DISSERTATION SUBMITTED FOR THE MASTER'S DEGREE IN MEDICAL BIOCHEMISTRY

TITLE

A STUDY OF MALONDIALDEHYDE AND FERRIC REDUCING ABILITY OF PLASMA IN PREGNANT AND NON-PREGNANT WOMEN

SUBMITTED

BY

ANKITA VERMA

2023

DEPARTMENT OF BIOCHEMISTRY

INTEGRAL INSTITUTE OF MEDICAL SCIENCES AND RESEARCH

FACULTY OF HEALTH AND MEDICAL SCIENCES INTEGRAL

UNIVERSITY LUCKNOW-226026, U.P

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DISSERTATION

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In partial fulfilment of the requirement for the award of degree of

Master of Science In
Medical Biochemistry

By

ANKITA VERMA

Enrollment No: 2000100621

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Professor and Head
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CERTIFICATE

This is to certify that **Miss Ankita Verma,** a student of **M.Sc. Medical Biochemistry**. Integral University has completed her dissertation titled **"A STUDY OF MALONDIALDEHYDE AND FERRIC REDUCING ABILITY OF PLASMA IN PREGNANT AND NON-PREGNANT WOMEN"** successfully. She has completed this work in the Department of Biochemistry, Integral Institute of Medical Sciences and Research, Integral University under my supervision. The dissertation was a compulsory part of her M.Sc. degree.

I wish her good luck and a bright future.

Guide

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I hereby declare that Integral Institute of Medical Sciences & Research Integral University, Lucknow shall have the right to preserve, use and disseminate this dissertation in print/electronic format for academic/ research purposes.

I will publish the research paper related to my dissertation only with the consent of my guide.

Date:

Place: Lucknow **Ankita Verma**

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I was able to overcome every barrier that stood out in the achievement of my thesis work.

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Date:

Place: *Lucknow, Uttar Pradesh, India* ***ANKITA VERMA***

CONTENTS

LIST OF ABBREVIATIONS

MDA	Malondialdehyde
FRAP	Ferric Reducing Ability of Plasma
ROS	Reactive Oxygen Species
RNS	Reactive Nitrogen Species
TBA	Thiobarbituric Acid
TBARS	Thiobarbituric Acid Reactive Substances
TCA	Trichloroacetic Acid
LPO	Lipid Peroxidation
AOA	Antioxidant Activity

INTRODUCTION

Pregnancy is a physiological condition in women characterized by fetal growth. It occurs when a fertilized egg develops into a zygote and then a fetus within the womb within a period of about 9 months. Many metabolic and physiological systems are significantly altered during pregnancy, which is a stressful condition **(Scott et al., 1994).**

The physiological change of pregnancy is characterized by a sharp rise in the amount of energy and oxygen required to ensure proper fetal growth and development. Thus, oxidative stress is likely to occur throughout pregnancy for both the mother and fetus **(Mutainati et al.,2013).** Late in the pregnancy, increased lipid peroxidation and decreased antioxidant activity contribute to the development of pregnancy complications (**Rejitha J et al., 2014).**

As a result, during this time, extraordinary and dramatic changes happen to support the mother and encourage the development and upkeep of the fetus **(Qanungo, S. et al. 2000)**. Due to the high energy demand and increased tissue oxygen needed during a normal pregnancy, the level of oxidative stress has increased. All cells and tissues undergo lipid peroxidation, an oxidative process, at low levels **(Kagan V. E et al 1988).**

The condition of a disturbed balance between ROS and the mechanisms for detoxification and repair is what is known as oxidative stress. Each live cell produces ROS (reactive oxygen species) through the natural process of respiration, and a ROS substance is made up of a molecule of oxygen and an electron that is unpaired. (**Halliwell B et al., 1987).** ROS interacts with numerous important cytoplasmic chemicals and structures, changing how these organelles function biologically.

Excessive ROS and RNS synthesis result in "oxidative" or "nitrogenous" stresses, crucial in numerous pathological processes indicating neoplastic disorders, and neurological conditions. **(Halliwell B et al., 1987).** The pro-neoplastic activity of ROS results mainly from DNA damage, proteins, and lipids, and altering these components may enhance the likelihood of mutation **(Wiseman H et al., 1995).**

Factors that limit the effect or block the formation of free radicals also inhibit mutagenesis, the transformation phagocytic cells, and the degradation of DNA **(Halliwell B et al., 1987).**

Numerous types of DNA damage resulting from the activity of endogenous and external agents are promptly recognized. A sophisticated network of signal transduction pathways is later activated as a reaction to DNA damage **(Barzilai A et al., 2004).** The human ovum is relatively capable of healing oxidative damage to DNA (**Menezo Y et al., 2010).**

Unfortunately, however, data on the DNA quality in human oocytes are scant, largely presumably due to a shortage of testable materials. On the other hand, mature sperm typically aren't capable of repairing DNA damage **(Barratt C.L et al., 2010).** The ability of the developing sperm to repair any possible DNA damage diminishes over the course of spermatogenesis **(Olsen A.K et al., 2000).** The perfect replication of the genome is of utmost relevance in a single germinal cell (**Menezo et al., 2010).**

There are three ways to approach DNA repairs for both somatic and germinal cells. Activating apoptotic pathways is the initial step. In this situation, the ratio of pro- to antiapoptotic factors in the ovum will determine the viability of the cells. The second option is damage tolerance, which might result in mutation in somatic and embryonic cells and, as a result, might lead to more birth defects and carcinogenesis in the following generation. Repairing the harm is the third choice **(Menezo et al., 2010).** Naturally, variations in the capacity for repair affect both the degree of oxidative DNA damage and an individual's vulnerability to disease **(Jaiswal M et al., 2000).**

An imbalance between the production of reactive free radicals, also known as ROS, and cellular antioxidant capability is known as oxidative stress (**Kumar Dabla, P. et al., 2015**). Free radicals such as superoxide (O_2) are examples of ROS, H_2O_2, OH’, and O^{2-} are examples including non-radical intermediates. Nitric oxide (NO’), which has a modest level of reactivity, and its product ONOO’, are

examples of RNS. The above species originate from enzymemediated processes such as the respiratory chain, phagocytosis, and prostaglandin formation, and are free of enzymes reactions (such as oxygen

chemical reactions with organic molecules or exposure to ionizing radiations), which primarily occur in the mitochondria but also occur in peroxisomes and the endoplasmic reticulum **(A. Phaniendra et al., 2015).**

OXIDATIVE STRESS IN PLACENTATION

For the placenta to develop and mature, the invasion, differentiation, and growth of the trophoblast in the maternal deciduous cells must be synchronized **(B. Huppertz et al., 2009).**

During pregnancy the trophoblast cells experience very low O2 concentration as a result of reduced O2 pressure occurring within the placenta. Because it promotes the cellular response to hypoxia this is physically low oxygen status is a crucial regulator of placental function **(Davies et al., 2015).**

The oxygen consumption of the mitochondria is downregulated in low-oxygen environments (G.J. Burton et.al 2017). While the endometrial glands' glucose consumption is sufficient to maintain the embryo and placenta access to a steady supply of ATP **(A.I. Frolova et al., 2011).** As a result, throughout the first trimester, the conceptus' metabolic needs are still sufficiently supplied in an environment with 2.5% oxygen **(A.I. Frolova et al., 2011).**

REVIEW

OF

LITERATURE

PREGNANCY

Pregnancy is a normal physiological phenomenon accompanied by a dynamic change in women's bodies that causes their susceptibility to oxidative stress. Oxidative stress is a condition that results from an imbalance between pre-oxidant and antioxidant defense systems **(Shah et al., 2004)**

The enormous increase in energy and oxygen necessary for the fetus's appropriate development and expansion makes pregnancy a stressful state **(Mutinati M, et al., 2013).** Pregnancy is a physiological development during which both the mother and the fetus suffer oxidative stress. Poor pregnancy outcomes, including fetal development restriction, can be caused by deficiencies of certain antioxidant activities linked to the micronutrients selenium, copper, zinc, and manganese (**Giles GI et al., 2002** Lipid peroxidation and reduced antioxidant activity, result from energy equilibrium and possibly aid in the emergence of problems **(C.H. Fall et al., 2003).**

EPIDEMIOLOGY

Live birth declined from 90.2% to 88.9%. Half of the Indian states/UTs reported lower live birth rates for 2019–21 than the national average (88.9%). In Telangana, the rate of abortions among adolescent women increased by 11.0% between 2019 and 21 and 0.7% between 2015 and 16 **(Kuppusamy et al., 2021).**

Antenatal Period

A) Embryonic phase

The term "*embryo*" refers to the developing organism from fertilization until the end of eight weeks.

(B) Fetal phase :-.The fetal period lasts from the first day of the third month's ninth week till delivery. Germinal phase, embryonic, and fetal phase are the three divisions of the prenatal period according to embryology **(Vishram S., 2012).**

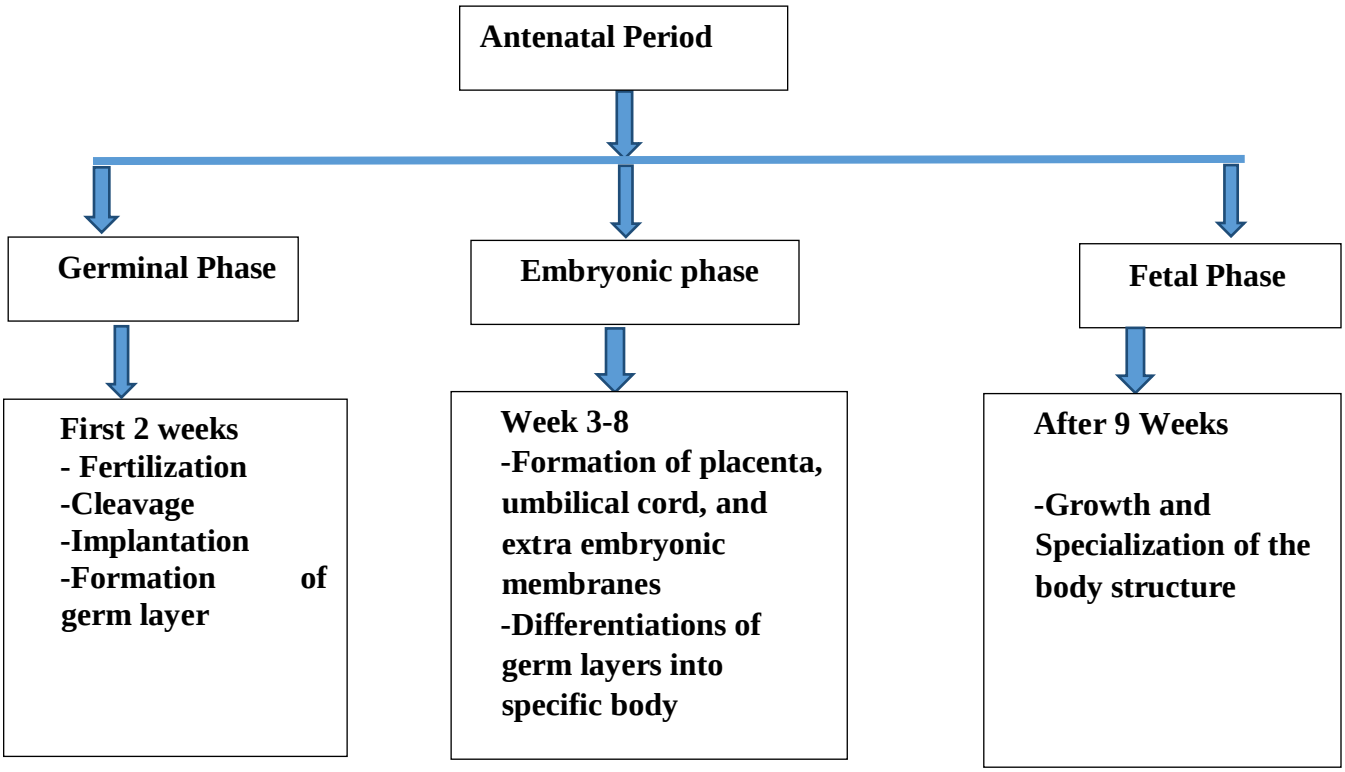


Fig: 1 Prenatal development periods **(Vishram S., 2012).**

PREGNANCY TERMS

1- First trimester

Minute breathing is increased by 40% during the first trimester **(Campbell LA, et al., 2001).** In eight weeks, the womb will reach the size of a lemon. During this trimester, several indicators of pregnancy and issues, including nausea and the development of breasts, also occur.

