight.s Research report Fluoride. 2015;48:160-8.

12. Hillman D, Bolenbaugh DL, Convey M. Hypothyroidism and anemia related to fluoride in dairy cattle. J Dairy Sci 1979;62:416–23.

13. Pasricha S. Anaemia in pregnancy-not just iron deficiency. Acta Haematologica. 2013;130:279-80.

14. Ho C, Yuan C, Yeh S. Serum ferritin, folate and cobalamin levels and their correlation with anemia in normal full-term pregnant women. European Journal of Obstetrics and Gynecology. 1987;26:7-13.

15. Ersoy IH, Alanoglu EG, Koroglu BK, Varol S, Akcay S, Ugan Y, Ersoy S, Tamer MN. Effect of Endemic Fluorosis on Hematological Parameters. Biological Trace Element Research. 2010;138:22-27.

16. Diouf M, Cisse D, Lo C, Ly M, Faye D, Ndiaye O. Pregnant women living in areas of endemic fluorosis in Senegal and low birthweight newborns: Case-control study. Revue d Epidemiologie et de Sante Publique. 2012;60:103-108.

17. Sukumar S,Verma Y,Swamy, A Study of Endemic Fluorosis in Domestic Ruminants. Indian Jounal of Vetenary Sciences . 2018 Jan-Mar; 13: 11–15.

18. Opydo-Szymaczek J, Borysewicz-Lewicka M. Urinary fluoride levels for assessment of fluoride exposure of pregnant women in Poznan, Poland. Fluoride. 2005;38:312-317.

19. Valdez Jiménez L, López Guzmán OD, Cervantes Flores M, Costilla-Salazar R, Calderón Hernández J, Alcaraz Contreras Y, Rocha-Amador DO. In utero exposure to fluoride and cognitive development delay in infants. Neurotoxicology. 2017;59:65-70.

20. Mudaliar AL, Krishna Menon MK, Chapter 23, Anaemia in Pregnancy, Krishna Menon MK, Palaniappan B (Eds), clinical Obstetrics, 9th Edition, Chennai, Oriental Longman 1990; 170-79.

21. Sharma JB. Chap 7. Nutritional anaemia during pregnancy in non industrialised countries, studd J (ed) progress in Obstetrics and Gynaecology, Churchill Livingstone, 2003 ; 15 : 103-22.

22. Cunningham FG, Leveno KJ, Bloom SL, Hauth JC, Gulstrap L, Wenstrom KD. Chapter 51. Haematological Disorders in Williams Obstetrics, 25nd Edition, Newyork, McGraw Hill 2005 ; 1143-67.

23. Nynke Vanden Broek. Anaemia in pregnancy in developing countries. Br J Obstet Gynaecol 1998; 105 : 385-90.

24. Ratnam SS, Bashker Rao K, Arulkumaran S. Anaemia in pregnancy. In : Obstetrics and Gynaecology for Postgraduates. Chap 5, Second Edition, Orient Longman, 1999 ; 1 : 46-55.

25. Sharma Neena. Iron deficiency in women : Role of Carbonyl Iron. Obs and Gynaec Today 2001; 6 : 399-03.

26. Mehta MB, Dodd NS. Effect of different levels of iron supplementation on maternal iron status and pregnancy outcome. The Ind J Nutr Dietet 2004 ; 41 : 467-77.

27. Sharma JB. Iron deficiency anaemia in pregnancy – still a major cause of maternal mortality and morbidity in India. Obs and Gynae Today 1999 ; 4 : 699-1.

28. Khandait WD, Ambadekar NN, Zodpey PS, Koram MR, Vasudeo ND. Risk factors for anaemia in pregnancy. J Obstet Gynecol India 2001 ; 51 : 42-44.

29. Ranju Agarwal. A Multicentric study of Anaemia : A step in conquering anaemia. Obs and Gynae Today 2003 ; 8 : 620-23.

30. Agarwal P, Chaturvedi B. Clinical study of fetomaternal outcome in pregnant anaemic patients. Obs and Gynae Today 2004 ; 9 : 426-28.

31. Awasthi A, Thakur R. Dave A, Goyal V. Maternal and perinatal outcome in cases of moderate and severe anaemia. J Obstet Gynecol India 2001 ; 57 : 62-65.

32. Shrivastava M, Shrivastava A, Nahar SL. Anaemia in Pregnancy (A study of 178 cases of anaemia). J Obst Gyn and Female Welfare 1999 ; 5 : 23-29.

33. Thangaleela T and Vijayalakshmi P. Impact of anaemia in pregnancy. The Ind J Nutr Dietet 1994 ; 31 : 251-56.

34. Rangnekar AG, Darbari Rashmi. Fetal outcome in pregnancy anaemia. J Oobstet Gynaecol India 1993 ; 43 : 72-176.

35. William JS, Gary R Thurnau. Iron deficiency Anaemia in Pregnancy. Clin Obstet Gynecol 1995 ; 38 : 443-53.

36. Arias F. Haematological problems during pregnancy in practical guide to high risk pregnancy and delivery. Chapter 13, 3rd edition, MOSBY 2016 ; 245-62.

37. Ian Donald. Practical Obstetrics Problems. Anaemia. , 7th edition, 2016; 196-21.

38. Vanden Broek NR, Letsky EA, White SA, Shenkin A. Iron status in pregnant women : which measurements are valid . Br J Haematol 1998 ; 103 : 817-24.

39. PL Halloran C, Trevino R, Felitti V, Beutler E. Human transferrin G277S mutation ; a risk factor for iron deficiency anaemia. Br J Haematol 2001 ; 116 : 329-33.

40. Sharma JB. Textbook of obstetrics.Medical disorders during pregnancy, 3rd edition,2016 ; 483-4.

41. Neena Sharma. Iron absorption: IPC therapy superior to conventional Iron salts. Obs and Gynae Today 2000 ; 5 : 15-19.

42. Sharma JB. The choice between carbonyl iron and Iron polymaltose in treating Anaemia. Obs and Gynae Today 2001 ; 6 : 629-32.

