COMPARATIVE STUDY OF VITAMIN D LEVELS IN TERM HYPERTENSIVE AND NORMOTENSIVE PREGNANT WOMEN.

By

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ABSTRACT

BACKGROUND Hypertensive disorders of pregnancy comprise of preeclampsia, eclampsia and gestational hypertension, chronic hypertension both essential and secondary and preeclampsia superimposed on chronic hypertension. Vitamin D plays a pivotal role in implantation and placental function due to its diverse action in angiogenic, immunomodulatory and anti-inflammatory effect:

OBJECTIVE:

To compare the maternal vitamin D and calcium levels of term normotensive and hypertensive pregnant women.

MATERIALS AND METHODS : A hospital based cross sectional prospective comparative study was conducted to compare maternal Vitamin D and calcium levels of 120 term hypertensive and normotensive pregnant women. The Study Group consisted of 60 term hypertensive pregnant women in labour defined as BP>140/90 mmHg and the Control Group comprised of 60 term normotensive pregnant women in labour defined as BP<140/90 mmHg.

Discussion : In this study, 12 (20%) patients in hypertensive Group had very severe deficiency vitamin D (<5 ng/ml) as compared to 4 (6.7%) patients in normotensive Group while 34 (56.7%) patients in Study Group had severe deficiency of vitamin D as compared to 18 (30%) patients in Control. 35 (58.3%) patients in Study Group had calcium deficiency (<8.5 mg/dl) while 25 (41.7%) patients had normal calcium levels. 18 (30%) patients in Control Group had calcium deficiency (<8.5 mg/dl) while 42 (70%) patients had normal calcium levels. The mean serum calcium levels was significantly lower in Study Group as compared to Control Group (8.05 ± 1.44 vs. 9.66 ± 1.70 mg/dl) ($p<0.05$).

CONCULSION : From the present study, it was concluded that Vitamin D and calcium levels were lowered in patients of pregnancy induced hypertension as compared to normotensive pregnant women. Predictive and prognostic value of these parameters may be helpful in the early diagnosis of PIH may contribute to alleviate maternal morbidity and preterm birth outcomes.

Key Words : hypertension, Vitamin D, calcium

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LIST OF ABBREVATIONS

PIH	: Pregnancy induced hypertension
HELLP	: Haemolysis elevated liver enzymes low platelet count
NICU	: Neonatal intensive care unit
HDP	: Hypertensive disorders in pregnancy
BP	: Blood pressure
SBP	: Systolic blood pressure
DBP	: Diastolic blood pressure
PE	: Preeclampsia
RAAS	: Renin angiotensin aldosterone system
BMI	: Body mass index
SGA	: Small for gestational age
IUFD	: Intrauterine fetal death
VGEF	: Vascular endothelial growth factor
PTH	: Parathyroid hormone
UV	: Ultraviolet radiation
HTN	: Hypertension
GDM	: Gestational Diabetes Mellitus
IL	: Interlukein
RCT	: Randomised Control trial
TNF	: Tumor necrosis factor
SSC	: Secondary school certificate

HSC : Higher Secondary School Certificate

LSCS : Lower segment ceaserean section

FTVD : Full term vaginal delivery

SGA : Small for gestational age

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INTRODUCTION

Hypertensive disorders during pregnancy remain the most common medical complications, leading to a majority of adverse perinatal and maternal outcome; despite the numerous efforts have been made at early diagnosis, prevention and treatment.

The incidence of various hypertensive disorders of pregnancy varies widely from 5 to 15%. PIH is a pregnancy specific syndrome that can virtually affect every organ system. It is defined as rise of blood pressure ≥ 140/90 mmHg after 20 wks of gestation in women with previously normal blood pressure with or without proteinuria. Proteinuria is the excretion of ≥ 300 mg/24 hrs or ≥ +1 on dipstick. It is a challenge to be addressed and overcome if there is to be any significant improvement in maternal and perinatal health.

Although the cause of PIH still remains unknown, its manifestation begins early in pregnancy. Many pathophysiological changes takes place that gain momentum across gestation and gradually become clinically apparent and ultimately lead to multi-system involvement with a clinical spectrum which varies from barely noticeable to one of devastating deterioration.

Maternal complications like antepartum haemorrhage, eclampsia, HELLP syndrome, disseminated intravascular coagulopathy, acute renal failure, intracerebral haemorrhage and even maternal death can occur. Long term complications like persistent hypertension and cardiovascular morbidity are also not rare.

Fetal complications like intra - uterine growth restriction, preterm delivery, sudden intra - uterine fetal death, still births, preterm and low birth weight babies, increased need for NICU care, increased neonatal morbidity and mortality are prevalent.

Thus early detection and appropriate management of the pregnancy induced hypertension may improve the outcome for both the mother and the baby.

Fetal wellbeing is monitored with simple methods like daily fetal kick count, non stress test and fetal biophysical profile which are an essential part of the management of pregnancy induced hypertension.

Occurrence of hypertension in pregnancy is a challenge to the obstetrician because despite best available treatment, maternal and fetal morbidity and mortality is still high.