2- Second Trimester

A woman's second trimester of pregnancy lasts for 13 to 28 weeks. Compared to the first trimester, this is the period when women experience more energy. Additionally, the muscular organ that houses the growing fetus grows as much as 20 times its typical size throughout pregnancy. The growing uterus has produced a noticeable "baby bump" at the conclusion of this trimester **(Stacey T, et al., 2011).**

3-Third Trimester

The last weight gain, which is the largest growth throughout the pregnancy, occurs. The uterus grows and takes up a growing percentage of the female abdomen. As the fetus turns lower in preparation for birth, the woman's abdomen will change shape and drop. Fetal development can be disruptive to women and rather robust. In spite of the reduced vena cava pressure caused by the larger uterus, found that the infants benefit from greater oxygenation in the lateral position **(Stacey T, et al., 2011).**

Oxidative Stress

The alteration in the prooxidant and antioxidant balance leads to some potential damage defined as oxidative stress **(Lobo et al., 2010).** Oxidative stress is a state that can result in harm to tissues because of excess free radical **(Agrawal A, et al., 2005).**

Increased levels of oxidative stress during pregnancy can result in pre-eclampsia, gestational diabetes, and fetal hypoxia. Many tissues have a supraphysiological rise in oxidative stress in pregnancy. It is especially concentrated in the human placenta, which causes vascular dysfunction and impairs the supply of nutrients to the developing fetus, leading to changes in fetal size for the duration

of pregnancy, fetal growth retardation, macrosomia, or premature delivery **(Marcoleta et al., 8 330024).**

Free radical generation is a physiologically normal process, but excessive production causes lipid peroxidation and encourages maternal vascular dysfunction because reactive oxygen species (ROS) like superoxide encourage endothelial activation. Antioxidant substances in the human body scavenge those free radicals, reducing oxidative damage **(Tiwari AKM, et al., 2010).**

Free radicals cause lipids to undergo oxidative alteration, which starts a process known as lipid peroxidation (LPO), which leads to the loss of lipid-rich regions like cell membranes or myelin sheaths and the production of highly reactive aldehydes like malondialdehyde(MDA) **(Ortiz, et al., 2013).** The result of oxidizing polyunsaturated fatty acids (PUFA) is MDA. As a result, it acts as an accurate oxidant marker of the peroxidation of lipids caused by oxidative stress **(Del Rio, et al., 2005) .**
ROS, RNS, and RSS are families of molecules that these free radicals belong to **(Giles GI et al., 2002).** The human body naturally produces free radicals as a consequence of metabolism and oxidation **(Lobo V et al., 2010).**

Mechanism

ROS are byproducts of the metabolism of oxidation that are crucial to cellular function. They are linked to other pathological disorders as well. UV radiation, alcohol, ischemia-reperfusion injury, persistent infections, and inflammatory disorders are the causes of excessive ROS generation (A Bhattacharyya et al., 2014). When there is a larger concentration of hydrogen peroxide (H_2O_2) in the cell, catalase removes it from the **body (L. W. Obereley et al., 2005)**. SOD enzyme is involved in the break down superoxide anion into less toxic products **(E.P. Harris et al., 1992).** The body's tissues include glutathione reductase, which functions similarly to GPx. Glutathione reductase enzymes employ NADPH in its antioxidants responsibilities **(J Fuji et al., 2011).**

a) Scenarios of Oxidative Stress in Pregnancy:-

The fetus needs enough nutrition and oxygen during a typical pregnancy to support its growing tissues and organs. These processes produce ROS that have an impact on the development of fetal growth. The equilibrium between ROS and antioxidants could be preserved to offer a healthy environment for the mother's body and the developing fetus **(J. Fuji et al., 2011).**

The body goes through a lot of physiological changes while pregnant. The researchers made the assumption that there was proof of ROS generation due to accelerated metabolism, high oxygen consumption, and utilization of fatty acids. Increased generation of hydrogen peroxide is caused by increased metabolic syndrome during the third trimester of pregnancy (**Duhig et al., 2016).**

b) Within the Placenta, Uterus, and Ovary

ROS has an effect on pregnancy in almost every stage. The key modulator of ovarian cell activity is recognized to be ROS. The advantages of ROS have already been addressed.

Leukocytes, macrophages, and cytokines are the generators of ROS in the follicles. ROS concentration is an indicator of ovulation. There is evidence that ROS suppressors interrupt the ovulatory phase (Kshkolnik et al., 2011).

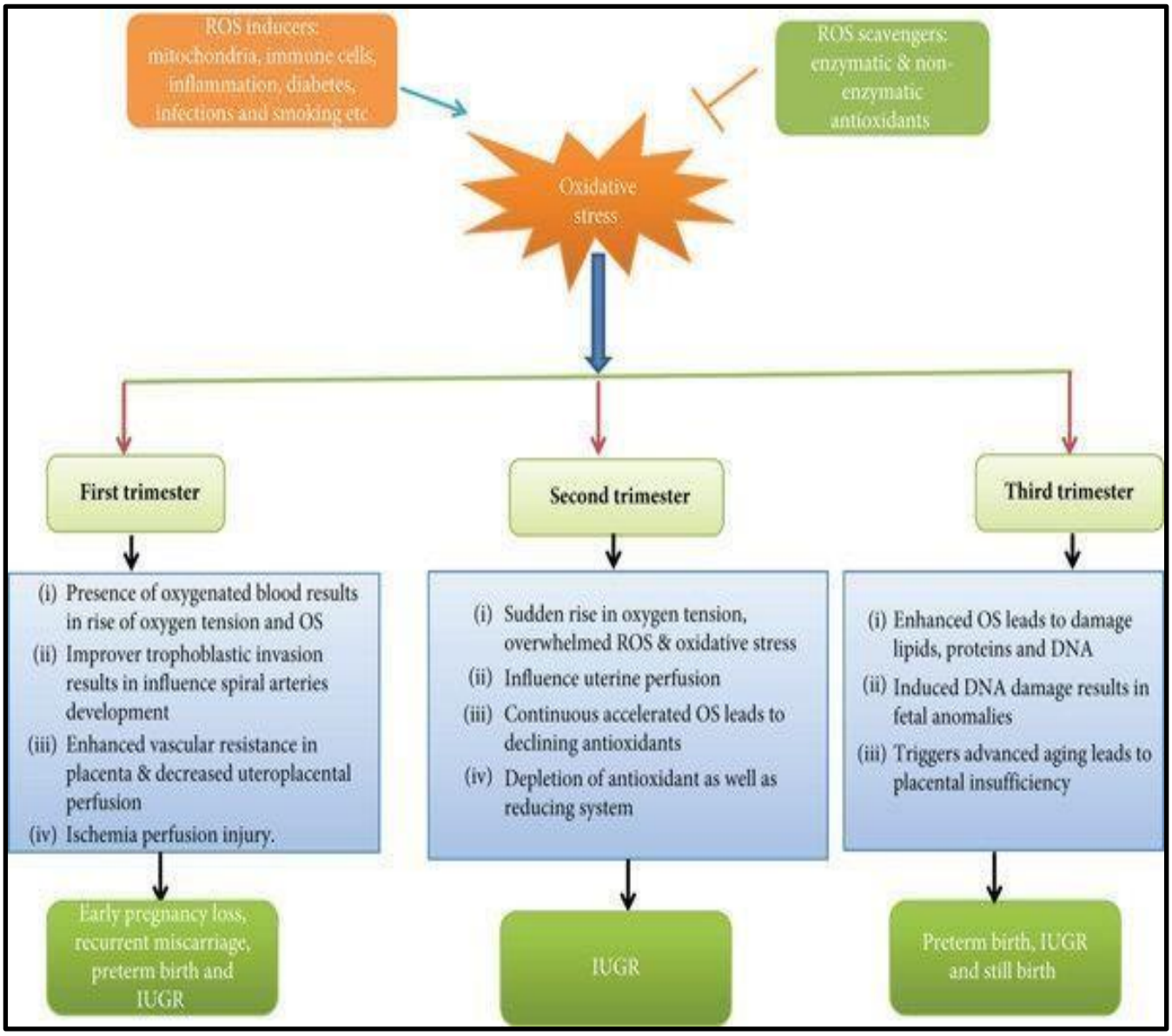


Fig: 2.Impact of Oxidative Stress on Pregnancy Outcomes [Hussain et al., *2021*]

ANTIOXIDANT -

The body has developed several strategies to combat the damaging effects inside the cell, including damage prevention, oxidative damage repair, physical defense against damage, and—perhaps most importantly—antioxidant defense mechanisms**(Pereira, R. D et al., 2015).**

Antioxidants are the first line of defense against stress, according to the free radical oxidative stress theory. The network of compartmentalized antioxidant enzyme and non-enzyme-containing molecules that make up endogenous antioxidant defences is typically dispersed throughout the cell. Antioxidant enzymes are involved in a complex series of reactions that reduced the toxicity of ROS into more stable forms **(Hussain, et al., 2021).**

The primary antioxidant enzyme receive support from secondary enzymes in collaboration with small molecular weight antioxidant that serve as cofactors in the reactions. ROS scavenger such as non-enzymatic antioxidant are essential. Effective free radical elimination require the combined action enzymatic and non- enzymatic antioxidants. **(Ďuračková, Z et al., 2010).**

It is known that even a straightforward pregnancy generates free radicals**(Casanueva E et al., 2003)** leading to oxidative stress. Due to these free radicals, pregnant women are more susceptible to infections, which in turn combine with oxidative stress to result in a plethora of perinatal, maternal, and even congenital diseases **(Klufio CA et al., 1992)** and abnormalities **(Palan PR et al .,2004).**

Needless to say, low antioxidant levels has been known to a variety of unfavourable pregnancy outcomes, including sluggish or defective fetal and childhood development **(Evans P et al., 2001).** Programs to lower free radical generation and raise antioxidant levels in pregnant women should therefore be part of prenatal care.

Benzie and Strain created the FRAP assay in order to gauge plasma's lowering power back in 1996. As a novel technique for determining the "antioxidant power," the FRAP assay, a straightforward, automated test that measures the ferric-reducing capacity of plasma, is described.

A colourful ferrous - tripyridyltriazine complex is produced when ferric-to-ferrous ion reduction occurs at low pH. The conversion of ferric tripyridyltriazine to ferrous tripyridyltriazine serves as a proxy for the total antioxidant capability **(Benzie et al., 2003).**

In the last decades, lots of work had been done on OS and antioxidants in pregnant women. From the above findings, the present study sought to clarify the roles of oxidative stress and antioxidant profile during progression of pregnancy.

AIM

AND

OBJECTIVES

Aim

The aim of this study was to determine the levels of MDA and FRAP in pregnant and non-pregnant women.

OBJECTIVES

1. To determine the level of MDA in pregnant and non-pregnant women.
2. To determine the ferric-reducing ability of plasma FRAP in pregnant and non-pregnant women.
3. To find the correlation between malondialdehyde and ferric-reducing ability of plasma in pregnant and non-pregnant women, if any.

MATERIALS

AND

METHODS

Research Question: - Is there any association between Malondialdehyde and Ferric reducing the ability of plasma in pregnant women compared to non-pregnant women?

Statistical Hypothesis:-

Null Hypothesis (H_0): There is no significant association between Malondialdehyde and Ferric reducing the ability of plasma in pregnant women compared to non-pregnant women.