43. Sharma JB. Sodium Feredetate – a novel iron supplement in pregnancy. Obs and Gynae Today 2005 ; 10 : 270-73.

44. Wintrobe's. Clinical haematology. 10th ed. Eds. G. Richard Lee et al. Williams and Wilkins 1999; Iron Deficiency and Iron deficiency anaemia, 979-10.

45. Rai Uma. Severe anaemia in Pregnancy and Role of partial exchange and maternal and perinatal outcome. Obs and Gynae Today 1999 ; 4 : 288-290.

46. Campbell AB. Megaloblastic anaemia in pregnancy. Clin Obstet Gynecol 1995 ; 38 : 455-462.

47. Bhatt RV. Anaemia in pregnancy : Early diagnosis and treatment. J Ind Med Assoc 1995 ; 93 : 80-82.

48. Sharma JB. Iron deficiency anemia in pregnancy – still a major cause of maternal mortality and morbidity in India. Obs Gynae Today 1999;IV:693-701.

49. https://www.nhp.gov.in

50. Guidelines for Fluorosis, District fluorosis office, Kolar District, NRHM 2018.

51. FRRDF, 1999. State of art report on the extent of fluoride in drinking water and the resulting endemicity in India. Fluorosis Research and rural Development Foundation, New Delhi, India.
52. Susheela AK. Fluorosis and associated health issues. Ind J Pract Pediatrics 2015;17:138-146.
53. Patsouri K. Re-Examining the Fluoride Intake During Pregnancy: A Necessity or not for the incoming Member. Int J Dent Med Res 2015;1:150-56.
54. Susheela AK, Toteja GS. Prevention & control of fluorosis & linked disorders: Developments in the 21st Century - Reaching out to patients in the community & hospital settings for recovery. Indian J Med Res 2018;148:539-47.
55. MacArthur JD. Fluoride, Premature Birth and Impaired Neurodevelopment. Townsend Letter.2018.
56. Susheela AK Anemia in pregnancy: an easily rectifiable problem :Guest editorial Fluoride 2010;43:104–07 Susheela AK. Anemia in pregnancy: an easily rectifiable problem. Fluoride 2010;43:104-7.
57. Susheela AK, Bhatnagar M, Vig K, Mondal NK. Excess fluoride ingestion and thyroid hormone derangements in children living in Delhi, India. Fluoride 2005;38:98-108.
58. Apelberg BJ, Witter FR, Herbstman JB, Calafat AM, Halden RU, Needham LL, et al. Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth. Environ Health Perspectives 2007;115:1670-6.
59. Karadeniz A, Altintas L. Effects of *Panax Ginseng* on fluoride-induced haematological pattern changes in mice. Fluoride 2008;41:67-71.

60. Machaliński B, Zejmo M, Stecewicz I, Machalinska A, Machoy Z, Ratajczak MZ, et al. The influence of sodium fluoride on the progenitor cells: preliminary report. Fluoride. 2000;33:168-73.
61. Choubisa SL. Prevalence of fluorosis in some villages of Dungarpur district of Rajasthan. Indian J Env Health 1996;38:119-26.
62. Aghaei M, Derakhshani R, Raoof M, Dehghani M, Mahvi AH, Kerman, et al. Effect of fluoride in drinking water on birth height and weight: an ecological study in Kerman Province, Zarand County, Iran. Fluoride 2015;48:160-8.
63. He H, Cheng ZS. Effects of fluorine on the human fetus. Fluoride 2008;41940:321-5.
64. Doull J, Boekelheide K, Farishian BG, Isaacson RL, Klotz JB, Kumar JV, et al., Committee on Fluoride in Drinking Water, Board on Environmental Studies and Toxicology, Division on Earth and Life Studies, National Research Council of the National Academies. Fluoride in drinking water: a scientific review of EPA's standards. Washington, DC: The National Academies Press; 2006. p. 264.

ANNEXURES

CASE PROFORMA

NAME: IP NO:

AGE: DOA:

OCCUPATION: DOD:

ADDRESS:

EDUCATION:

HUSBAND S OCCUPATION:

SOCIOECONOMIC STATUS:

CHIEF COMPLAINTS:

HISTORY OF PRESENT ILLNESS:

H/O amenorrhea yes/no
H/o bleeding /PV spotting yes/no
H/o pain abdomen yes/no

OBSTETRIC HISTORY:

Marital life: Consanguinity:

Gravida: Para: living: Abortion: Dead:

Details of previous pregnancy:

Details of present pregnancy:

MENSTRUAL HISTORY:
Last menstrual period: Age of menarche:

Expected delivery date:

Period of gestation:

Period of gestation according to early scan:

Past menstrual cycles:

PAST HISTORY:

HTN/DM/BA/TB/BLEEDING DISSORDERS/EPILEPSY/THYROID DISORDER/CARDIAC DISEASE/ALLERGY

H/O blood transfusions:

H/O Surgeries or hospitalization:

PERSONAL HISTORY:

Sleep and appetite:
Diet:
Source of drinking water
Bowel and bladder:

FAMILY HISTORY:

DRUG HISTORY:

GENERAL EXAMINATION:
General condition: Fair/ moderate/ Poor

Built: Nourishment:

Ht: cmsWt: kgs BMI:

Pallor: Icterus:
Cyanosis: Clubbing:
Lymphadenopathy: Edema:

VITALS:
Pulse rate:
Respiratory rate:
Blood pressure
Temperature:

SYSTEMIC EXAMINATION:
Cardiovascular system:
Respiratory system:
Central nervous system:

Per abdomen: Uterus size:
Relaxed / Irritable / Acting
Presentation: cephalic/ Breech/ other
FHS:

LOCAL EXAMINATION:
Per speculum
Per vaginum:Effacement:
Dilatation:
Station:
Membranes:
Pelvis:

PROVISIONAL DIAGNOSIS:

INVESTIGATIONS:
Blood group and Rh typing:
CBC: HB: HIV:
PCV: HbsAG:
RBC: VDRL:
WBC:
PLT:
RBS:
Serum fluoride
Serum ferritine
Serum Folate
Serum B12
Urine analysis: Albumin-