Hypertension complicating 5% to 8% of all pregnancies is a major leading cause of fetal and maternal morbidity for which the pathogenesis remains unclear. Essential hypertension, which is associated with the insulin resistance, may play a vital role in hypertension in pregnancy.[1] Insulin resistance is a pathological situation characterized by lack of the physiological response of peripheral tissue to insulin action leading to metabolic and hemodynamic changes like glucose intolerance, diabetes mellitus, hypertension, hyperinsulinemia, hyperlipidemia etc.[2]

Pre-eclampsia is the most common form of high blood pressure and is characterized by the occurrence of new-onset hypertension plus new-onset proteinuria and is found to be linked with higher degrees of insulin resistance than characteristic of normal pregnancy. The usual onset of pre-eclampia and gestational hypertension is seen in late pregnancy, a time when the insulinresistance of pregnancy is maximal supporting a plausible association.[1]

There are various proposedmechanisms, by which insulin resistance might increase blood pressure in pregnancy .Itincludes activation of sympathetic nervous system, retention of renal sodium, increased cation transport, and associated endothelial dysfunction. [1]

Metabolic abnormalities are also observed in pregnant women which is associated with insulin resistance to a greater extent than in normotensive pregnant women. Some of these includes glucose intolerance, hyperlipidemia, hyperinsulinemia and increased alfa- tumor necrosis factor, fasting insulin, HOMA-IR and lower levels of sex hormone binding globulin.[1, 2, 3] These findings were suggestive of the fact that insulin resistance may be associated in the pathological processof hypertensionin pregnancy and approaches that upgrade insulin sensitivity require further studies that might have benefit in the prevention or treatment of this syndrome.[1]

Hypertensive disorders in pregnancy (HDP) arethe most common complication encountered during pregnancy. From various epidemiological studies, the incidence was reported to be different in various populations which is influenced by nulliparity, age, and race and is largely affected by the definition applied. In 2006, the incidence of HDP was reportedto be 5.38%, among Indians while preeclampsia to be 44%, eclampsia for 40% followed by7% of HELLP (hemolysis, elevated liver enzymes, and low platelet count) syndrome respectively.[4]It was also noted that maternal and perinatal deaths accounted for 5.5% and 37.5% of deliveries, respectively.[4] HDP comprises of preeclampsia and eclampsia. The former is a multisystem disorder of unknown etiology that affects approximately 4%–5% of pregnancies[5, 6]whereas the latter is the development of seizures in women with severe preeclampsia. The incidence of eclampsia is estimated to be 0.3%–0.9% with a maternal mortality rate of 0.5%–10.0%.[7]

The exact mechanism of HDP is not completely clear. It is a complex multifactorial disease and the central pathogenesis seems to involve the systemic activation leading to endothelial dysfunction. This disease is exhibited as raised blood pressure (BP),

proteinuria, systemic inflammatory response, and deposition of antiangiogenic factors, which is supposed to produce the disease by depriving the glomerular endothelial cells of essential growth factors. The termination in pregnancy alters the clinical manifestations of the disease, suggesting that trophoblastic invasion has a major role in the occurence of preeclampsia.[8] Matthy's et al in a study reported that preeclampsia is the result of excessive placental secretion of soluble fms-like tyrosine kinase-which may contribute to endothelial dysfunction, hypertension, and proteinuria.[5] From a multicenter study, it was observed that due to chronic hypertension approximately 30% of HDP cases were involved followed by 70% of caseswhich were noted to be due to gestational hypertension/preeclampsia.[9]

The metabolism of maternal vitamin D is changed in pregnancy, leading to raised circulating levelsof both the vitamin D binding protein (VDBP)[10]and the active metabolite, 1,25-dihydroxyvitaminD (1,25(OH)2D). At term, expectant mothers have around twice the concentration of 1, 25(OH) 2D in comparison with non-pregnant women, of which about 50% is supposed to be promoted by theplacenta and/or decidual tissue. The exact function of this rise in 1, 25(OH) 2D has notbeen fully understood, but it has been proposed from current concepts that the surge in 1, 25(OH) 2D is a physiologicalresponse to permit immune tolerance through pathways of vitamin D at the maternal-foetal interface, thereby reinforcing proper placentation.[11]

The deficiency of Vitamin D has been reportedworldwide among gravidae population, and thecapacity of vitamin D to prevent pregnancy-related complications is a current issue which needs focus, however,conclusive verification from randomised trials to support a role for vitamin D in perinatal healthis still not confirmed.[12]

Women suffering from PE have been shown to experience variations in the metabolism of calcium andvitamin D. In contrast with the normal placenta, mRNA expression for the vitaminD-metabolising enzymes CYP2R1, CYP27B1, CYP24A1, and the vitamin D receptor (VDR) havebeen increased and decreased in placentas of women with PE, giving direct evidencefor damaged vitamin D metabolic homeostasis in the preeclamptic placenta.

The possible mechanism for this disruption and its association with PE development is not fully understood. It is hypothesized that low-circulating 1, 25(OH) 2D leads to an imbalance in immune function, resulting in ashift towards a pro-inflammatory environment and distorted implantation.