Alternate Hypothesis (H_1): There is a significant association between Malondialdehyde and Ferric reducing the ability of plasma in pregnant women compared to non-pregnant women.

METHODOLOGY

Type of Study: A case-control study

Study design: Prospective

Place of Study: -Department of Biochemistry, Integral Institute of Medical Science and Research, Lucknow (U.P).

Collaborating Department: -Department of Obs & Gynaecology Department, OPD at IIMS&R, Integral University, Lucknow

SUBJECTS SELECTION

Selection of Control:

INCLUSION CRITERIA FOR CONTROL

- Apparently Healthy non-pregnant women
- Age group between 18-40 years
- Individuals who have agreed to sign the consent form

Selection of Cases:

INCLUSION CRITERIA

- Pregnant women.
- Age group between 18-40 years
- Patients who have agreed to sign the consent form

EXCLUSION CRITERIA

- Anaemia (Hb of 8.0 g/dl or less)
- Any Chronic diseases
- Smokers

Enrollment of Participants:

Cases were enrolled from pregnant women attending the Integral Hospital, Obs & Gynaecology Department.

Data collection

Details from the subjects were obtained using data collection proforma after taking written consent

Sampling Method – Non – probability, Purposive sampling

Collection of sample:-

Under aseptic conditions, 4 ml venous blood was obtained 2 ml in PLAIN and 2 ml in EDTA vials for the determination of malondialdehyde and FRAP assay respectively. EDTA was used as an anticoagulant to help preserve our sample for FRAP assay.

Sample Storage

The sample was refrigerated at - 20°c at the central clinical laboratory for preservation.

Sample Size.

$$n = (\frac{r+1}{r}) \frac{\sigma^2 (Z_\beta + Z_{\alpha/2})^2}{(d\text{ifference})^2}$$

Reference: **Charan J and Biswas; 2013**

n = sample size in the case group.

r = ratio of controls to cases.

σ = standard deviation of the outcome variables.

Difference = effect size (the difference in means of cases and controls)

Zβ = Represent the desired power (typically .84% for 80% power)

Zα/2 = Represents the desired level of statistical significance

For 95% CI **Zα/2** = 1.96, for 80% power **Zβ** = 0.84

Expected mean difference between Cases and controls is **= 0.066**

Standard Deviation is (σ) =0.13

$$n = 2 \frac{(0.13)^2 (0.84 + 1.96)^2}{(0.066)^2}$$

Therefore, n = 60.8 ≈ 60

n1 (Cases) = **30**

n2 (Controls) = **30**

Mother Article: (Patil, S. B., Kodliwadmath, M. V., & Kodliwadmath, S. M. (2007). Study of oxidative stress and enzymatic antioxidants in normal pregnancy. *Indian journal of clinical biochemistry: IJCB, 22*(1), 135–137)

ETHICS REVIEW

Permission from the Institutional Ethics Committee was taken (IEC/IIMS&R/2023/64).

STATISTICAL ANALYSIS

Statistical analysis was performed using IBM-SPSS software (version 16), Graph Pad (Prism 6.0) and Microsoft – Excel (version 2013). All the data were expressed as mean ± standard deviation. An unpaired t-test was performed to compare the study parameters between cases and controls. Karl Pearson's correlation analysis was employed to determine the relationship between variables.

p-value <0.05 was considered statistically significant.

LABORATORY INVESTIGATION

DETERMINATION OF MALONDIALDEHYDE BY SATOH K. (1978) METHOD

Principle: - Deproteinized serum is treated with Thiobarbituric acid (TBA) at 90°c for about 10 minutes giving a pink colour product. This estimates the thiobarbituric acid reactive substance measured at 535 nm and reads on a spectrophotometer.

Reagents

1. Tri chloroacetic acid (TCA)
2. Thiobarbituric acid (TBA)
3. 0.25N Hydrochloric Acid
4. 1N Sodium Hydroxide
5. Tetramethoxypropane

MDA Procedure

- 0.8 ml of serum + 1.2 ml of TCA-TBA-HCL Reagent
- Mix and keep in a boiling water bath for 10 minutes
- Cool and add 2 ml of NaOH
- O.D is taken at 535 nm against a blank which contains normal saline in place of serum.

Calculation

$$\textbf{Sample (µmol/L)} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times 10$$

DETERMINATION OF ANTIOXIDANT STATUS BY FRAP ASSAY [Benzie, I. F., & Strain, J. J. (1996)]

Principle: In FRAP assay antioxidants are used as reductants using a colorimetric method where ferric tripyridyltriazine to ferrous tripyridyltriazine i.e colorless to blue colour is observed at 593 nm. The readings are according to the reducing power of the electrons donating antioxidants.

Reagents:

1. Tripyridyltriazine
2. Ferric chloride
3. Dilute Hydrochloric acid
4. Acetate buffer
5. Standard used iron 2 sulfate

Procedure -

1. Add the above reagent into the glass tube (standard is added last).
2. For blank: 2 ml FRAP reagent + 1 ml Distilled water + 2 ml FRAP reagent.
3. Into a cuvette add 100 µL sample + 900 µL Distilled water + 2 ml FRAP reagent.
4. Invert tubes to mix properly.
5. Transfer the cuvette to a spectrophotometer.
6. Zero the spectrophotometer using blank at 593 nm and then read at OD.

Calculation

$$\text{FRAP value of sample (µmol/L)} = \frac{\text{Absorbance of Sample} \times \textit{FRAP value of Standard}}{\text{Absorbance of Standard}}$$

Draw standard curve Absorbance vs Fe2+ conc (X) and obtain the equation for a fit Line.

Use sample absorbance values that have been zeroed (i.e., Absorbance samples – Absorbance blank) to express FRAP in molarity.

OBSERVATION

&

RESULTS

MALONDIALDEHYDE

The difference in the level of between cases and controls was statistically significant (p value= 0.0001)

(Table-1 & figure-3)

Table-1 Mean and standard deviation of the study groups

MALONDIALDEHYDE(µmol/L)					
Groups	**n**	**Mean**	**Standard Deviation**	**p-value**	**Significance**
Controls	**30**	**1.92**	**± 0.35**	**0.0001**	**Statistically Significant**
Case	**30**	**3.39**	**± 0.68**		

p-value <0.05 is considered statistically significant

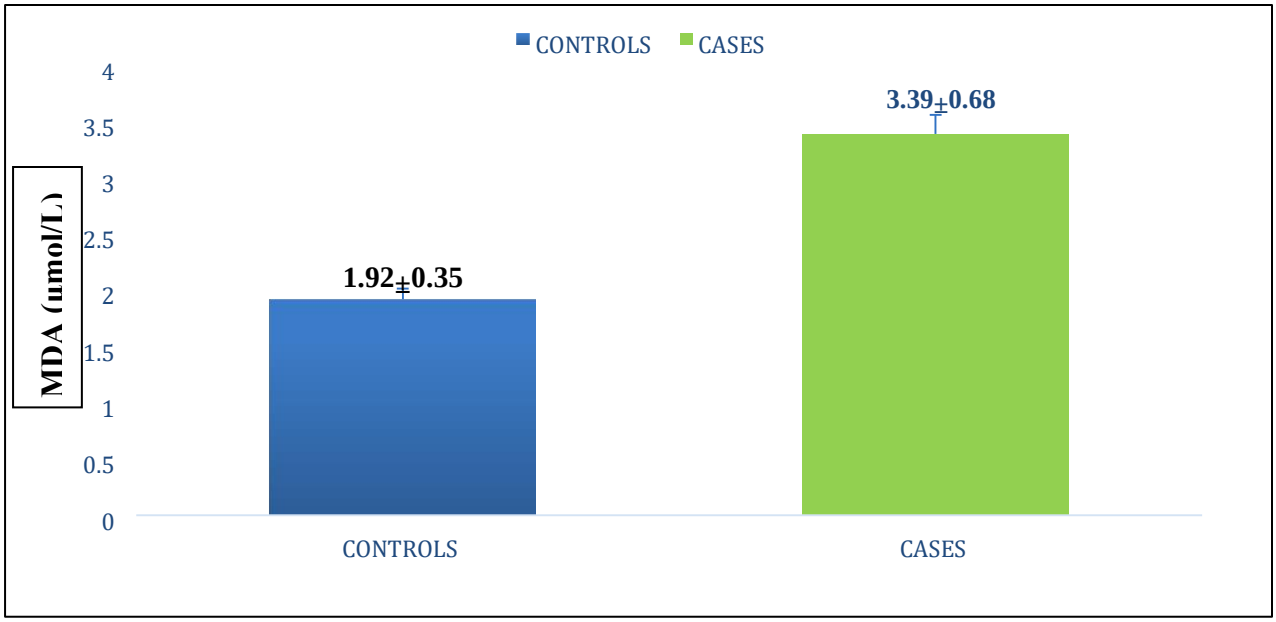


FIG: 3 Comparison of malondialdehyde in between controls and cases

FERRIC REDUCING ABILITY OF PLASMA

The difference in the level of FRAP between cases and controls was statistically significant (p-value= 0.0001) (Table-2 & figure-4)

Table-2 Mean and standard deviation of the study groups.

FERRIC REDUCING ABILITY OF PLASMA (µmol/L)					
Groups	n	Mean	Standard Deviation	p-value	Significance
Controls	30	1276.33	± 88.7	0.0001	Statistically Significant
Cases	30	1074.33	± 53.46		

p-value <0.05 is considered statistically significant

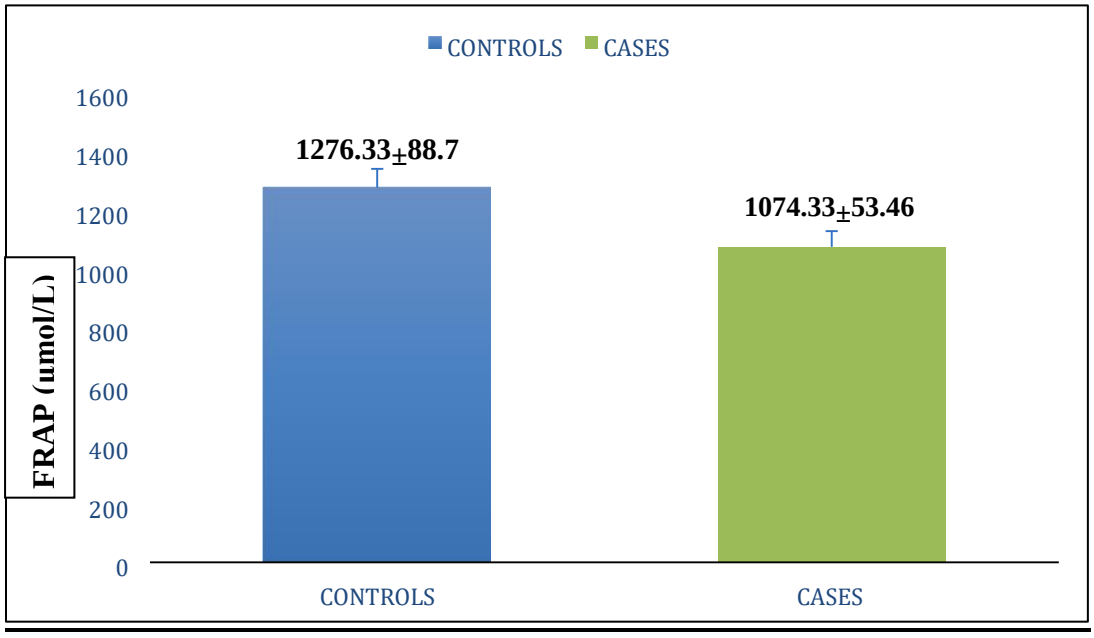


Fig: 4 Comparison of FRAP in between controls and cases

KARL PEARSON'S CORRELATION COEFFICIENT BETWEEN THE STUDY PARAMETERS IN CASE

Table-3 Karl Pearson's correlation coefficient between MDA and FRAP

		MDA(µmol/L)	**FRAP (µmol/L)**
MDA(µmol/L)	Pearson Correlation	1	.423*
	Sig. (2-tailed)		.020
	n	30	30
FRAP (µmol/L)	Pearson Correlation	.423*	1
	Sig. (2-tailed)	.020	
	n	30	30