Sugar-
Microscopy-
Urine fluoride

OBSTETRICS SCAN:

DELIVERY DETAILS:
Mode of delivery: Vaginal delivery/ Caesarean section

CAESAREAN-
Indication:

DETAILS OF NEONATE:
Sex: Date: Time:
Birth weight:
APGAR : 1'- 5'-
Admission to NICU:

MATERNAL COMPLICATIONS:
Hypertension
Convulsions
Premature rupture of membranes
Antepartum hemorrhage
Postpartum hemorrhage
Uterine hyperstimulation

FETAL COMPLICATIONS:
Respiratory distress
Low birthweight
Admission to NICU
CONDITION AT DISCHARGE:
Mother:

Baby:

PATIENT CONSENT FORM

Case no:

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I have understood that I have the right to refuse consent or withdraw it at any time during the study and this will not affect my treatment in any way. I consent voluntarily to participate in this study

CORRELATION OF FLUORIDE LEVELS WITH ANAEMIA AND MATERNAL AND PERINATAL OUTCOME IN PREGNANT WOMEN

Name of Participant_____

Signature/ thumb print of Participant _____
Date _____

Statement by the researcher/person taking consent:
I have accurately read out the information sheet to the potential participant and to the best of my ability made sure that the participant understands that the following will be done:

I confirm that the participant was given an opportunity to ask questions about the study and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.
Name of Researcher/person taking the consent_____

Signature of Researcher /person taking the consent_____

Date _____
Name and Address of Principal Investigator: Dr.Sneha singh
R.L Jalappa Hospital ,Tamaka, Kolar.

ತಿಳುವಳಿಕೆಯ ಒಪ್ಪಿಗೆ ಪತ್ರ

ಅಧ್ಯಯನ ಶೀರ್ಷಿಕೆ:– "CORRELATION OF FLUORIDE LEVELS WITH ANAEMIA AND MATERNAL AND PERINATAL OUTCOME IN PREGNANT WOMEN

ಶ್ರೀ/ಶ್ರೀಮತಿ ಆದ ನಾನು ಈ ಮೇಲಿನ ಸಂಶೋಧನ ವಿಷಯದ ಬಗ್ಗೆ ನನಗೆ ಅರ್ಥವಾಗುವರೀತಿಯಲ್ಲಿ ನನ್ನದೇ ಭಾಷೆಯಲ್ಲಿ ತಿಳಿಸಿರುತ್ತಾರೆ. ಈ ಸಂಶೋಧನಾ ವಿಷಯದಲ್ಲಿ ನಾನು ಒಬ್ಬ ವಿಷಯಿಯಾಗಿ ಭಾಗವಹಿಸಲು ನನ್ನ ಸಂಪೂರ್ಣವಾಗಿಒಪ್ಪಿಗೆಇರುತ್ತದೆ. ಈ ಸಂಶೋಧನಾಉದ್ದೇಶವನ್ನು ಪೂರ್ಣವಾಗಿಅರಿತಿರುತ್ತೇನೆ. ಈ ಸಂಶೋಧನೆಗೆ ನನ್ನಿಂದಯಾವುದೇಆರ್ಥಿಕತೆಯಅವಶ್ಯಕತೆಇರುವುದಿಲ್ಲ. ನಾನು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ನನ್ನ ಸಹಕಾರವನ್ನು ಹಿಂಪಡೆದು ಈ ಸಂಶೋಧನೆಯಿಂದ ಹೊರಹೋಗುವ ಹಕ್ಕನ್ನು ಹೊಂದಿರುತ್ತೇನೆ. ಇದರಿಂದ ನನ್ನಚಿಕಿತ್ಸೆಗೆಯಾವುದೇರೀತಿಯತೊಂದರೆಯಾಗುವುದಿಲ್ಲ. ಮುಖ್ಯವಾಗಿ ನನ್ನಿಂದ ಪಡೆದ ಈ ಮಾಹಿತಿಯು ಸಂಶೋಧನೆಗೆ ಮಾತ್ರ ಸೀಮಿತವಾಗಿರುತ್ತದೆ. ಮತ್ತು ಈ ಮಾಹಿತಿಯುಎಲ್ಲೂ ಸೋರಿಕೆಯಾಗದಂತೆಎಚ್ಚರಿಕೆವಹಿಸುವುದಾಗಿ ತಿಳಿವಳಿಕೆ ನೀಡಿರುತ್ತಾರೆಂದು ನಾನು ದೃಢಪಡಿಸಿಕೊಂಡು ಒಪ್ಪಿಗೆ ನೀಡಿರುತ್ತೇನೆ.

ರೋಗಿಯ ಸಹಿ/ ಸಾಕ್ಷಿ ಸಹಿ. ಸಂಶೋದಕನ ಸಹಿ

ಬೆರಳಚ್ಚು.

ತಾಳ್ಮೆ ಮಾಹಿತಿ SHEET

ಪ್ರೆಗ್ನೆಂಟ್ ಮಹಿಳೆಯರಲ್ಲಿ ಅನೆಮಾ ಮತ್ತು ಮೆಟರ್ನಲ್ ಮತ್ತು ಪೆರಿನಾಟಲ್ ಒಟ್ಯುಟೋಮ್ಗಳೊಂದಿಗೆ ಫ್ಲೂರೈಡ್ ಮಟ್ಟಗಳ ಅಧ್ಯಯನ ಶೀರ್ಷಿಕೆ ಶೀರ್ಷಿಕೆ