Increased tumour necrosis factor (TNF)-α stimulates catabolism of 1, 25(OH) 2D, leading to decreased circulating calcium levels which are seen in PE-diagnosed pregnant women. To elucidate that malplacentation results mainly from the pro-inflammatory environment induced by Th1 cytokine activity would be considered an inadequateinterpretation, however, it is likely that vitamin D may lead to various innate and adaptive immune responses in placental and decidual tissues. Despite the recent advancements in vitro studies of vitamin D and PIH,it is still difficult to explain at a clinical level,owing to the uncertainty of the mechanism of PE, alongside the different roles of vitamin D inimmune function.[14]

Maternal vitamin D deficiency in pregnancy has been associated not only with an increased risk of pre-eclampsia but also with an increased maternal and perinatal morbidity and mortality across the globe.[15] In addition, Two meta-analysis done found significantly higher risks pre-eclampsia in women with vitamin D deficiency and early pregnancy has also been associated with elevated risk for gestational diabetes mellitus along with caesarean section.[16]

Few health organizations have recommended that vitamin D supplementation during pregnancy, should range from from 200 to 400 IU/d (5 to 10 μg/d)[17]. These doses may not lead to optimal serum 25(OH) D levels during pregnancy. However, there is debate regarding the 25(OH) D levels that are considered adequate or optimal for overall health and during pregnancy. The Institute of Medicine in United States has determined that levels > 50 nmol/L or 20 ng/mL are adequate, although many researchers consider that optimal levels should be higher (greater than 75 nmol/L or 30 ng/mL). It has been recommended that regular supplemental dose of vitamin D of 1000–1600 IU (25–40 μg/d) might be required to produce and maintain what many considered to be optimal levels in the body.[18]

It has been suggested that preeclampsia and eclampsiaresults from breakdown of tolerance to the developingfetus after maternal immune maladaptation.[19] Vitamin D is a seco-steroid prohormone which has direct effect on molecular pathways such as trophoblastic invasion and immunomodulation.

During pregnancy, Vitamin D may play a role inimplantation and placental function due to angiogenicand anti-inflammatory effects. It is important in directingimmune responses at the fetal-placental interface as wellas immunological adaptation to reduce the risk of inflammation and infection.

Preeclampsia and eclampsia of varying degrees ofseverity form a considerable portion of admission inhospitals. Despite the considerable morbidity and mortality, the cause of preeclampsia and eclampsia has remained enigmatic.

Hence the present study was done at our tertiary care centre to compare maternal vitamin D and calcium levels of term normotensive and Hypertensive pregnant women patients.

AIMS AND OBJECTIVES

To compare maternal vitamin D and calcium levels of term normotensive and Hypertensive pregnant women attending the OPD or admitted as in patients at Shri. B. M. Patil.Medical College Hospital and Research Centre, Vijayapur, Karnataka between October 2016 to October 2018.

REVIEW OF LITERATURE

HISTORICAL ASPECTS

Over the centuries, there have been many theories to explain the etiology of pregnancy induced hypertension. In fact Zweifel in 1916 called it a disease of theories.[20]

Fits occurring in pregnancy were recognised and recorded as early as 4th century BC by Hippocrates. The condition was termed as eclampsia, a Greek word which implies a sudden development.

Until 1843 very little was known about eclampsia when Lever of Guys hospital found that many women who had fits also had albumin in their urine. However it was not until the discovery of the sphygmomanometer that it was found associated with raised blood pressure.[21]

Even though clinical manifestations of eclampsia-preeclampsia have been known since very long the pathophysiology of this syndrome remained unknown for nearly two millennia. These were later recognised as a result of endothelial injury. Renal glomerular capillary endotheliosis, swelling of endothelial cytoplasm and obliteration of endothelial fenestrae was originally observed by Mayer in 1924,[22] described further by Bell[23] and later refined by Spargo et al.[24] The latter investigator was the first to apply electron microscopy in this field and to introduce the term "glomerular capillary endotheliosis" as a characteristic feature of preeclampsia.

BRIEF OVERVIEW OF HYPERTENSION IN PREGNANCY

CLASSIFICATION

The classification of hypertensive disorders complicating pregnancy (HPD) by the Working Group of the NHBPEP (2000) is as follows.

There are 5 types of hypertensive disease:

1) Gestational hypertension

BP ≥ 140/90 mm Hg for first time during pregnancy

No proteinuria

BP returns to normal < 12 weeks' postpartum

Final diagnosis made only postpartum

2) Preeclampsia

BP ≥ 140/90 mm Hg after 20 weeks' gestation

Proteinuria ≥ 300 mg/24 hours or ≥ 1+ dipstick

3) Eclampsia

Seizures that cannot be attributed to other causes in a woman with preeclampsia

4) Superimposed Preeclampsia (on chronic hypertension)

New-onset proteinuria ≥ 300 mg/24 hours occurs in hypertensive women but no proteinuria takes place before 20 weeks' gestation but sudden increase in proteinuria or platelet count < 100,000/mm3 in women with hypertension and proteinuria occurs before 20 weeks' gestation.

5) Chronic Hypertension

BP ≥ 140/90 mm Hg before pregnancy or diagnosed before 20 weeks' gestation not attributable to gestational trophoblastic disease or Hypertension first diagnosed after 20 weeks' gestation and persistent after 12 weeks' postpartum.