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

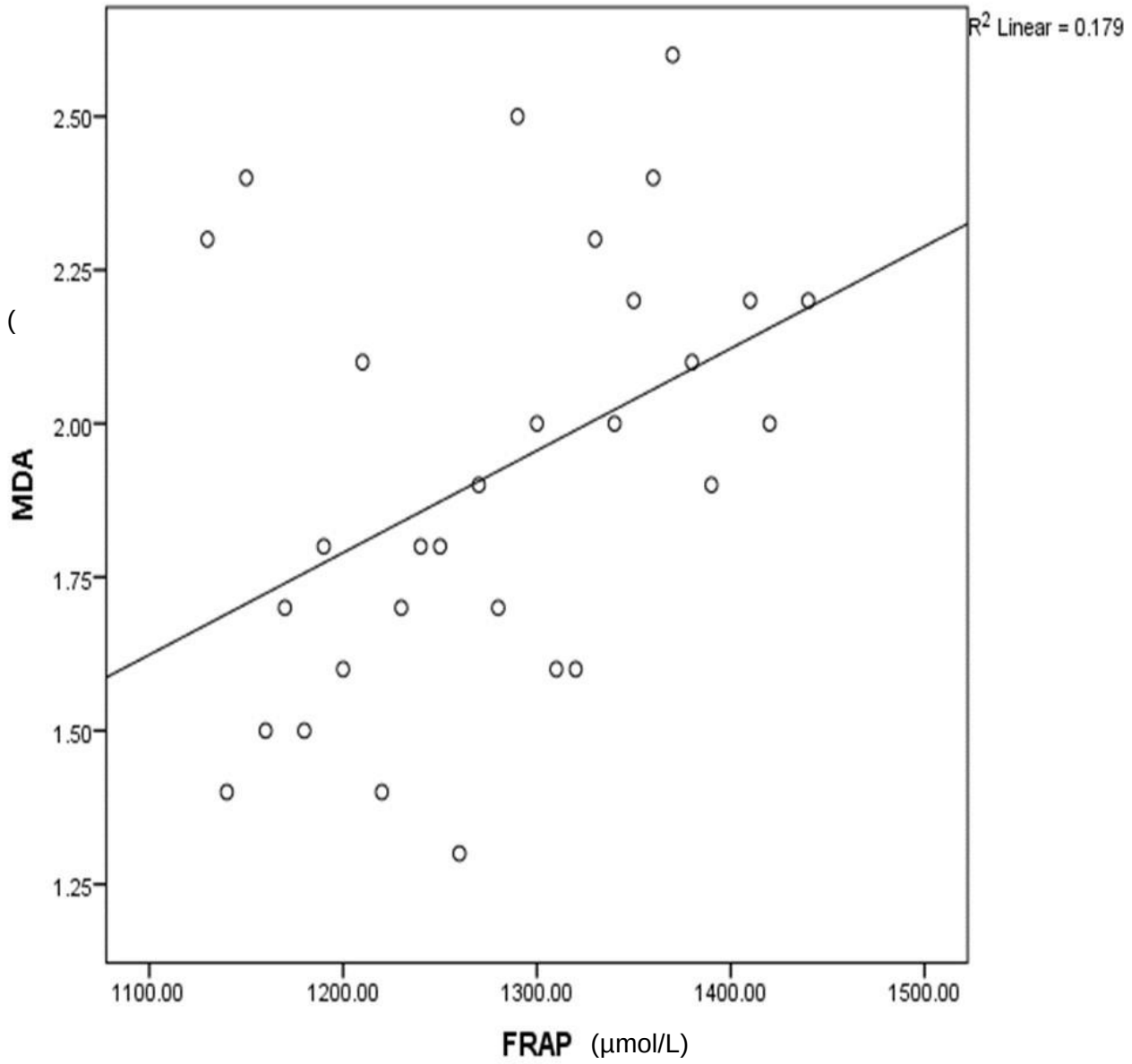


Fig: 5 Scatter diagram showing a correlation between MDA and FRAP

RESULT

- A significant increase ($p<0.0001$) was found in the levels of Malondialdehyde in Pregnant women when compared to Non-Pregnant women. (Table :1)
- A significant decrease ($p<0.0001$)was found in the levels of FRAP in Pregnant women when compared to non-pregnant women (Table: 2)
- The level of MDA was observed to be increased between cases 3.39±0.68 (µmol/L), compared with controls 1.92±0.35 (µmol/L).
- The level of FRAP was found to be lower among cases 1074.33±53.46 (µmol/L) compared with controls 1276.33±88.7(µmol/L).
- The Data shows a positive correlation between MDA and FRAP in cases.
- The results obtained show no significance between MDA and FRAP in pregnant women.

DISCUSSION

Due to the elevated metabolic load and increased tissue oxygen requirements, oxidative stress increases throughout a typical pregnancy. MDA is a suitable marker for the evaluation of free radical-induced harm to tissues since it is a stable by-product of free radicals created by lipid peroxidation in the body**(Sachdev et al., 2008).**

In the investigation, pregnant women and non-pregnant women had their marker values compared to MDA and FRAP. According to this study, the average plasma level of MDA in pregnant women is 3.39±0.68 (µmol/L), while in non-pregnant women, the value is 1.92±0.35 (µmol/L), value and FRAP 1074.3±53.46 (µmol/L) in 1276.3±88.7(µmol/L), in pregnant women and non-pregnant women respectively.

Because they are fragile and fleeting, reactive oxygen species can be hard to directly quantify. It has been utilized for indirect measurement of their ability to trigger lipid peroxidation. The development of a typical pregnancy has been accompanied by a rise in lipid peroxidation markers (MDA) **(Wickens D et al.,1981).**

Chamy et al., 2006deduced that healthy pregnant women have greater lipid peroxidation levels than normal pregnant women. In response, the body tips antioxidant defence system to restore haemostatics balance .Consequently, oxidative equilibrium might endure the entire pregnancy.

According **to Walsh S. W et al,** the maternal antioxidant system regulates placental lipid formation during a healthy pregnancy. ROS serve as signal transducers in physiology, but their overproduction can lead to a variety of health issues in people. While the body's own mechanisms play a critical part in regulating the amounts of these free radicals, the antioxidant levels that serve as a counterweight to these oxidative radicals themselves deteriorate.

The aim of the study was to investigate the difference in levels of MDA within pregnant women compared to non-pregnant women.

Reduced AOA is a sign of an issue with the antioxidant system and may be caused by fewer individual antioxidants. In a normal pregnancy, we observe a drop in each person's antioxidant status. According to this hypothesis, the lower AOA found in our research is due to a fall in the number of specific antioxidants in pregnancy (**Bainbridge et al., 2005).**

The dynamic equilibrium between different antioxidants is what it is. Therefore, even though total antioxidant capacity might decrease while individual antioxidant levels increase during pregnancy **(Adiga, U et al., 2009).**

In our investigation, there was a statistically significant decrease in FRAP and a spike in malondialdehyde.

SUMMARY

AND

CONCLUSIONS

SUMMARY

MDA and FRAP levels in pregnant women individuals are intended to be evaluated in the present study.

The following summary was drawn**:**

1. FRAP levels significantly decreased while MDA levels were elevated.
2. Pregnant women have significantly higher MDA levels.
3. Pregnant women have significantly lower levels of the enzyme antioxidant FRAP than non-pregnant women.
4. MDA and FRAP have a positive correlation or MDA is higher and FRAP is lower in pregnant women, according to Karl Pearson's Coefficient of association.

Therefore, the finding that pregnant women have lower levels of FRAP and higher levels of Malondialdehyde support the idea that pregnancy causes oxidative stress.

Conclusion -

In conclusion, it is a proven fact that oxidative stress occurs in pregnancy, the results of which can lead to complications such as pre-eclampsia or worse miscarriage if left unchecked.

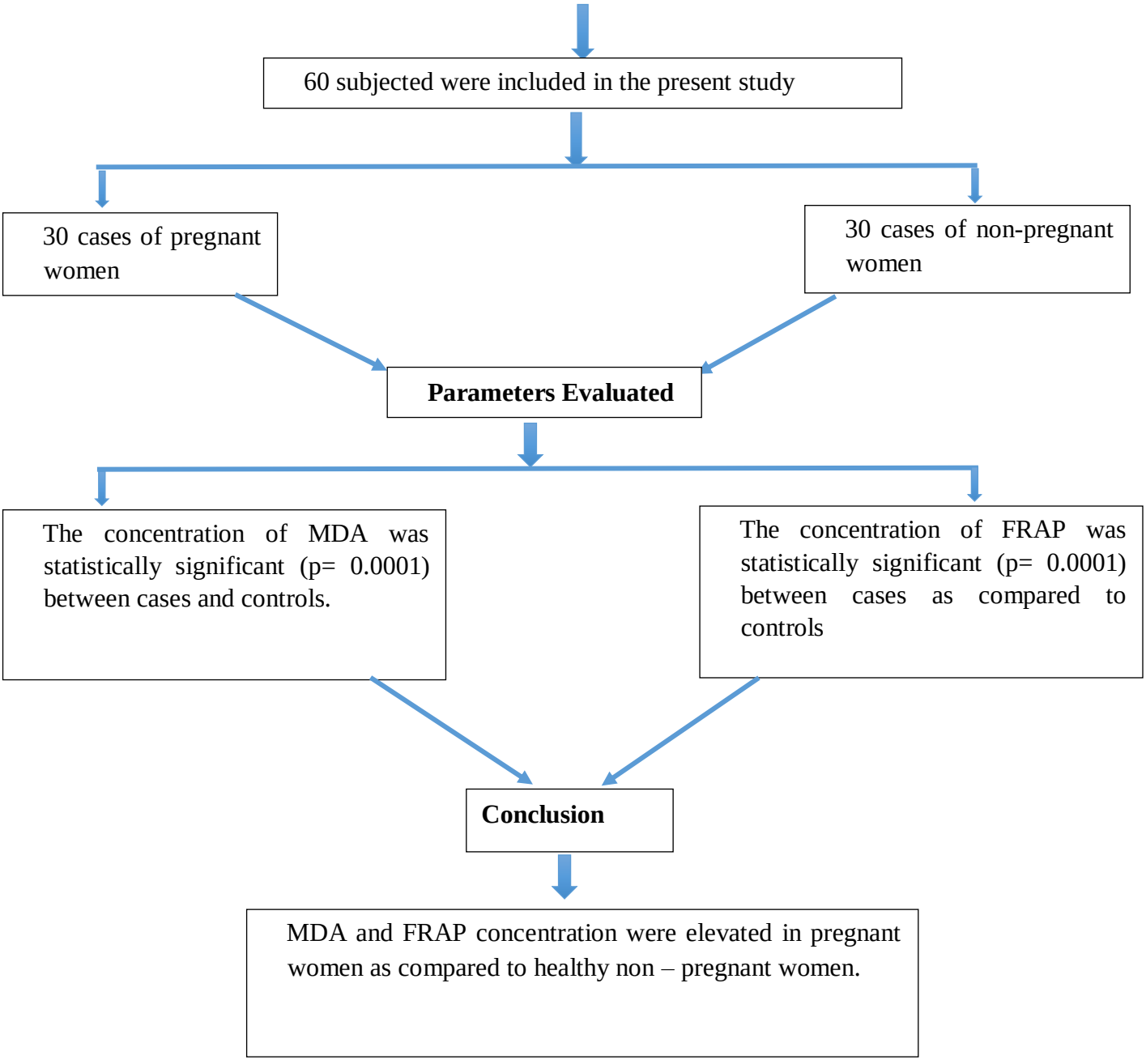
MDA is a preferred biomarker for oxidative stress. According to S.B. Patil. et al, (2007) with increasing stages of pregnancy, there is increased oxidative stress.