ಅಧ್ಯಯನ ಸ್ಥಳ: ಶ್ರೀ ಎಲ್.ಜಲ್ಲಪ್ಪ ಆಸ್ಪತ್ರೆ ಮತ್ತು ಸಂಶೋಧನಾ ಕೇಂದ್ರ ಶ್ರೀ ದೇವರಾಜೂರ್ಸ್ ಮೆಡಿಕಲ್ ಕಾಲೇಜ್ಗೆ ಜೋಡಿಸಲಾಗಿದೆ. ತಮಾಕಾ, ಕೋಲಾರ
ವಿವರಗಳು-
ರೋಗಿಯ ಇಲಾಖೆಗೆ ಹೊರಬರುವ ಅಂಟನಾಟಲ್ ಪ್ರಕರಣಗಳು ಮತ್ತು ಕಾರ್ಮಿಕ ವಾರ್ಡ್ನಲ್ಲಿ ಒಪ್ಪಿಕೊಳ್ಳಲಾಗುವುದು
ಈ ಅಧ್ಯಯನದ ರೋಗಿಗಳು ಸಂಪೂರ್ಣ ಸಾಮಾನ್ಯ ದೈಹಿಕ ಪರೀಕ್ಷೆ, ಪ್ರಸೂತಿ ಪರೀಕ್ಷೆ, ಸಂಪೂರ್ಣ ರಕ್ತ ಎಣಿಕೆ, ವೈರಲ್ ಸೆರಾಲಜಿ, ಮೂತ್ರ ವಾಡಿಕೆಯ ಮತ್ತು ಯಾದೃಚ್ಛಿಕ ರಕ್ತದ ಸಕ್ಕರೆ ಮಟ್ಟಗಳು, ಸೀರಮ್ ಫೆರಿಟಿನ್, ಸೀರಮ್ ಬಿ 12, ಸೀರಮ್ ಫೋಲೇಟ್, ಸೀರಮ್ ಫ್ಲೋರೈಡ್ ಮತ್ತು ಮೂತ್ರ ಫ್ಲೋರೈಡ್ ಮಟ್ಟಗಳು. ಭ್ರೂಣದ ಆರೋಗ್ಯವನ್ನು ಕಾರ್ಡಿಯೋಟೊಕಾಗ್ರಫಿ ಮತ್ತು ಬಯೋಫಿಸಿಕಲ್ ಪ್ರೊಫೈಲ್ನೊಂದಿಗೆ ಒಂದು ಪ್ರಸೂತಿಯ ಅಲ್ಟ್ರಾಸೌಂಡ್ ಎಂದು ನಿರ್ಣಯಿಸಲು ಸಹ ಮಾಡಲಾಗುತ್ತದೆ. ಕೆಳಗಿನ ಮಾಹಿತಿಯನ್ನು ಓದಿ ಮತ್ತು ನಿಮ್ಮ ಕುಟುಂಬ ಸದಸ್ಯರೊಂದಿಗೆ ಚರ್ಚಿಸಿ. ಅಧ್ಯಯನದ ಬಗ್ಗೆ ನೀವು ಯಾವುದೇ ಪ್ರಶ್ನೆಯನ್ನು ಕೇಳಬಹುದು. ನೀವು ಅಧ್ಯಯನದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳಲು ಒಪ್ಪಿಕೊಂಡರೆ ನಾವು ನಿಮ್ಮಿಂದ (ಮಾಹಿತಿ ಪ್ರಕಾರ) ಮಾಹಿತಿಯನ್ನು ಅಥವಾ ನಿಮ್ಮ ಅಥವಾ ಎರಡಕ್ಕೂ ಜವಾಬ್ದಾರರಾಗಿರುವ ವ್ಯಕ್ತಿಗಳನ್ನು ಸಂಗ್ರಹಿಸುತ್ತೇವೆ. ಸಂಬಂಧಿತ ಇತಿಹಾಸವನ್ನು ತೆಗೆದುಕೊಳ್ಳಲಾಗುವುದು.ಯಾವುದೇ ವಿಶೇಷ ತನಿಖೆಗಳಿಗೆ ನಿಮಗೆ ಶುಲ್ಕ ವಿಧಿಸಲಾಗುವುದಿಲ್ಲ. ಸಂಗ್ರಹಿಸಿದ ಈ ಮಾಹಿತಿಯನ್ನು ಪ್ರೌಢಪ್ರಬಂಧ ಮತ್ತು ಪ್ರಕಟಣೆಗಾಗಿ ಮಾತ್ರ ಬಳಸಲಾಗುತ್ತದೆ.
ನಿಮ್ಮಿಂದ ಸಂಗ್ರಹಿಸಿದ ಎಲ್ಲಾ ಮಾಹಿತಿಯನ್ನು ಗೌಪ್ಯವಾಗಿರಿಸಲಾಗುವುದು ಮತ್ತು ಯಾವುದೇ ಹೊರಗಿನವರಿಗೆ ಬಹಿರಂಗಪಡಿಸಲಾಗುವುದಿಲ್ಲ. ನಿಮ್ಮ ಗುರುತನ್ನು ಬಹಿರಂಗಪಡಿಸಲಾಗುವುದಿಲ್ಲ. ಈ ಅಧ್ಯಯನವು ಸಾಂಸ್ಥಿಕ ನೀತಿಶಾಸ್ತ್ರ ಸಮಿತಿಯಿಂದ ಪರಿಶೀಲಿಸಲ್ಪಟ್ಟಿದೆ ಮತ್ತು ನೀವು ಸಂಸ್ಥೆಯ ಎಥಿಕ್ಸ್ ಸಮಿತಿಯ ಸದಸ್ಯರನ್ನು ಸಂಪರ್ಕಿಸಲು ಮುಕ್ತವಾಗಿರುತ್ತೀರಿ. ಈ ಅಧ್ಯಯನಕ್ಕೆ ಒಪ್ಪಿಗೆ ನೀಡಲು ಯಾವುದೇ ಕಡ್ಡಾಯವಿಲ್ಲ. ನೀವು ಭಾಗವಹಿಸಲು ಬಯಸದಿದ್ದರೆ ನೀವು ಪಡೆಯುವ ಕಾಳಜಿ ಬದಲಾಗುವುದಿಲ್ಲ. ಈ ಅಧ್ಯಯನದಲ್ಲಿ ನೀವು ಸ್ವಯಂಪ್ರೇರಣೆಯಿಂದ ಒಪ್ಪಿಕೊಳ್ಳುವುದಾದರೆ ಮಾತ್ರ ಹೆಬ್ಬೆರಳು ಅನಿಸಿಕೆಗೆ ನೀವು ಸಹಿ / ನೀಡಬೇಕಾಗಿದೆ.