The following are the indications of severity of hypertensive disorders during pregnancy1

Abnormality	**Mild**	**Severe**
Diastolic blood pressure	< 100 mm Hg	≥ 110 mm Hg
Proteinuria	≤ 2+	≥ 3+
Headache	Absent	Present
Visual disturbances	Absent	Present
Upper abdominal pain	Absent	Present
Oliguria	Absent	Present
Convulsion (eclampsia)	Absent	Present
Serum creatinine	Normal	Elevated
Thrombocytopenia	Absent	Present
Liver enzyme elevation	Minimal	Marked
Fetal growth restriction	Absent	Obvious
Pulmonary edema	Absent	Present

NORMAL ADAPTATIONS EXPECTED IN PREGNANCY

Different physiologic changes occur in the context of a normal healthy pregnancy. Mostly up-regulation of the renin-angiotensin-aldosterone system (RAAS) which begins at the time of the luteal phase of the menstrual cycle and is co-incident with increased estrogen and progesterone levels.[25]

Before fertilization begins, elaboration of each of these hormones continues; renin up to eight times, angiotensin up to four times, and aldosterone up to ten to twenty times normal levelsin order to support gestation.[26]

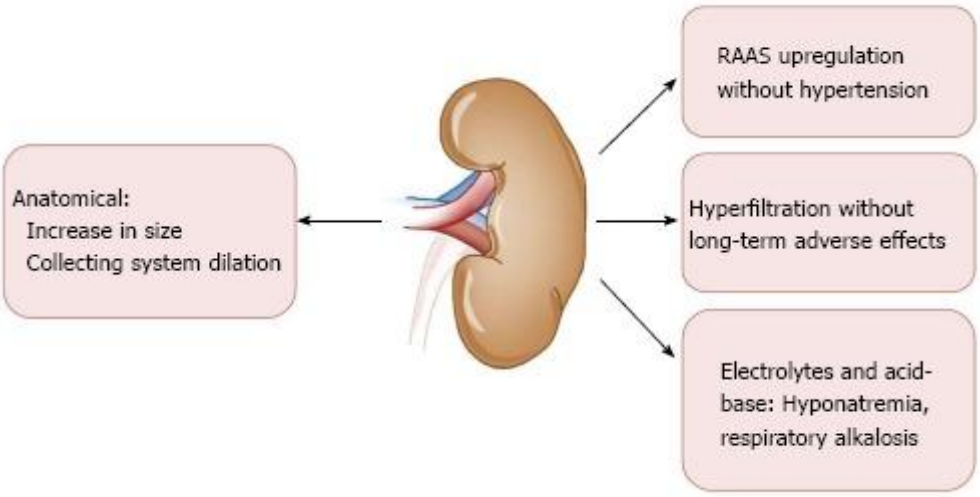


Figure 1 : Physiological changes in kidney during pregnancy.

In this context, healthy women do not suffer from hypertension, however, because of either to reducedestrogen-mediated vascular responsiveness to such RAAS components, or possibly due to to the counteracting vasodilatory effect of prostacyclins and the gestational hormone relaxin secreted by the ovaries.[27]This systemic vasodilation by hormones leads to decreased systolic blood pressure by about 10-15 mmHg.[28]

In mid-pregnancy, the vasodilation results into an early and robust increase in glomerular filtration-initially by about 25% and progressing up to 50% because of the dilation of the renal vasculature. There is an even more increase in renal plasma flow, which is found to be around 60%.

The latter is a state of hyperfiltration that does not create any pathologic injury as seen in other states of hyperfiltration for example in diabetic kidney disease.[29]When compared to the increased encountered in other hyperfiltration conditions, pregnancy is found to be associated with reduction in the fraction of single nephron filtration.

Up-regulation of RAAS contributes to retention of sodium and fluid which facilitates expansion of plasma volume within the dilated vasculature whereas intravascular expansion in volume leads to hyponatremia and mild dilutional anemia with serum sodium concentrations reduced by approximately 4-5 meq/L in some cases.[30]

In ultrasonography, it was found that increased renal length and volume is frequently appreciated along with mild non-obstructive hydronephrosis because of compression in the uterine of the ureters. Pelviectasis on the right side is typically more pronounced, perhaps owing to dextrorotation of the uterus and exaggeration by the relative protection of the left ureter provided by the sigmoid colon which may be symptomatic in approximately 30% of pregnant women with low-grade obstruction which can predispose to urinary tract infections.[31]

Incidence and Prevalence

The prevalence of preeclampsia varies in different populations and in different ethnic groups.

Preeclampsia has remained a significant public health threat in both developed and developing countries contributing to maternal and perinatal morbidity and mortality globally.[32] The exact incidence is not known. Different studies have shown preeclampsia affecting 5%–8% of all pregnancies. Globally, pre-eclampsia and eclampsia account for 10%–15% of maternal deaths. The majority of deaths in developing countries result from eclampsia.[33]

Definition of hypertensive disorders of pregnancy

Confusion still prevails over the terminology and classification of the hypertensive disorders of pregnancy. The latest recommendation from The National High Blood Pressure Education Program Working Group 2000 has proposed the term gestational

hypertension to replace the term pregnancy-induced hypertension in describing cases in which elevated blood pressure without proteinuria develops in a woman after 20 weeks of gestation and blood pressure levels return to normal in postpartum period. In pregnant women, hypertension is defined as a systolic blood pressure level of 140mmHg or higher, or a diastolic blood pressure level of 90 mmHg or higher, which occurs after 20 weeks of gestation in a woman with previously normal blood pressure. Pre-eclampsia is a syndrome defined by hypertension and proteinuria which may also be associated with a myriad of other signs and symptoms such as visual disturbances, headache and epigastric pain. Laboratory abnormalities may include hemolysis, elevated liver enzymes and low platelet counts (HELLP syndrome). Proteinuria isdefined as the presence of 0.3g or more of protein in a 24-hour urine specimen (or 1+ or greater in random urine dipstick).[34]

The diagnostic criteria for superimposed pre-eclampsia include —new-onset proteinuria in a woman evincing hypertension before 20 weeks of gestation, a sudden increase in proteinuria if this is already present in early gestation, a sudden increase in hypertension, or the development of the HELLP syndrome. Women with chronic hypertension who develop headache, visual signs or epigastric pain may also have superimposed pre-eclampsia.