Though increase OS triggers a reduced antioxidant response, FRAP cannot be sufficiently used to diagnose total antioxidant capacity it should be accompanied by other well-established protocols such as SOD activity and glutathione reduction.

More research is needed to be conducted along these parameters for them to be more useful clinically in the diagnosis.

FLOW CHART OF RESEARCH WORK

In our study, we performed **a study of malondialdehyde and ferric reducing ability of plasma in pregnant and non- pregnant women**.



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ANNEXURES

ANNEXURE I (A)

INFORMED CONSENT FORM (FOR CASE)

1. I am Ankita Verma ,M.SC in Medical Biochemistry 3 year student at IIMS&R Lucknow.
2. For this study, I will take your 4 ml blood sample for the determination of MDA and FRAP.
3. The blood is only subjected to the determination of serum MDA and FRAP, not for any other purpose.
4. There will be no charges /fees/any consideration will be given or taken for the study.
5. Your identity will be confidential and information and the result of your blood test will not be revealed to any other except you if you desire.
6. This study has nothing to do with your treatment nor is it going to hamper the same if you refuse to participate.
7. The study has nothing to do with your current treatment but may improve the knowledge and understanding of the disease process and that knowledge may or not be helpful in the future.
8. After knowing all the above details, would you like to participate in our study? Yes/No

Name of the patient Signature of the M.SC student

Signature:

CONSENT FORM

I……..…age.....W/O, D/O, S/O.....R/O.....……. Here I state that I have been duly informed about the study titled "**A STUDY OF MALONDIALDEHYDE AND FERRIC REDUCING ABILITY OF PLASMA IN PREGNANT AND NON-PREGNANT WOMEN**", and its prospects and consequences. I hereby give informed and written consent for the collection of my blood sample for the above-said study only.

Signature/thumb impression of the patient:

Signature/thumb impression of the witness Signature of M.Sc student

सूचित सहमति प्रपत्र (FOR CASES)

1. मैं आईआईएमएसआर लखनऊ में मेडिकल बायोकैमिस्ट्री तृतीय वर्ष में अंकिता वर्मा एमएससी हूं।

2. मैं आपके इलाज करने वाले डॉक्टर पैनल से जुड़ा नहीं हूं।

3. आप किसी बीमारी से पीड़ित नहीं हैं और आपका ऐसा कोई इलाज नहीं चल रहा है।

4. इस अध्ययन के लिए, मैं प्लाज्मा की मैलोन्डियलडिहाइड और फेरिक-घटाने की क्षमता के निर्धारण के लिए आपका 4 मिलीलीटर रक्त का नमूना लूंगा।

5. रक्त केवल सीरम एमडीए और एफआरएपी के निर्धारण के अधीन है, अध्ययन के लिए नहीं।

6. अध्ययन के लिए कोई शुल्क/फीस नहीं दी जाएगी या कोई विचार नहीं किया जाएगा।

7. आपकी पहचान गोपनीय रहेगी और जानकारी तथा आपके रक्त परीक्षण का परिणाम गोपनीय रहेगा

यदि आप चाहें तो आपके अलावा किसी अन्य पर प्रकट नहीं होंगे

8. यदि आप भाग लेने से इनकार करते हैं तो अध्ययन में कोई बाधा नहीं आएगी।

9. अध्ययन आपके लिए फायदेमंद नहीं होगा लेकिन रोग प्रक्रिया के बारे में आपके ज्ञान और समझ में सुधार हो सकता है और वह ज्ञान भविष्य में सहायक हो भी सकता है और नहीं भी।

10. उपरोक्त सभी विवरण जानने के बाद, क्या आप हमारे अध्ययन में भाग लेना चाहेंगे? हां नहीं

सहमतिपत्र

मैं.....उम्र.....W/O, D/O, S/O..... .....आर/ओ.....यहां मैं बताता हूं कि मुझे "**ए स्टडी ऑफ़ मालोंडियालहाइड और फेरिक रिड्यूसिंग एबिलिटी ऑफ़ प्लाज्मा इन प्रेगनेंट एंड नॉन प्रेगनेंट वूमेन**" इसकी संभावनाएं और परिणाम। मैं इसके द्वारा केवल उपरोक्त अध्ययन के लिए अपने रक्त के नमूने के संग्रह के लिए सूचित और लिखित सहमति देता हूं।

रोगी के हस्ताक्षर/अंगूठे का निशान:

गवाह के हस्ताक्षर/अंगूठे का निशान एम.एससी छात्र के हस्ताक्षर

INFORMED CONSENT FORM (FOR CONTROL)

1. I am Ankita Verma M.SC in Medical Biochemistry 3rd year at IIMSR Lucknow.
2. I'm not associated with your treating doctor panel.
3. You are not suffering from any disease and you are not undergoing any such treatment.
4. For this study, I will take your 4 ml blood sample for the determination of the Malondialdehyde and Ferric- reducing ability of plasma.
5. The blood is only subjected to the determination of serum MDA and FRAP, not else for the study.
6. There will be no charges /fees/any consideration will be given or taken for the study.
7. Your identity will be confidential and information and the result of your blood test will not be revealed to any other except you if u desire
8. The study is not going to hamper you if you refuse to participate.
9. The study will not be beneficial for you but may improve your knowledge and understanding of the disease process and that knowledge may or may not be helpful in the future.
10. After knowing all the above details, would you like to participate in our study? Yes/No

Name of subject Signature of the M.sc student

Signature:

CONSENT FORM

I……..…age.....W/O, D/O, S/O.....R/O.....……. Here I state that I have been duly informed about the study titled "**A STUDY OF** MALONDIALDEHYDE AND FERRIC REDUCING ABILITY OF PLASMA IN **PREGNANT AND NON-PREGNANT WOMEN"**, and its prospects and consequences. I hereby give informed and written consent for the collection of my blood sample for the above said study only.

Signature/thumb impression of the patient:

Signature/thumb impression of the witness Signature of M.Sc student

सूचित सहमति प्रपत्र (CONTROLS)

1. मैं आईआईएमएसआर लखनऊ में मेडिकल बायोकैमिस्ट्री तृतीय वर्ष में अंकिता वर्मा एमएससी हूं।

2. मैं आपके इलाज करने वाले डॉक्टर पैनल से जुड़ा नहीं हूं।

3. आप किसी बीमारी से पीड़ित नहीं हैं और आपका ऐसा कोई इलाज नहीं चल रहा है।

4. इस अध्ययन के लिए, मैं प्लाज्मा की मैलोन्डियलडिहाइड और फेरिक-घटाने की क्षमता के निर्धारण के लिए आपका 4 मिलीलीटर रक्त का नमूना लूंगा।

5. रक्त केवल सीरम एमडीए और एफआरएपी के निर्धारण के अधीन है, अध्ययन के लिए नहीं।

6. अध्ययन के लिए कोई शुल्क/फीस नहीं दी जाएगी या कोई विचार नहीं किया जाएगा।

7. आपकी पहचान गोपनीय रहेगी और जानकारी तथा आपके रक्त परीक्षण का परिणाम गोपनीय रहेगा

यदि आप चाहें तो आपके अलावा किसी अन्य पर प्रकट नहीं होंगे

8. यदि आप भाग लेने से इनकार करते हैं तो अध्ययन में कोई बाधा नहीं आएगी।

9. अध्ययन आपके लिए फायदेमंद नहीं होगा लेकिन रोग प्रक्रिया के बारे में आपके ज्ञान और समझ में सुधार हो सकता है और वह ज्ञान भविष्य में सहायक हो भी सकता है और नहीं भी।

10. उपरोक्त सभी विवरण जानने के बाद, क्या आप हमारे अध्ययन में भाग लेना चाहेंगे? हां नहीं

सहमतिपत्र

मैं.....उम्र.....W/O, D/O, S/O..... .....आर/ओ.....यहां मैं बताता हूं कि मुझे "**ए स्टडी ऑफ़ मालोंडियालहाइड और फेरिक रिड्यूसिंग एबिलिटी ऑफ़ प्लाज्मा इन प्रेगनेंट एंड नॉन प्रेगनेंट वूमेन**" इसकी संभावनाएं और परिणाम। मैं इसके द्वारा केवल उपरोक्त अध्ययन के लिए अपने रक्त के नमूने के संग्रह के लिए सूचित और लिखित सहमति देता हूं।

रोगी के हस्ताक्षर/अंगूठे का निशान:

गवाह के हस्ताक्षर/अंगूठे का निशान

एम.एससी छात्र के हस्ताक्षर

ANNEXURE II (A)

INTEGRAL INSTITUTE OF MEDICAL SCIENCES AND RESEARCH, LUCKNOW

1. INCLUSION AND EXCLUSION CRITERIA

INCLUSION CRITERIA	YES	NO
1. Pregnant women	☐	☐
2. Age group between 18 – 40 years	☐	☐
3. Signed consent form	☐	☐

EXCLUSION CRITERIA

1. Anemia (Hb of 8.0 g/dl or less)	☐	☐
2. History of any Chronic disease	☐	☐
3. Smokers	☐	☐

Subject is eligible for the study if all **INCLUSION** criteria are **YES** and all **EXCLUSION** criteria are NO

INVESTIGATOR'S STATEMENT

I have verified the data entered in the case report form and have determined that it is complete, accurate, and compatible with the source documents.

Investigator’s name and signature Date

_____ _____ _____

ANNEXURE 11 (B)

WORKING PROFORMA

1. Registration No.:

2. Date

3. OPD ☐ **IPD** ☐

2. Contact No:

3. Name:

Age ☐ **Sex**: a) Male ☐ b) Female ☐

4. Husband's Name:

5. Place of Residence: a) Urban ☐ b) Rural ☐

6. Address:

1. Marital status: a) Unmarried b) Married c) Divorced d) Widow

8. Education_____

9. Occupation_____

10. Diet: a) Vegetarian ☐ b) non-Vegetarian ☐

11. Height:

12. Weight:

III.FAMILY HISTORY

1. MOTHER **2.FATHER**

a). Mother suffers from any chronic disease: b). Father suffers from any chronic disease

YES NO UNKNOWN **YES NO UNKNOWN**

3. No. of siblings ☐

IV. MEDICAL HISTORY

1. Duration of Pregnancy:- ☐

	YES	NO
2. Any chronic Disease:	☐	☐
3. Smoker/tobacco consumer:	☐	☐
4. Alcohol consumer:	☐	☐
5. Gravida:	☐	☐

INSTITUTIONAL ETHICS COMMITTEE (IEC)

IIMS&R INTEGRAL UNIVERSITY, LUCKNOW

IEC/IIMS&R/2023/64

CERTIFICATE

This is to certify that research work entitled "**A Study of Malondialdehyde and Ferric Reducing Ability of Plasma in Pregnant and Non-Pregnant women**" submitted by **Ankita Verma, Dr.Roshan Alam** for ethical approval before the Institutional Ethics Committee IIMS&R.

The above mentioned research work has been approved by Institutional Ethics Committee, IIMS&R with consensus in the meeting held on **30th December 2022**.