ಹೆಚ್ಚಿನ ಮಾಹಿತಿಗಾಗಿ
ಡಾ. ಸ್ನೇಹಾ ಸಿಂಗ್
ಪೋಸ್ಟ್ ಪದವಿ
ಪ್ರಸೂತಿ ಮತ್ತು ಸ್ತ್ರೀರೋಗ ಶಾಸ್ತ್ರ ಇಲಾಖೆ, SDUMC, ಕೋಲಾರ.me māhiti SHEET

PATIENT INFORMATION SHEET

CORRELATION OF FLUORIDE LEVELS WITH ANAEMIA AND MATERNAL AND PERINATAL OUTCOME IN PREGNANT WOME

Principal investigators: Dr. Sneha Singh/ Dr. Munikrishna M I, Dr. Sneha Singh, a post graduate student at the Sri Devaraj Urs Medical College, will be conducting a study titled CORRELATION OF FLUORIDE LEVELS WITH ANAEMIA AND MATERNAL AND PERINATAL OUTCOME IN PREGNANT WOMEN for my dissertation under the guidance of Dr. Munikrishna M, Professor,Department of Obstetrics and Gynecology. Participants of this study i,e pregnant women with anaemia will be included in a comparative observational where in all the pregnant women who visit outpatient department and labour ward to RLJH hospital will be admitted and examined thoroughly by clinical examination and biochemical parameteters will be done. Maternal and Perinatal outcome will be documented. Participants included in this study won't have any financial compensation to participate in this research project. All the data will be kept confidential and will be used only for research purpose by this institution.

Name and signature of the investigator

Date

KEY TO MASTER CHART

IP.No : In-patient hospital number

Study group :