THEORIES OF HYPERTENSION IN PREGNANCY

Pathogenesis of hypertension in pregnancy

Despite increasing knowledge of the pathophysiology of pre-eclampsia its etiology is still obscure .Several models for its pathogenesis have been proposed.

Placental ischemia

Although the cause of pre-eclampsia remains undefined the condition is now assumed to be a disease related to the placenta.[35] Pre-eclampsia can develop with abdominal pregnancy and the presence of a fetus is not required, as pre-eclampsia can occur with hydatidiform mole. Pre-eclampsia is generally considered to be a consequence of an inadequate uteroplacental circulation, thought to be due to failure of trophoblastic invasion of the spiral arteries. In early pregnancy trophoblast cells invade the placental bed, leading to remodelling of spiral arteries into maximally dilated low-resistance vascular channels, unable to constrict upon vasoactive stimuli. Endovascular trophoblast invasion has been reported to occur in two waves; the first into the decidualsegments of the spiral arteries at 8 to 10 weeks of gestation and the second into myometrial segments at 16 to18 weeks of gestation.[36]

There is a failure of cytotrophoblasts to undergo transformation of their phenotype to endothelial cell characteristics and this is likely to have a negative effect on the cytotrophoblast endovascular invasion which may be involved in development of hypertension in pregnancy. Moreover, the severity of hypertension may be related to the degree of trophoblastic invasion.[37]

Immunology and genetics

Epidemiological studies strongly suggest that immune maladaptation is involved in the etiology of pre-eclampsia. The disorder develops mainly in first pregnancies,

suggesting that exposure to paternal antigen is protective.[38] Even a prior abortion may provide protection against the disease. The protective effect of multiparity is lost with change of paternity. A previous pregnancy with the same father and a longer period of sexual cohabitation with the father before conception reduce the risk of pre-eclampsia.[39]

The conception of an indirect immunologic basis for preeclampsia is also supported by the finding that the pre-eclampsia risk is increased in pregnancies with donor insemination or with oocyte donation.[40] Candidates for mediators of immune maladaptation in pre-eclampsia include cytokinesespecially tissue necrosis factor alpha and interleukin-2 and -6.

Although genetic influences have long been regarded as etiologically important in preeclampsia, no single gene has been identified which would explain the inheritance of the disorder. Redman and colleagues (2009) recently reviewed the possible role of immune maladaptation in the physiology of preeclampsia. Early in pregnancy destined to be pre-eclamptic, extravillous trophoblast express as reduced amounts of immunosuppressive human leukocyte antigen G.This may contribute to defective placental vascularizationin stage 1. During normal pregnancy, T-helper (Th) lymphocytes are produced so that type 2 activity is increased in relation to type 1-termed type 2 bias.Th2 cells promote humoral immunity , whereas Th1 cells stimulate inflammatory cytokine secretion. Beginning in the early second trimester in women who develop preeclampsia, Th1 action is increased and the Th1/Th2 ratio changes.

Contributors to an enhanced immunologically mediated inflammatory reaction are stimulated by placental microparticles, as well as adipocytes (Redman and Sargent, 2008).[41]

Maternal risk factors

There are numerous maternal constitutional factors predisposing to the disorder. In patients with pre-existing vascular disease, chronic hypertension and autoimmune disorders such as systemic lupus erythematosus and antiphospholipid syndrome the risk is increased ten times, in chronic renal insufficiency 20 times.[42] Women with thrombophilias are more likely to develop pre-eclampsia.In a series of women with severe, early-onset pre-eclampsia, 25% had functional protein S deficiency, 18% evidenced hyperhomocysteinemia, and 29% had detectable anticardiolipin IgG and IgM antibodies. Predisposing factors also include obesity, pregestational diabetes andincreased insulin resistance Women with multiple gestations are more likely to develop pre-eclampsia.[43]

Endothelial cell dysfunction

Abnormal placentation and resulting poor placental perfusion may be the impetus for the endothelial changes evidenced in pre-eclampsia. An immunohistologicstudy has evidenced morphologic changes in the endothelialisation of uteroplacental vessels 23 and an electro microscopic study of the uteroplacental arteries noted gross endothelial damage, massive intramural fibrin deposition, luminal thrombosis and vessel rupture with hemorrhage in pre-eclampsia. Healthy endothelial cells maintain vascular integrity, prevent platelet adhesion and influence the tone of the underlying vascular smooth muscle. Endothelial cell dysfunction may result from a variety of factors, including physical tear forces, hypoxia, lipid peroxides and infiammatory cytokines. When activated by a chronic pathologic process endothelial cells lose these functions and produce procoagulants, vasoconstrictors and mitogens, causing increased capillary permeability, platelet thrombosis and increased vascular tone. Many markers

of endothelial dysfunction have been reported in women who develop pre-eclampsia, suggesting that this is an endothelial cell disorder.[44]

Markers of endothelial cell dysfunction

PROSTAGLANDINS

Endothelial cells are the most important source of prostacyclin, which is a potent vasodialator, inhibitor of platelet aggregation and stimulator of renin secretion Prostacyclin production is increased eight to ten fold in normal pregnancy, whereas in pregnancy induced hypertension the increase is only one to two fold.