Dr.Q.S.Ahmed
(Member Secretary)
IRC/IEC
IIMS &R

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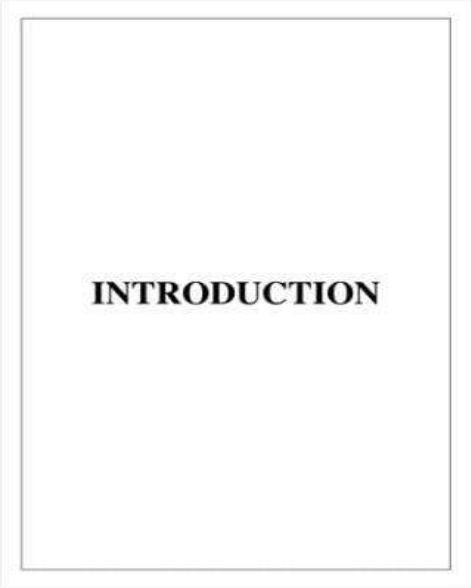

INTRODUCTION

ANKITA PLAG

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INTRODUCTION

Pregnancy is a physiological condition in women characterized by fetal growth. It occurs when a fertilized egg develops into a zygote and then a fetus within the womb within a period of about 9 months. Many metabolic and physiological systems are significantly altered during pregnancy, which is a stressful condition **(Scott et al., 1994).**

The physiological change of pregnancy is characterized by a sharp rise in the amount of energy and oxygen required to ensure proper fetal growth and development. Thus, oxidative stress is likely to occur throughout pregnancy for both the mother and fetus **(Mutainati et al., 2013).** Late in the pregnancy, increased lipid peroxidation and decreased antioxidant activity contribute to the development of pregnancy complications (**Rejitha J et al., 2014).**

As a result, during this time, extraordinary and dramatic changes happen to support the mother and encourage the development and upkeep of the fetus **(Qanungo, S. et al. 2000)**. Due to the high energy demand and increased tissue oxygen needed during a normal pregnancy, the level of oxidative stress has increased. All cells and tissues undergo lipid peroxidation, an oxidative process, at low levels **(Kagan V. E et al 1988).**

The condition of a disturbed balance between ROS and the mechanisms for detoxification and repair is what is known as oxidative stress. Each live cell produces ROS (reactive oxygen species) through the natural process of respiration, and a ROS substance is made up of a molecule of oxygen and an electron that is unpaired. **(Halliwell B et al., 1987).** ROS interacts with numerous important cytoplasmic chemicals and structures, changing how these organelles function biologically.

Excessive ROS and RNS synthesis result in "oxidative" or "nitrogenous" stresses, crucial in numerous pathological processes indicating neoplastic disorders,

and neurological conditions. **(Halliwell B et al., 1987).** The pro-neoplastic activity of ROS results mainly from DNA damage, proteins, and lipids, and altering these components may enhance the likelihood of mutation **(Wiseman H et al., 1995).** Factors that limit the effect or block the formation of free radicals also inhibit mutagenesis, the transformation phagocytic cells, and the degradation of DNA **(Halliwell B et al., 1987).**

Numerous types of DNA damage resulting from the activity of endogenous and external agents are promptly recognized. A sophisticated network of signal transduction pathways is later activated as a reaction to DNA damage **(Barzilai A et al., 2004).** The human ovum is relatively capable of healing oxidative damage to DNA **(Menezo Y et al., 2010).**

Unfortunately, however, data on the DNA quality in human oocytes are scant, largely presumably due to a shortage of testable materials. On the other hand, mature sperm typically aren't capable of repairing DNA damage **(Barratt C.L et al., 2010).** The ability of the developing sperm to repair any possible DNA damage diminishes over the course of spermatogenesis **(Olsen A.K et al., 2000).** The perfect replication of the genome is of utmost relevance in a single germinal cell **(Menezo et al., 2010).**

There are three ways to approach DNA repairs for both somatic and germinal cells. Activating apoptotic pathways is the initial step. In this situation, the ratio of pro to anti apoptotic factors in the ovum will determine the viability of the cells. The second option is damage tolerance, which might result in mutation in somatic and embryonic cells and, as a result, might lead to more birth defects and carcinogenesis in the following generation. Repairing the harm is the third choice **(Menezo et al., 2010).** Naturally, variations in the capacity for repair affect both the degree of oxidative DNA damage and an individual's vulnerability to disease **(Jaiswal M et al., 2000).**

An imbalance between the production of reactive free radicals, also known as ROS, and cellular antioxidant capability is known as oxidative stress (**Kumar Dabla, P. et al., 2015**). Free radicals such as superoxide (O_2) are examples of ROS, H_2O_2, OH', and O^{2-} are examples including non-radical intermediates. Nitric oxide (NO'), which has a modest level of reactivity, and its product ONOO', are examples of RNS. The above species originate from enzyme mediated processes such as the respiratory chain, phagocytosis, and prostaglandin formation, and are free of enzymes reactions (such as oxygen chemical reactions with organic molecules or exposure to ionizing radiations), which primarily occur in the mitochondria but also occur in peroxisomes and the endoplasmic reticulum (**A. Phaniendra et al., 2015).**

OXIDATIVE STRESS IN PLACENTATION

For the placenta to develop and mature, the invasion, differentiation, and growth of the trophoblast in the maternal deciduous cells must be synchronized **(B. Huppertz et al., 2009).**

During pregnancy the trophoblast cells experience very low O2 concentration as a result of reduced O2 pressure occurring within the placenta. Because it promotes the cellular response to hypoxia this is physically low oxygen status is a crucial regulator of placental function **(Davies et al., 2015).**

The oxygen consumption of the mitochondria is downregulated in low-oxygen environments (G.J. Burton et.al 2017). While the endometrial glands' glucose consumption is sufficient to maintain the embryo and placenta access to a steady supply of ATP (**A.I. Frolova et al., 2011**). As a result, throughout the first trimester, the

conceptus' metabolic needs are still sufficiently supplied in an environment with 2.5% oxygen **(A.I. Frolova et al., 2011).**

REVIEW OF LITERATURE

PREGNANCY

Pregnancy is a normal physiological phenomenon accompanied by a dynamic change in women's bodies that causes their susceptibility to oxidative stress. Oxidative stress is a condition that results from an imbalance between pre-oxidant and antioxidant defense systems **(Shah et al., 2004)**

The enormous increase in energy and oxygen necessary for the fetus's appropriate development and expansion makes pregnancy a stressful state **(Mutinati M, et al., 2013).** Pregnancy is a physiological development during which both the mother and the fetus suffer oxidative stress. Poor pregnancy outcomes, including fetal development restriction, can be caused by deficiencies of certain antioxidant activities linked to the micronutrients selenium, copper, zinc, and manganese **(Giles GI et al., 2002** Lipid peroxidation and reduced antioxidant activity, result from energy equilibrium and possibly aid in the emergence of problems **(C.H. Fall et al., 2003).**

EPIDEMIOLOGY

Livebirth declined from 90.2% to 88.9%. Half of the Indian states/UTs reported lower live birth rates for 2019–21 than the national average (88.9%). In Telangana, the rate of abortions among adolescent women increased by 11.0% between 2019 and 21 and 0.7% between 2015 and 16 **(Kuppusamy et al., 2021).**

Antenatal Period

A) Embryonic period

The term "*embryo*" refers to the developing organism from fertilization until the end of eight weeks.

(B) Fetal phase- .The fetal period lasts from the first day of the third month's ninth week till delivery. Germinal phase, embryonic, and fetal phase are the three divisions of the prenatal period according to embryology **(Vishram S., 2012).**

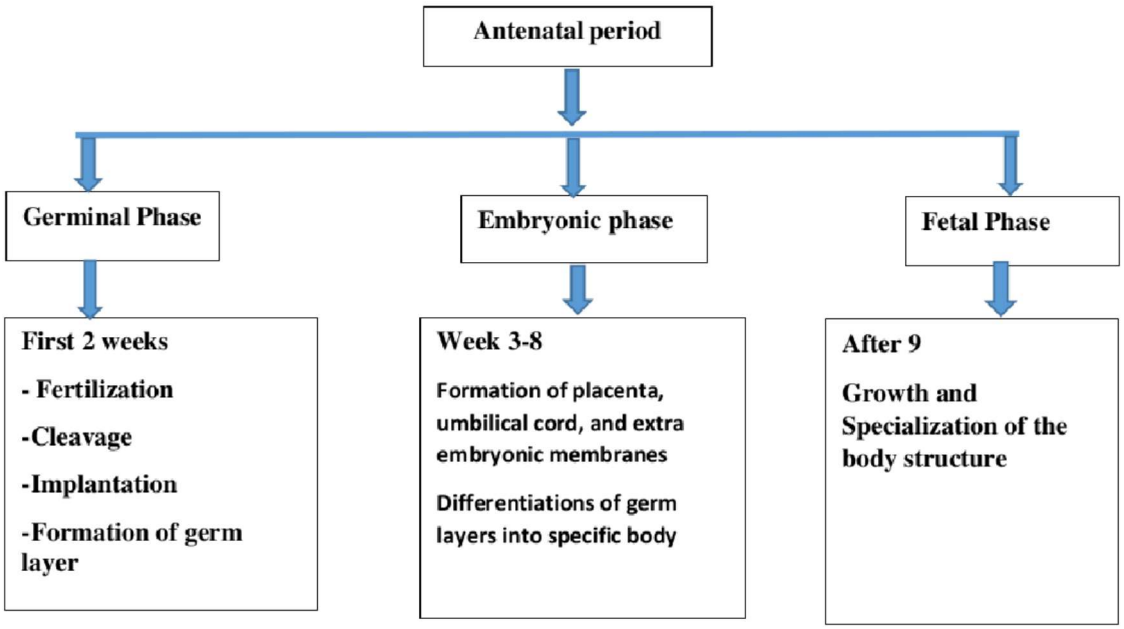

Fig: 1 Antenatal development periods **(Vishram S et al., 2012).**

PREGNANCY TERMS

1- First trimester

Minute breathing is increased by 40% during the first trimester **(Campbell LA, et al., 2001).** In eight weeks, the womb will reach the size of a lemon. During this trimester, several indicators of pregnancy and issues, including nausea and the development of breasts, also occur.

2- Second Trimester

A woman's second trimester of pregnancy lasts for 13 to 28 weeks. Compared to the first trimester, this is the period when women experience more energy. Additionally, the muscular organ that houses the growing fetus grows as much as 20 times its typical size throughout pregnancy. The growing uterus has produced a noticeable "baby bump" at the conclusion of this trimester **(Stacey T, et al., 2011).**

3-Third Trimester

The last weight gain, which is the largest growth throughout the pregnancy, occurs. The uterus grows and takes up a growing percentage of the female abdomen. As the fetus turns lower in preparation for birth, the woman's abdomen will change shape and drop. Fetal development can be disruptive to women and rather robust. In spite of the reduced vena cava pressure caused by the larger uterus, found that the infants benefit from greater oxygenation in the lateral position **(Stacey T, et al., 2011).**

Oxidative Stress

The alteration in the prooxidant and antioxidant balance leads to some potential damage defined as oxidative stress **(Lobo et al., 2010).** Oxidative stress is a state that can result in harm to tissues because of excess free radical **(Agrawal A, et al., 2005).**

Increased levels of oxidative stress during pregnancy can result in pre-eclampsia, gestational diabetes, and fetal hypoxia. Many tissues have a supraphysiological rise in oxidative stress in pregnancy. It is especially concentrated in the human placenta, which causes vascular dysfunction and impairs the supply of nutrients to the developing fetus, leading to changes in fetal size for the duration of pregnancy, fetal growth retardation, macrosomia, or premature delivery **(Marcoleta et al., 8 330024).**

Free radical generation is a physiologically normal process, but excessive production causes lipid peroxidation and encourages maternal vascular dysfunction because reactive oxygen species (ROS) like superoxide encourage endothelial activation. Antioxidant substances in the human body scavenge those free radicals, reducing oxidative damage (**Tiwari AKM, et al., 2010).**

Free radicals cause lipids to undergo oxidative alteration, which starts a process known as lipid peroxidation (LPO), which leads to the loss of lipid-rich regions like cell membranes or myelin sheaths and the production of highly reactive aldehydes like malondialdehyde(MDA) **(Ortiz, et al., 2013).**

The result of oxidizing polyunsaturated fatty acids (PUFA) is MDA. As a result, it acts as an accurate oxidant marker of the peroxidation of lipids caused by oxidative stress (**Del Rio, et al., 2005**) .