0: Control - nonanaemic study group

1: Case -anaemic study group

Obstetric score

1 : Primigravida

2 : Gravida 2

3 : Gravida 3

4 : Gravida 4

POG : Period of gestation

1: 28-32 WKS

2: 32+1-36 WKS

3: 36+1-WKS and above

SES: SOCIOECONOMIC STATUS

1: UPPER INCOME

2: MIDDLE

3: LOWER

HEMOGLOBIN

1: MILD ANEMIA

2: MODERATE ANEMIA

3: SEVERE ANEMIA

4: NON ANEMIC

PERIPHERAL SMEAR

1: NCNC – NORMOCYTIC NORMOCHROMIC ANEMIA

2: MCHC- MICROCYTIC HYPOCHROMIC ANEMIA

3: DM – DIMORPHIC ANEMIA

4: MA- MEGALOBLASTIC ANEMIA

SYMPTOMS

1: ASYMPTOMATIC

2: FATIGIABLITY

3: DYSPNEA

4: PEDAL EDEMA

SIGNIFICANT PAST OBS H/O

1: NOT SIGNIFICANT
2: H/O BLOOD TRASFUSION
3: H/O IRON SUCROSE INFUSION

REQUIRED OF BLOOD TRANSFUSION

1: YES
2: NO

MODE OF DELIVERY

1: NORMAL DELIVERY
2: INSTRUMENTAL
3: LOW SEGMANT CESAREAN SECTION

INDICATION FOR LSCS

1: FETAL DISTRESS
2: PREVIOUS LSCS
3: OLIGOHYDRAMNIOS
4: BREECH
5: CPD- CEPHALOPELVIC DISPROPORTION

OUTCOME OF DELIVER PATIENT

1: TERM
2: PRETERM

BIRTH WEIGHT

1: 1-1.45KG
2: 1.5 -2.49
3: 2.5-3.49
4: >3.5

APGAR

1: <7/10
2: >7/10

NICU ADMISSION

1: YES
2: NO

REASON FOR NICU ADMISSION

1: RESPIRATORY DISTRESS SYNDROME
2: JAUNDICE
3: MECONIUM ASPIRATION SYNDROME
4: PRETERM CARE

PERINATAL MORTALITY

1: YES
2: NO

MATERANAL COMPLICATIONS

1: WOUND GAPING
2: POSTPARTUM HEMORRHAGE
3: FAILED LACTATION
4: SUB INVOLUTION OF UTERUS
5: NO COMPLICATIONS

MASTER CHART

Sl.no	Group	HOS.NO	Group	Age(year)	Age distribution	Booked	Parity	Gest Age(wks)	ADDRESS	SE status	HB	ps	symptoms	sig past obs h/o	required blood transfusion	Mode of delivery	indication of LSCS	Fetal out come	Birth weight(kg)	APGAR 1'min	NICU Admission	cause of nicu adm	Perinatal Mortality	Maternal Complications	Serum fluoride	Urine fluoride	Serum ferittin (ng/ml)	S.Folate(ng/ml)	S.Vitamin B12(pg/ml)
1	Control	622585	0	24	2) 21-25	1	1	3	5	2	4		1	1	2	1		1	3	2	2		2	5	0.5	0.6	55.9	2.46	159
2	Control	625607	0	26	3) 26-30	1	2	2	4	2	4		1	1	2	3	2	2	3	2	2		2	5	0.3	1.2	29.6	11.8	230
3	Control	611568	0	24	2) 21-25	2	1	3	1	1	4		1	1	2	3	1	1	3	2	2		2	5	0.32	0.84	7.15	5.05	160
4	Control	611521	0	23	2) 21-25	2	2	3	2	2	4		1	1	2	1		1	3	2	2		2	5	0.24	0.92	46.15	3.68	172
5	Control	611390	0	24	2) 21-25	1	2	3	5	3	4		1	1	2	2		1	3	2	2		2	5	0.06	0.28	50.6	6.82	229
6	Control	611573	0	29	3) 26-30	2	2	3	4	2	4		1	1	2	3	2	1	3	2	2		2	1	0.42	2.1	14.2	2.02	310
7	Control	597214	0	22	2) 21-25	2	1	3	1	3	4		1	1	2	1		1	3	2	2		2	5	0.06	0.54	12.76	2.12	246
8	Control	621972	0	28	3) 26-30	1	2	3	5	2	4		1	1	2	3	2	1	3	2	2		2	5	0.2	0.64	45	3.8	221
9	Control	622838	0	25	2) 21-25	2	1	3	3	3	4		1	1	2	3	1	1	3	2	2		2	5	0.06	1.4	45.6	2.2	306
10	Control	615933	0	26	3) 26-30	1	2	3	4	3	4		1	1	2	2		1	3	2	2		2	5	0.05	0.84	14.8	3.6	240
11	Control	579895	0	24	2) 21-25	2	3	3	1	2	4		1	1	2	1		1	3	2	2		2	5	0.24	0.94	18.46	3.8	200
12	Control	558066	0	24	2) 21-25	2	4	3	2	2	4		1	1	2	3	2	1	3	2	2		2	5	0.06	0.47	10.1	2.6	160
13	Control	596024	0	23	2) 21-25	2	1	3	1	1	4		1	1	2	3	1	1	3	2	2		2	5	0.44	0.36	44.45	7.85	300
14	Control	582638	0	24	2) 21-25	2	1	3	4	1	4		1	1	2	2		1	3	2	2		2	5	0.46	1.4	14.5	3.11	400
15	Control	582821	0	27	3) 26-30	2	2	3	5	2	4		1	1	2	3	2	1	3	2	1	1	2	5	0.3	1.9	44.5	4.85	193
16	Control	581091	0	19	1) <=20	2	1	3	2	1	4		1	1	2	1		1	3	2	2		2	5	0.05	0.67	14.02	5	202
17	Control	572275	0	24	2) 21-25	2	2	3	1	1	4		1	1	2	1		1	4	2	2		2	5	0.3	1.08	9.48	4.26	500
18	Control	515104	0	20	1) <=20	2	1	3	4	3	4		1	1	2	1		2	3	2	1	1	2	5	0.06	0.05	9.6	3.77	197
19	Control	584309	0	21	2) 21-25	2	1	2	1	3	4		1	1	2	3	3	2	2	2	1	3	1	5	0.3	0.72	14.8	5.83	400
20	Control	584969	0	23	2) 21-25	2	1	3	5	1	4		1	1	2	3	4	1	3	2	1	1	2	1	0.07	1.05	11.3	3.66	310
21	Control	584681	0	20	1) <=20	2	1	2	4	1	4		1	1	2	3	5	2	2	2	1	2	2	5	0.4	1	18.1	6.67	245
22	Control	582496	0	24	2) 21-25	2	1	3	3	2	4		1	1	2	3	1	1	3	2	2		2	5	0.06	0.68	14.2	8.48	300
23	Control	595628	0	24	2) 21-25	2	1	3	2	2	4		1	1	2	1		1	3	2	1	1	2	5	0.1	0.64	50	9.26	564
24	Control	595665	0	40	4) >30	2	3	2	4	2	4		1	1	2	3	2	2	2	2	1	2	2	5	0.1	0.81	53.9	10	200
25	Control	595674	0	26	3) 26-30	2	2	3	2	2	4		1	1	2	3	2	1	3	2	2		2	5	0.04	0.69	54.1	10	200
26	Control	582652	0	25	2) 21-25	2	1	3	2	2	4		1	1	2	1		1	3	2	1	1	2	5	0.04	2.1	13.6	6.34	172
27	Control	596867	0	19	1) <=20	2	1	3	2	3	4		1	1	2	1		1	3	2	2		2	5	0.06	0.78	14.59	2.99	159
28	Control	586921	0	25	2) 21-25	2	2	3	3	1	4		1	1	2	3	2	1	3	2	2		2	5	0.05	0.96	12.1	8.1	170
29	Control	595674	0	26	3) 26-30	2	2	3	2	2	4		1	1	2	3	2	1	2	2	1	1	2	5	0.44	2.1	16	7.8	160
30	Control	593577	0	24	2) 21-25	2	3	2	4	3	4		1	1	2	2		2	1	1	1		2	5	0.3	0.62	14.6	11.3	159
31	Control	582638	0	19	1) <=20	2	1	3	2	2	4		1	1	2	1		1	3	2	2		2	5	0.05	0.86	15.01	4.46	269
32	Control	608377	0	27	3) 26-30	2	1	3	2	3	4		1	1	2	1		2	3	2	2		2	5	0.16	0.89	37.9	6.2	348
33	Control	599732	0	24	2) 21-25	2	1	3	5	2	4		1	1	2	2		1	3	2	2		2	5	0.4	0.63	10.9	3.08	159
34	Control	586478	0	24	2) 21-25	2	1	3	1	2	4		1	1	2	3	3	1	3	2	2		2	5	0.31	0.82	16.6	13.7	200
35	Control	611597	0	26	3) 26-30	2	2	3	2	2	4		1	1	2	1		1	2	2	2		2	5	0.3	1.4	15.4	4.83	250
36	Control	621614	0	25	2) 21-25	2	2	3	2	3	4		1	1	2	3	2	1	3	2	2		2	5	0.1	0.92	18.9	12.6	403
37	Control	626531	0	19	1) <=20	2	2	1	3	2	4		1	1	2	1		2	1	1	1	4	2	5	0.1	0.6	20.2	18.5	205
38	Control	578929	0	20	1) <=20	2	1	3	4	1	4		1	1	2	3	1	1	3	2	1	4	1	5	0.09	1.2	18	6.8	169
39	Control	570007	0	23	2) 21-25	2	1	2	2	1	4		1	1	2	1		2	3	2	2		2	5	0.09	2.28	15.05	7.2	170