ENDOTHELIN-1

Endothelin-1, an endothelium-derived peptide, is a potent vasoconstrictor in the human uterine artery, and the effect is mediated by receptors on the smooth muscle cells. Most studies have demonstrated an increase in endothelin in the plasma and in placental tissue in pre-eclamptic women.[45]

NITRIC OXIDE

The production of nitric oxide (NO), a potent vasodilator synthesised by endothelial cells, is elevated in normal pregnancy but the effects of nitric oxide in preeclampsia are unclear

VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF),AND PLACENTAL GROWTH FACTOR (PlGF)

The circulating levels of two angiogenic growth factors, VEGF and PlGF, may play an important role in the pathogenesis of pre-eclampsia. Studies have shown deprivation of VEGF and PlGF to be involved in the condition. Soluble fms-like tyrosine kinase 1 (sFlt1) is a variant of the VEGF receptor. Flt1 lacks segments usually binding the protein with a cell membrane and acting as a potent VEGF and

PlGF antagonist. Increased amounts of sFlt1 reduce free VEGFand PlGF in the blood of patients withpre-eclampsia, and this altered balance causes endothelial dysfunction.[8]

VASCULAR CELL ADHESION MOLECULE 1 (VCAM-1)

Soluble adhesion molecules such as the vascular cell adhesion molecule 1 (VCAM-1), are known to be increased in the serum of patients with pre-eclampsia, indicating that these molecules are possible markers of endothelial cell activation In conclusion, endothelial dysfunction has been shown to be an early pathogenic feature of pre-eclampsia and many markers of endothelial activation precede clinically evident disease and disappear with resolution of the disease. The cause of endothelial dysfunction is not known, but the initiating event has been postulated to be reduced placental perfusion

Oxidative stress

The maternal response to reduced placental perfusion is influenced by maternal constitutional factors –genetic, behavioral or environmental The similarities between risk factors in pre-eclampsia and atherosclerosis support the conception that oxidative stress, which is pathogenically important in atherosclerosis could also be the link between reduced placental perfusion and maternal constitutional factors in pre-eclampsia.[46]

The reduced placental perfusion in association with the reduction in uterine blood flow known to accompany postural changes and uterine contractions could lead to intermittent intervillous hypoxia. Upon reperfusion, free radicals would be generated. The impact of this oxidative stress would be accentuated by maternal constitutional factors (e.g. decreased levels of antioxidants, lipoproteins). It has been shown that

malondialdehyde, a marker of lipid peroxidation, is increased in women with pre-eclampsia. Activated neutrophils, stable products ofoxidative stress (e.g. malondialdehyde), oxidised fragments of syncytiotrophoblast entering the systemic circulation, or cytokines could be the factors transferring oxidative stress from the intervillous space to the systemic circulation.

Inflammatory theory

Endothelial cell dysfunction is one aspect of the generalised systemic maternal inflammatory response. Syncytiotrophoblasts normally shed redundant placental debris into the maternal circulation, and this process depends on apoptosis. Syncytiotrophoblast microfragments are detected in increased amounts in preeclampsia. It has been proposed that increased oxidative stress in the placenta leads to an overload of debris by stimulating apoptosis or necrosis or both. Continual clearance of this debris causes the systemic inflammatory response, which is present in all pregnant women in the third trimester. Pre-eclampsia may occur if the burden of debris is abnormally high, or if the woman's response to the process is excessive.

GENETIC FACTORS

Pre-eclampsia is a multifactorial, polygenic disorder.

From their recent review, Ward and Lindheimer (2009) found that more than 70 genes have been studied for their possible association with pre-eclampsia. Seven of those that have been investigated widely are listed in table-Genes Frequently Studied for Their Association with Preeclampsia syndrome. [47]

Table1: Genes Frequently Studied for Their Association with Preeclampsia syndrome

Chromosome location	Gene	Primary Polymorphism Studied	Number of Studies	Presumed Biological Association with Preeclampsia
1p36.3	*MTHFR*	C677T	27	Vascular diseases
1q23	*F5*	Leiden	21	Thrombophilia
1q42–q43	*AGT*	M235T	17	Blood pressure regulation
6p21.3	*HLA*	Various	14	Immunity
7q36	*NOS3*	Glu298Asp	16	Vascular endothelial function
11p11–q12	*F2*	G20210A	12	Blood coagulation
17q23	*ACE*	I/D at intron 16	13	Blood pressure regulation

INSULIN RESISTANCE:

Insulin resistance (IR) was first proposed in 1936.[48] Decreased insulin sensitivity or increased insulin resistance is defined as the decreased biological response of a nutrient to a given concentration of insulin at the target tissue, e.g. liver, muscle, or adipose tissue.