ROS, RNS, and RSS are families of molecules that these free radicals belong to **(Giles GI et al., 2002).** The human body naturally produces free radicals as a consequence of metabolism and oxidation **(Lobo V et al., 2010).**

Mechanism

ROS are byproducts of the metabolism of oxidation that are crucial to cellular function. They are linked to other pathological disorders as well. UV radiation, alcohol, ischemia-reperfusion injury, persistent infections, and inflammatory disorders are the causes of excessive ROS generation (A Bhattacharyya et al., 2014). When there is a larger concentration of hydrogen peroxide (H_2O_2) in the cell, catalase removes it from the **body (L. W. Obereley et al., 2005)**. SOD enzyme is involved in the break down superoxide anion into less toxic products **(E.P. Harris et al., 1992).** The body's tissues

include glutathione reductase, which functions similarly to GPx. Glutathione reductase enzymes employs NADPH in its antioxidants responsibilities **(J Fuji et al., 2011).**

a) Scenarios of Oxidative Stress in Pregnancy:-

The fetus needs enough nutrition and oxygen during a typical pregnancy to support its growing tissues and organs. These processes produce ROS that have an impact on the development of fetal growth. The equilibrium between ROS and antioxidants could be preserved to offer a healthy environment for the mother's body and the developing fetus **(J. Fuji et al., 2011).**

The body goes through a lot of physiological changes while pregnant. The researchers made the assumption that there was proof of ROS generation due to accelerated metabolism, high oxygen consumption, and utilization of fatty acids. Increased generation of hydrogen peroxide is caused by increased metabolic syndrome during the third trimester of pregnancy (**Duhig et al., 2016).**

b) Within the Placenta, Uterus, and Ovary

ROS has an effect on pregnancy in almost every stage. The key modulator of ovarian cell activity is recognized to be ROS. The advantages of ROS have already been addressed.

Leukocytes, macrophages, and cytokines are the generators of ROS in the follicles. ROS concentration is an indicator of ovulation. There is evidence that ROS suppressors interrupt the ovulatory phase (**Kshkolnik et al., 2011).**

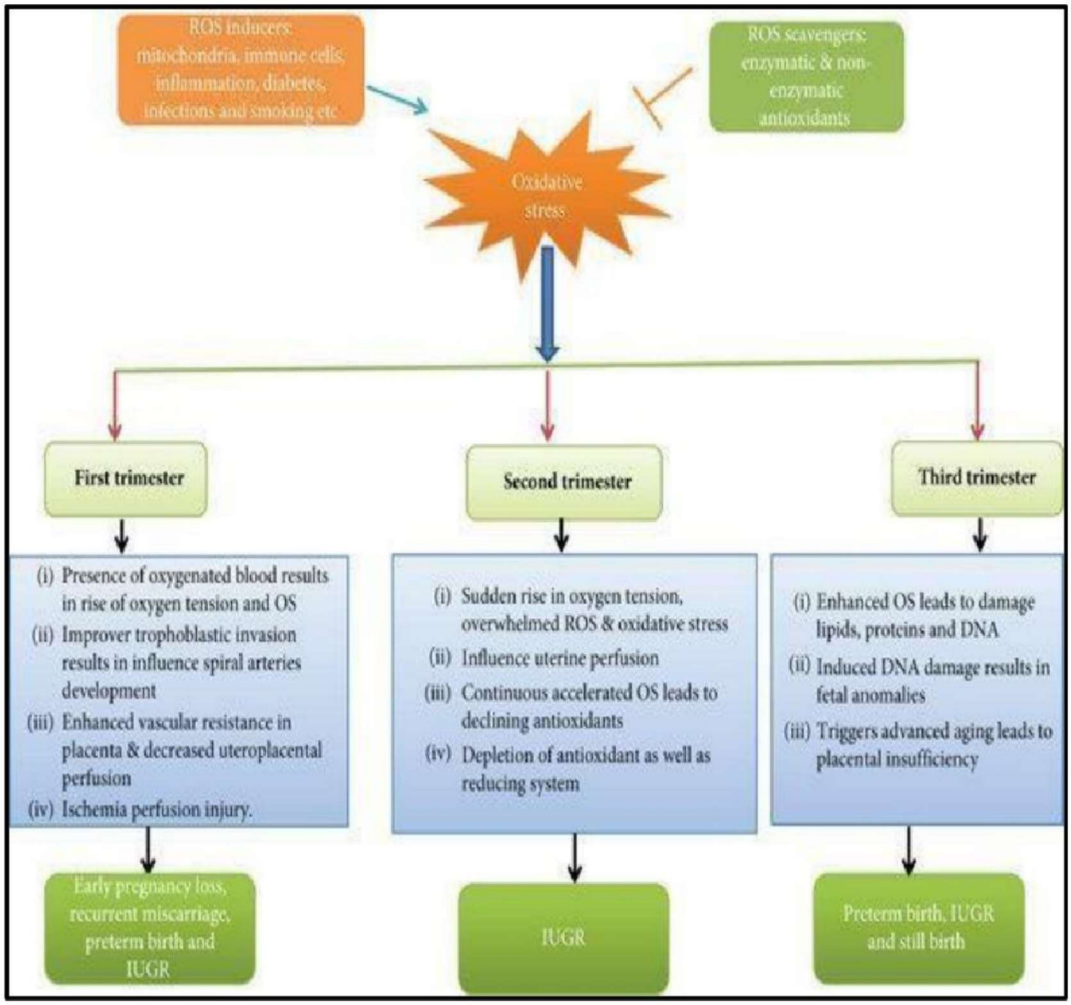

Fig: 2. Impact of Oxidative Stress on Pregnancy (Hussain et al., *2021*)

ANTIOXIDANT -

The body has developed several strategies to combat the damaging effects inside the cell, including damage prevention, oxidative damage repair, physical defense against damage, and—perhaps most importantly—antioxidant defense mechanisms **(Pereira, R. D et al., 2015).**

Antioxidants are the first line of defense against stress, according to the free radical oxidative stress theory. The network of compartmentalized antioxidant enzyme and non-enzyme-containing molecules that make up endogenous antioxidant defences is typically dispersed throughout the cell. Antioxidant enzymes are involved in a complex series of reactions that reduced the toxicity of ROS into more stable forms **(Hussain, et al., 2021).**

The primary antioxidant enzyme receive support from secondary enzymes in collaboration with small molecular weight antioxidant that serve as cofactors in the reactions. ROS scavenger such as non-enzymatic antioxidant are essential. Effective free radical elimination require the combined action enzymatic and non- enzymatic antioxidants. **(Ďuračková, Z et al., 2010).**

It is known that even a straightforward pregnancy generates free radicals **(Casanueva E et al., 2003)** leading to oxidative stress. Due to these free radicals, pregnant women are more susceptible to infections, which in turn combine with oxidative stress to result in a plethora of perinatal, maternal, and even congenital diseases **(Klufio CA et al., 1992)** and abnormalities **(Palan PR et al .,2004).**

Needless to say, low antioxidant levels has been known to a variety of unfavourable pregnancy outcomes, including sluggish or defective fetal and childhood development **(Evans P et al., 2001).** Programs to lower free radical generation and raise antioxidant levels in pregnant women should therefore be part of prenatal care.

Benzie and Strain created the FRAP assay in order to gauge plasma's lowering power back in 1996. As a novel technique for determining the "antioxidant power," the FRAP assay, a straightforward, automated test that measures the ferric-reducing capacity of plasma, is described. A colourful ferrous - tripyridyltriazine complex is produced when ferric-to-ferrous ion reduction occurs at low pH. The conversion of

ferric tripyridyltriazine to ferrous tripyridyltriazine serves as a proxy for the total antioxidant capability (Benzie et al., 2003).

In the last decades, lots of work had been done on OS and antioxidants in pregnant women. From the above findings, the present study sought to clarify the roles of oxidative stress and antioxidant profile during progression of pregnancy.

AIM AND OBJECTIVES

Aim

The aim of this study was to determine the levels of MDA and FRAP in pregnant and non-pregnant women.

OBJECTIVES

1. To determine the level of MDA in pregnant and non-pregnant women.
2. To determine the FRAP in pregnant and non-pregnant women.
3. To determine correlation between MDA and FRAP in pregnant and non-pregnant women, if any.

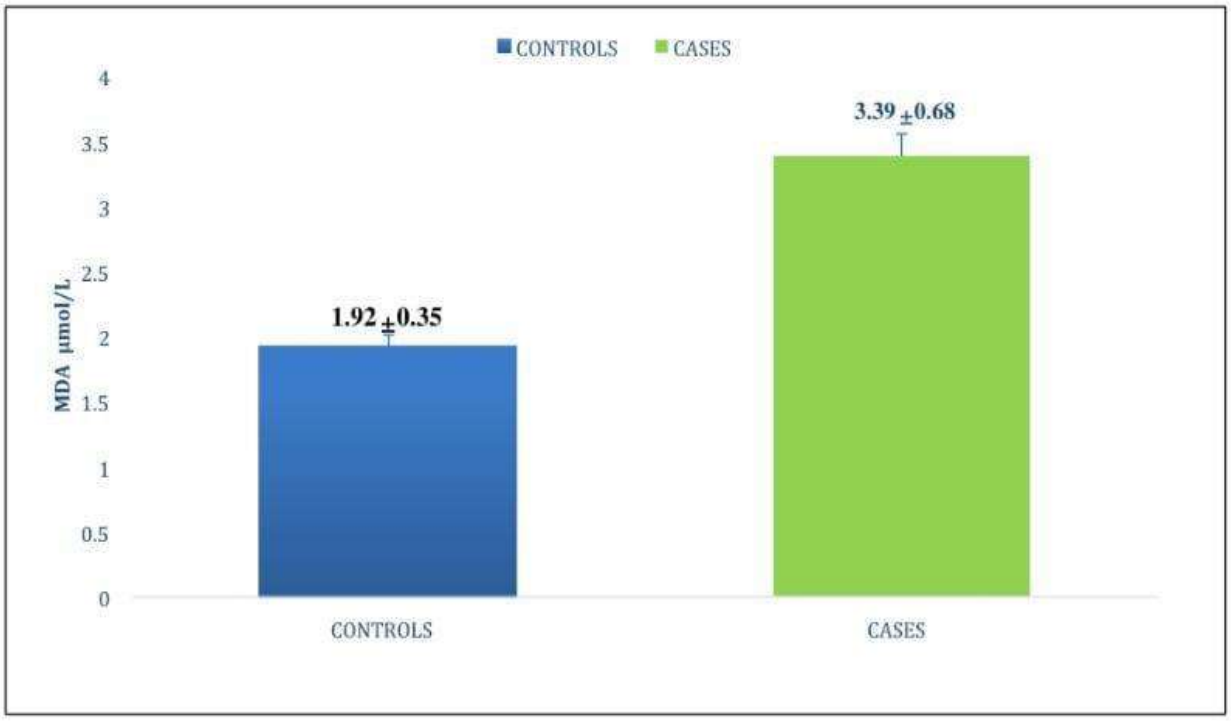

FIG: 3 Comparison of malondialdehyde in between controls and cases

MALONDIALDEHYDE

The difference in the level of between cases and controls was statistically significant (p value= 0.0001) (Table-1 & figure-3)

Table-1 Mean and standard deviation of the study groups

MALONDIALDEHYDE(µmol/L)					
Groups	**n**	**Mean**	**Standard Deviation**	**p-value**	**Significance**
Controls	**30**	**1.92**	**± 0.35**	**0.0001**	**Statistically Significant**
Case	**30**	**3.39**	**± 0.68**		

p-value <0.05 is considered statistically significant

FERRIC REDUCING ABILITY OF PLASMA

The difference in the level of FRAP between cases and controls was statistically significant

(p-value= 0.0001) (Table-2 & figure-4)

Table-2 Mean and standard deviation of the study groups.