40	Control	578929	0	20	1) <=20	2	1	3	2	1	4		1	1	2	3	3	1	3	2	2		2	5	0.32	0.71	32.1	10.14	170
41	Control	591657	0	20	1) <=20	2	2	3	4	1	4		1	1	2	1		1	1	1	1	1	2	5	0.06	0.55	7.15	5.05	300
42	Control	584684	0	30	3) 26-30	2	4	3	5	2	4		1	1	2	3	4	1	3	2	2		2	5	0.1	0.52	12	8.7	200
43	Control	584775	0	28	3) 26-30	2	3	3	3	2	4		1	1	2	3	3	1	2	2	1	1	2	5	0.31	1.21	12.5	11.96	160
44	Control	515104	0	20	1) <=20	2	1	3	1	2	4		1	1	2	1		1	2	2	1	1	2	5	0.09	0.49	18	7.8	230
45	Control	598499	0	20	1) <=20	2	1	2	2	2	4		1	1	2	3	5	1	3	2	2		2	5	0.06	1.4	12.4	3.1	170
46	Control	584775	0	28	3) 26-30	2	3	3	2	2	4		1	1	2	1		1	3	2	2		2	5	0.06	0.67	12.5	10.8	205
47	Control	631136	0	23	2) 21-25	2	2	3	4	1	4		1	1	2	3	3	1	2	2	2		2	5	0.06	1.62	10	3.6	160
48	Control	640973	0	26	3) 26-30	2	2	3	2	2	4		1	1	2	1		1	3	2	2		2	5	0.4	0.86	45.68	3.2	250
49	Control	609998	0	22	2) 21-25	2	1	3	4	1	4		1	1	2	3	5	1	3	2	2		2	5	0.23	0.34	24.82	2.92	170
50	Control	593609	0	24	2) 21-25	2	2	3	2	2	4		1	1	2	1		1	3	2	2		2	5	0.2	0.92	26.2	2.78	246
51	Control	616341	0	22	2) 21-25	2	3	3	4	2	4		1	1	2	1		1	3	2	2		2	5	0.3	0.85	9.09	16.4	180
52	Control	622838	0	28	3) 26-30	2	2	3	2	2	4		1	1	2	1		1	3	2	2		2	5	0.23	0.64	37.4	3.73	400
53	Control	59965	0	25	2) 21-25	2	1	3	4	2	4		1	1	2	1		1	3	2	2		2	5	0.07	0.85	20.2	11.5	169
54	Control	625607	0	26	3) 26-30	2	1	3	2	1	4		1	1	2	1		1	3	2	2		2	5	0.3	0.96	14	3.45	170
55	Control	655519	0	25	2) 21-25	2	1	3	4	2	4		1	1	2	1		1	3	2	2		2	5	0.1	0.89	6.31	6.34	240
56	Case	625604	1	28	3) 26-30	1	2	2	1	2	2	1	2	1	1	3	2	2	2	2	1	4	2	5	1	1.47	19	1.63	159
57	Case	622904	1	20	1) <=20	2	2	3	5	2	2	1	2	1	2	2		1	3	2	2		2	5	0.38	1	28.4	3.01	159
58	Case	619504	1	24	2) 21-25	2	2	3	1	3	3	2	3	3	1	1		1	3	2	1	1	2	5	0.78	1.76	15.7	6.23	232
59	Case	622969	1	29	3) 26-30	1	1	2	5	2	1	1	4	1	1	3	4	2	1	1	1	3	1	5	0.1	1.6	9.48	4.26	196
60	Case	620206	1	25	2) 21-25	2	2	3	3	2	2	1	2	1	2	3	2	1	2	2	1	1	2	5	0.69	1.5	37.9	2.26	348
61	Case	622918	1	19	1) <=20	2	1	3	4	3	3	2	3	1	1	3	4	1	2	2	1	1	2	1	0.8	2.1	10.37	3.68	204
62	Case	622906	1	22	2) 21-25	2	2	3	3	2	2	1	2	1	2	1		1	2	2	2		2	5	0.9	1.54	10.5	3	160
63	Case	622945	1	23	2) 21-25	2	1	2	2	3	2	1	2	1	2	1		1	3	2	2		2	5	0.12	2.52	11.9	2.3	170
64	Case	622521	1	23	2) 21-25	1	3	2	4	1	1	1	4	1	1	1		1	3	2	2		2	2	0.11	2.68	29.5	3.03	159
65	Case	585529	1	27	3) 26-30	2	4	3	2	2	2	2	2	3	1	3	1	1	3	2	1	1	2	2	0.13	1.2	32	1.14	159
66	Case	589491	1	24	2) 21-25	2	2	3	4	2	2	2	2	1	2	3	1	1	3	2	2		2	4	0.1	1.11	16.5	1.69	159
67	Case	593609	1	24	2) 21-25	1	2	3	4	2	1	1	4	1	2	3	1	1	3	2	1	3	2	5	0.11	1.6	13.9	1.18	159
68	Case	593962	1	20	1) <=20	1	3	3	4	2	1	1	4	1	2	1		1	3	2	1	2	2	5	0.14	0.46	9.6	3.77	117
69	Case	593997	1	24	2) 21-25	2	2	3	1	3	2	2	4	1	1	1		2	2	2	1	4	2	5	0.89	2.5	26.6	1.64	159
70	Case	523324	1	24	2) 21-25	2	3	2	5	3	2	2	2	1	2	3	2	2	2	2	1	4	2	5	0.88	1.9	32.8	3.49	159
71	Case	615337	1	30	3) 26-30	1	2	3	4	2	1	1	1	1	2	3	2	1	3	2	2		2	5	0.1	1.4	33.4	3.09	159
72	Case	615405	1	24	2) 21-25	2	3	2	2	2	2	2	2	3	1	3	2	2	2	2	1	1	2	5	0.9	1.8	16.1	8.1	159
73	Case	612414	1	34	4) >30	2	3	2	3	2	1	1	1	1	1	3	2	2	2	2	1	1	2	5	0.66	1.6	24	1.26	201
74	Case	597041	1	26	3) 26-30	2	1	3	4	3	2	3	4	2	2	1		1	3	2	2		2	5	0.14	2.8	30.6	1.43	230
75	Case	587721	1	23	2) 21-25	1	1	3	3	2	1	1	1	1	2	2		1	3	2	1	3	2	5	0.1	1	21	1.11	400
76	Case	620283	1	21	2) 21-25	1	2	3	2	2	2	2	4	1	2	1		1	3	2	2		2	5	0.12	1.2	17.2	8.16	170
77	Case	617137	1	25	2) 21-25	2	1	3	3	3	3	1	3	1	1	1		1	3	2	1	1	2	1	0.21	1.6	8.46	2.82	222
78	Case	616341	1	24	2) 21-25	2	3	3	3	3	2	2	4	3	2	1		1	3	2	2		2	3	0.2	0.11	14	7.28	176
79	Case	6104646	1	27	3) 26-30	2	4	3	2	3	3	3	4	3	1	2		1	4	2	1	1	2	5	0.34	2.45	15.4	3.13	140
80	Case	579077	1	19	1) <=20	2	2	2	2	3	3	3	3	2	1	1		2	2	2	1	1	2	5	0.9	1.8	16.2	1.1	160
81	Case	582611	1	22	2) 21-25	1	1	3	3	2	2	1	2	1	2	1		2	3	2	2		2	3	0.1	0.67	9.1	1.49	200
82	Case	532994	1	24	2) 21-25	2	3	3	2	2	2	2	3	2	1	3	1	1	3	2	2		2	3	0.1	2.89	12.5	1.45	159
83	Case	583592	1	23	2) 21-25	2	1	2	3	2	3	3	4	1	2	1		2	1	1	1	1	1	1	0.88	2.46	12.6	1.57	159
84	Case	585464	1	21	2) 21-25	2	1	2	2	2	2	1	4	1	1	1		2	2	2	1	1	2	5	0.67	1.89	27	1.47	159
85	Case	586921	1	20	1) <=20	1	2	3	4	2	1	2	1	1	1	2		2	2	2	1	4	2	5	0.9	1	10.2	1.84	205
86	Case	587721	1	23	2) 21-25	1	1	3	5	1	2	1	1	1	2	1		1	2	2	2		1	5	0.86	1.2	12.2	1.11	159