Insulin resistance has been established to play a major role in Type II diabetes mellitus and in the pathogenesis of hypertension, dyslipidemias, and coronary artery disease.

The resistance to insulin can be characterized as pre-receptor (insulin antibodies), receptor (decreased number of receptors on the cellsurface), or post- receptor (defects in the intracellular insulin signaling pathway).Normal pregnancy is characterised by insulin resistance which is greatest in the third trimester. This appears to be an adaptive response, diverting glucose and lipids to the developing fetus and thought to

be due to the combined effects of human placental lactogen, progesterone, oestradiol and cortisol, which act as counter-regulatory hormones to insulin.

Exaggeration of the insulin resistance normally seen in pregnancy is associated with gestational diabetes mellitus and hypertension in pregnancy.[36] In pregnancy, the decreased insulin sensitivity is best identified as a post-receptor defect resulting in the decreased ability of insulin to bring about GLUT4 (glucose transporter) mobilization from the interior of the cell to the cell surface.[49]

When pregnant women are compared with gravid women it was found that lower fasting, higher postprandial glucose values and hyperinsulinemia were observed and immediately after taking aglucose meal orally, prolonged hyperglycemia and hyperinsulinemia as well as greater suppression of glucagon was demonstrated in gravid women. This process cannot be explained by a decreased metabolism of insulin because its half-life is not changed during pregnancy. Rather, the motive of which is likely to ensure a sustained postprandial supply of glucose to the fetus which was consistent with a pregnancy-induced state of peripheral insulin resistance.

Generally, sensitivity towards insulin in late normal pregnancy was about 45 to 70 percent less than that of non-pregnant women.Insulin resistance was calculated from fasting maternal plasma glucose and insulin concentrations. Insulin resistance was calculated using the surrogate indices of homeostatic model assessment (HOMA) and also the quantitative insulin sensitivitycheck index (QUICKI).

Preeclampsia and gestational hypertension may be involved with higher degrees of insulin resistance than any other characteristics seen in normal pregnancy. In late pregnancy, usually beginning of preeclampia and gestational hypertension is seen; a

time when the insulin resistance is more which is a physiological characteristic and also supports a probable association.

Postulated mechanisms, through which insulin resistance might increase blood pressure in pregnancy, include sympathetic nervous system activation, renal sodium retention, increased cation transport, and associated endothelial dysfunction.

There are various serum markers of insulin resistance fasting insulin, serum cholesterol, SHBG,HOMA-IR, hs-CRP, TNF- alfa measured before 20 weeks of gestation may be helpful in diagnosis of hypertension in pregnancy.

So to study serum markers of insulin resistance in first 20 weeks of gestation and observe their correlation with onset of hypertension in pregnancy may be helpful in early detection of pre- eclampsia and gestational hypertension.

ETIOLOGY AND PATHOPHYSIOLOGY

Pregnancy-induced hypertension is having multifactorial etiology.

At present, numbers of hypotheses are the subject of extensive investigation, as follows:

Abnormal trophoblastic invasion — In normal implantation, the uterine spiral arteries undergo extensive remodelling as they are invaded by endovascular trophoblasts. In preeclampsia, however, there is incomplete trophoblastic invasion. In this case, decidual vessels, but not myometrial vessels, become lined with endovascular trophoblasts.[50]

In preeclamsia there is incomplete trophoblastic invasion. The magnitude of defective trophoblastic invasion of spiral arterioles was correlated with the severity of

hypertensive disorders.Very low-density lipoproteins versus toxicity-preventing activity — in compensation for increased energy demand during pregnancy, nonesterified fatty acids are mobilized. In women with low albumin concentrations, transporting extra nonesterified fatty acids from adipose tissues to the liver is likely to reduce albumin's antitoxic activity to a point at which very-low density lipoprotein toxicity is expressed.

Endothelial cell activation — Interaction between decidual leukocytes and invading cytotrophoblast cells is essential for normal trophoblast invasion and development. In response to placental ischemia various inflammatory mediators like interleukins, tumor necrosis factor -α, cytokines, proteolytic enzymes, reactive oxygen species etc. provoke endothelial injury. Immune maladaptation may cause shallow invasion of spiral arteries by endovascular cytotrophoblast cells and endothelial cell dysfunction.

Genetic imprinting — Development of preeclampsia-eclampsia may be based on a single recessive gene or a dominant gene with incomplete penetrance. Penetrance may be dependent on fetal genotype. The possibility of genetic imprinting should be considered in future genetic investigations of preeclampsia.[51]