FERRIC REDUCING ABILITY OF PLASMA (µmol/L)					
Groups	n	Mean	Standard Deviation	p-value	Significance
Controls	30	1276.33	± 88.7	0.0001	Statistically Significant
Cases	30	1074.33	± 53.46		

p-value <0.05 is considered statistically significant

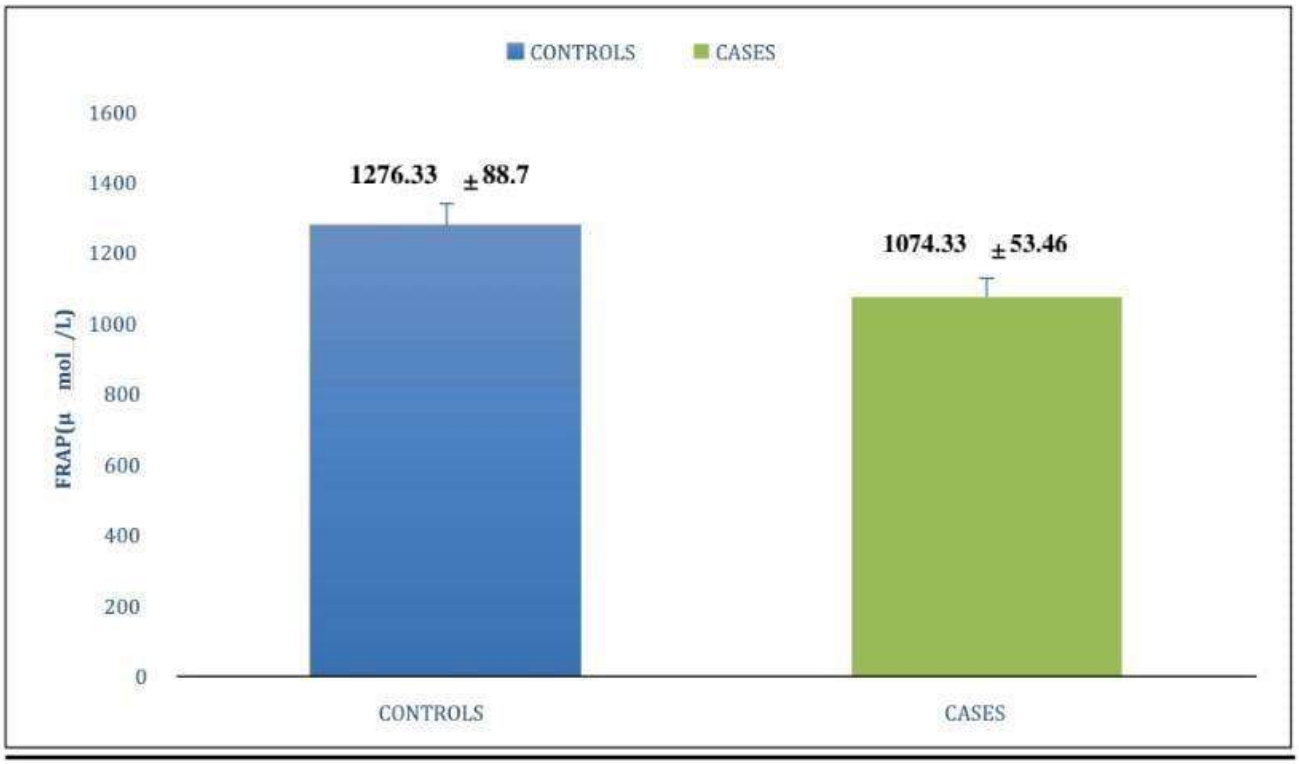

Fig: 4 Comparison of FRAP in between controls and cases

KARL PEARSON'S CORRELATION COEFFICIENT BETWEEN THE STUDY PARAMETERS IN CASE

Table-3 Karl Pearson's correlation coefficient between MDA and FRAP

		MDA(µmol/L)	FRAP (µmol/L)
MDA(µmol/L)	Pearson Correlation	1	.423*
	Sig. (2-tailed)		.020
	n	30	30
FRAP (µmol/L)	Pearson Correlation	.423*	1
	Sig. (2-tailed)	.020	
	n	30	30

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

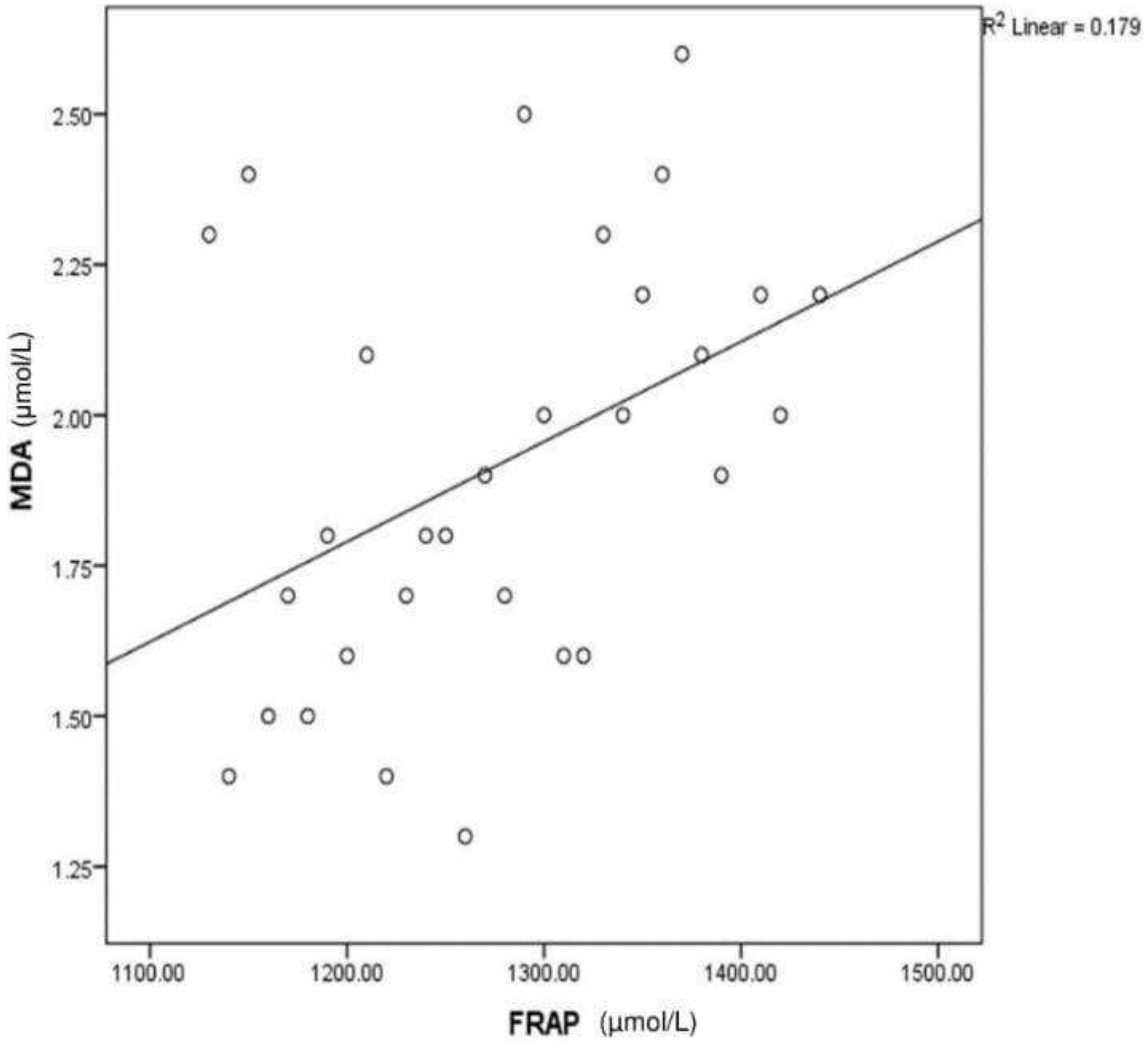

Fig: 5 Scatter diagram showing a correlation between MDA and FRAP

RESULT

- A significant increase ($p<0.0001$) was found in the levels of Malondialdehyde in Pregnant women when compared to Non-Pregnant women. (Table :1)
- A significant decrease ($p<0.0001$)was found in the levels of FRAP in Pregnant women when compared to non-pregnant women (Table: 2)
- The level of MDA was observed to be increased between cases 3.39±0.68 (µmol/L), compared with controls 1.92±0.35 (µmol/L).
- The level of FRAP was found to be lower among cases 1074.33±53.46 (µmol/L) compared with controls 1276.33±88.7 (µmol/L).
- The Data shows a positive correlation between MDA and FRAP in cases.
- The results obtained show no significance between MDA and FRAP in pregnant women.

DISCUSSION

Due to the elevated metabolic load and increased tissue oxygen requirements, oxidative stress increases throughout a typical pregnancy. MDA is a suitable marker for the evaluation of free radical-induced harm to tissues since it is a stable by-product of free radicals created by lipid peroxidation in the body **(Sachdev et al., 2008).**

In the investigation, pregnant women and non-pregnant women had their marker values compared to MDA and FRAP. According to this study, the average plasma level of MDA in pregnant women is 3.39±0.68 (μmol/L), while in non-pregnant women, the value is 1.92±0.35 (μmol/L), value and FRAP 1074.3±53.46 (μmol/L),in 1276.3±88.7(μmol/L), in pregnant women and non-pregnant women respectively.

Because they are fragile and fleeting, reactive oxygen species can be hard to directly quantify. It has been utilized for indirect measurement of their ability to trigger lipid peroxidation. The development of a typical pregnancy has been accompanied by a rise in lipid peroxidation markers (MDA) **(Wickens D et al., 1981).**

Chamy et al., 2006 deduced that healthy pregnant women have greater lipid peroxidation levels than normal pregnant women. In response, the body tips antioxidant defence system to restore haemostatics balance .Consequently, oxidative equilibrium might endure the entire pregnancy.

According **to Walsh S. W et al,** the maternal antioxidant system regulates placental lipid formation during a healthy pregnancy. ROS serve as signal transducers in physiology, but their overproduction can lead to a variety of health issues in people. While the body's own mechanisms play a critical part in regulating the amounts of these free radicals, the antioxidant levels that serve as a counterweight to these oxidative radicals themselves deteriorate. The aim of the study was to investigate the difference in levels of MDA within pregnant women compared to non-pregnant women.

Reduced AOA is a sign of an issue with the antioxidant system and may be caused by fewer individual antioxidants. In a normal pregnancy, we observe a drop in each person's antioxidant status. According to this hypothesis, the lower AOA found in our research is due to a fall in the number of specific antioxidants in pregnancy **(Bainbridge et al., 2005).**

The dynamic equilibrium between different antioxidants is what it is. Therefore, even though total antioxidant capacity might decrease while individual antioxidant levels increase during pregnancy **(Adiga, U et al., 2009).**

In our investigation, there was a statistically significant decrease in FRAP and a spike in malondialdehyde.

SUMMARY AND CONCLUSIONS

MDA and FRAP levels in pregnant women individuals are intended to be evaluated in the present study.
The following summary was drawn:

- FRAP levels significantly decreased while MDA levels were elevated.
- Pregnant women have significantly higher MDA levels.
- Pregnant women have significantly lower levels of the enzyme antioxidant FRAP than non-pregnant women.
- MDA and FRAP have a positive correlation or MDA is higher and FRAP is lower in pregnant women, according to Karl Pearson's Coefficient of association.
- Therefore, the finding that pregnant women have lower levels of FRAP and higher levels of Malondialdehyde supports the idea that pregnancy causes oxidative stress.

Conclusion -

- In conclusion, it is a proven fact that oxidative stress occurs in pregnancy, the results of which can lead to complications such as pre-eclampsia or worse miscarriage if left unchecked.
- MDA is a preferred biomarker for oxidative stress. According to S.B. Patil. et al, (2007) with increasing stages of pregnancy, there is increased oxidative stress.
- Though increase OS triggers a reduced antioxidant response, FRAP cannot be sufficiently used to diagnose total antioxidant capacity it should be accompanied by other well-established protocols such as SOD activity and glutathione reduction.

- More research is needed to be conducted along these parameters for them to be more useful clinically in the diagnosis.

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