87	Case	582232	1	30	3) 26-30	2	4	3	4	3	2	2	2	3	2	3	3	1	3	2	2		2	3	0.11	1.3	6.67	7.97	208
88	Case	576541	1	20	1) <=20	2	3	2	1	3	2	1	4	1	1	3	4	1	3	2	2		2	5	0.12	1.8	12.68	1.6	160
89	Case	588088	1	19	1) <=20	1	1	2	5	1	2	1	2	1	2	3	2	1	3	2	2		2	5	0.67	1.9	13.4	6.23	180
90	Case	593609	1	24	2) 21-25	1	2	3	4	2	1	2	1	2	2	3	1	1	3	2	2		2	5	0.1	1.22	33.4	2.43	140
91	Case	582680	1	20	1) <=20	1	2	3	5	2	2	2	1	1	1	3	2	1	2	2	2		2	5	0.93	2.2	13.5	1.52	124
92	Case	626531	1	19	1) <=20	2	2	1	2	3	2	1	1	1	1	1		2	1	1	1	1	1	5	0.9	2.21	11.1	1.5	176
93	Case	560334	1	28	3) 26-30	2	2	3	4	2	2	2	4	2	1	2		2	2	2	1	4	2	5	0.89	2.5	10.7	5.13	135
94	Case	596650	1	22	2) 21-25	1	1	3	4	2	1	1	1	3	1	3	5	1	3	2	1	1	2	4	0.11	1.14	16.6	1.37	128
95	Case	578929	1	20	1) <=20	2	1	3	1	3	3	4	4	3	2	1		1	2	2	1	1	2	5	0.88	2.32	5.45	8.73	166
96	Case	591657	1	20	1) <=20	2	2	3	2	1	1	2	1	1	1	2		1	3	2	1	3	2	5	0.13	0.46	16.2	8.73	120
97	Case	584684	1	30	3) 26-30	2	4	3	2	3	3	3	4	2	2	3	3	1	3	2	2		2	5	0.14	2.23	20.4	1.58	177
98	Case	584775	1	28	3) 26-30	1	3	2	4	2	1	1	1	1	1	3	4	2	1	1	1	1	2	5	0.89	2.7	9.16	3.37	229
99	Case	585529	1	27	3) 26-30	2	1	2	1	2	2	2	1	2	2	3	5	2	2	2	1	1	2	5	0.78	2	9.66	2.39	230
100	Case	598499	1	20	1) <=20	2	1	2	5	3	3	3	4	3	1	2		1	2	2	2		2	1	0.8	2.1	14	2.34	240
101	Case	589128	1	25	2) 21-25	2	3	2	4	2	2	2	1	1	2	1		2	2	2	1	4	2	5	0.9	1.8	14.44	3.25	150
102	Case	631136	1	23	2) 21-25	1	3	3	4	1	1	1	1	1	1	3	2	1	3	2	2		2	5	0.33	0.42	14.3	1.16	140
103	Case	642349	1	26	3) 26-30	1	2	3	5	3	3	4	1	1	2	3	5	1	3	2	2		2	5	0.14	0.46	8.04	6.86	125
104	Case	643274	1	21	2) 21-25	2	1	3	4	2	3	3	3	2	2	3	1	1	3	2	2		2	5	0.48	1.02	13.2	4.31	229
105	Case	642123	1	25	2) 21-25	2	2	2	3	2	2	4	1	3	1	1		2	2	2	2		2	5	0.99	1.7	15.6	9.1	120
106	Case	641557	1	22	2) 21-25	2	2	3	5	3	3	3	2	1	2	1		1	3	2	2		2	5	0.12	1.02	15.6	3.82	166
107	Case	605813	1	20	1) <=20	2	2	2	2	2	3	2	2	2	2	1		2	2	1	1	1	2	1	0.8	1.9	5.89	5.2	180
108	Case	640962	1	25	2) 21-25	1	3	2	4	3	2	3	2	1	1	2		1	3	2	2		2	5	0.11	2	9.4	4.4	240
109	Case	613184	1	24	2) 21-25	1	2	3	1	1	2	2	1	1	2	3	4	1	2	1	1	2	2	5	0.77	2.42	16.6	1.64	200
110	Case	641299	1	25	2) 21-25	2	3	3	2	3	3	4	1	1	2	1		1	3	2	1	3	2	1	0.2	2.62	10.99	3.09	120