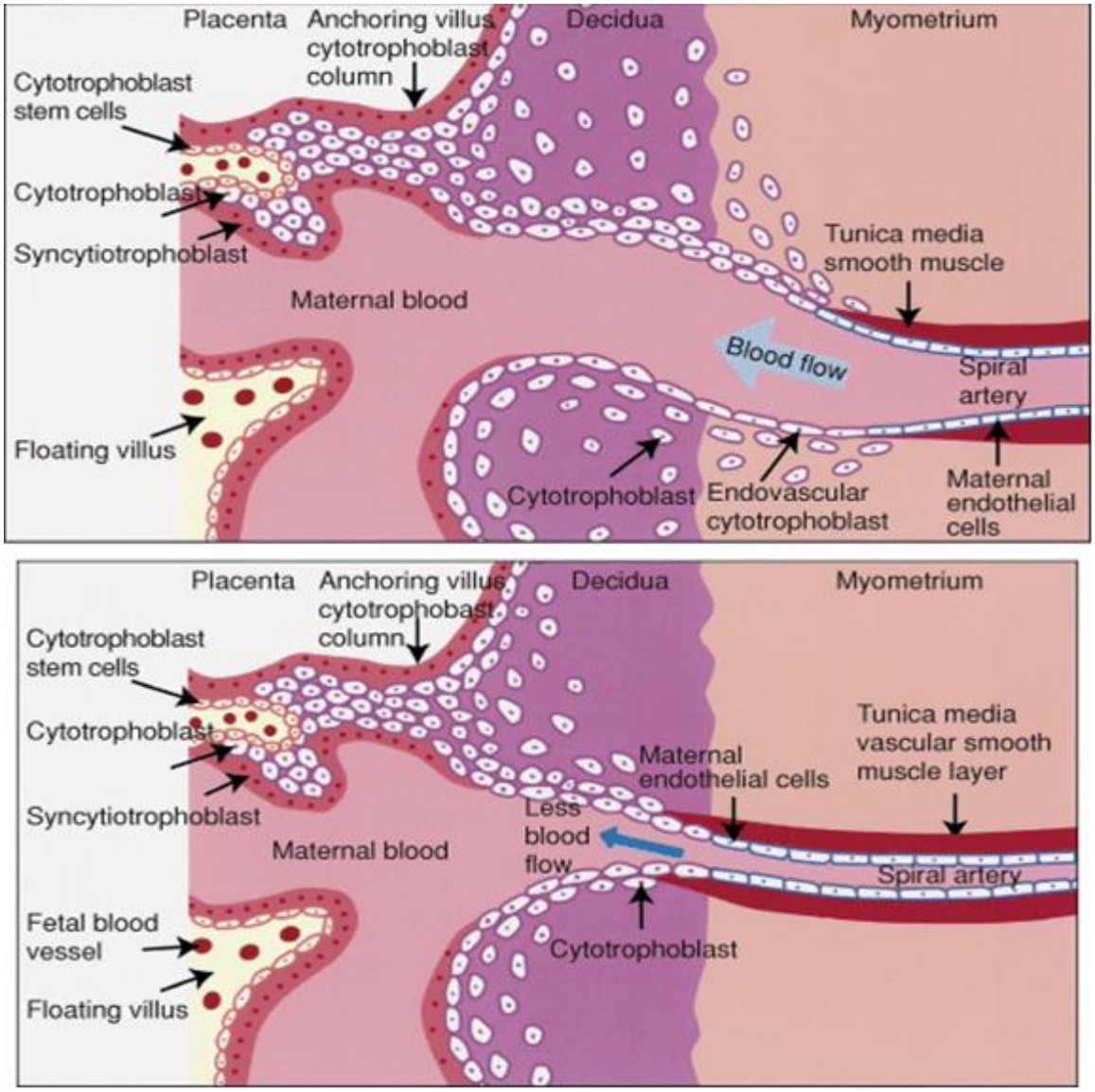


Figure 2 : Trophoblastic invasion of spiral arteries

The initiating event in PIH appears to be reduced uteroplacental perfusion as a result of abnormal cytotrophoblast invasion of spiral arterioles. Therefore preeclampsia is primarily a disorder of placental dysfunction leading to a syndrome of endothelial dysfunction with associated vasospasm. A wide variety of angiogenic molecules and proteolytic enzymes play critical roles in the establishment of placentation and development of placental circulatory system. For example, vascular endothelial growth factor, fibroblast growth factor, and placental growth factor are indispensable in the entire course of gestation.

In later stages of pregnancy, villous trophoblasts and fetal-side blood vessels of terminal villi form finely-differentiated vascular network to serve a fetus with sufficient amounts of oxygen and substances for exponential fetal growth.11 The arterial circulation in the placenta lacks autonomic innervation and is regulated by local signals such as pressure and flow.[52]

If implantation process is not proper, the placenta suffers from insufficient perfusion and secretes various kinds of pro-inflammatory molecules that damage maternal endothelial cells, and in consequence, vascular resistance is increased that further burdens maternal organs with hypertension as well as aggravates fetoplacental milieu.

In a study conducted by Levine RJ et al in 2006, rising circulating levels of soluble endoglin and ratios of soluble fms-like tyrosine kinase 1 (sFlt 1), an antiangiogenic protein, and placental growth factor, a proangiogenic protein, herald the onset of preeclampsia.[53]

Many of the other popular theories for new onset hypertension in pregnancy are as follows along with various studies.

Nitric oxide imbalance:

It has been postulated that low levels of nitric oxide in maternal and fetal vessels may result in endothelial dysfunction, resulting in hypertension and insufficient fetal-placental blood flow.

Calcium insufficiency:

Preeclampsia is increased in populations with low calcium intake. In addition, hypocalciuria is a common finding and is associated with lower 1,25 vitamin D levels

and higher parathyroid hormone levels than those seen in normal pregnancy. Therefore, it has been postulated that calcium supplementation would reverse this process and lower the incidence of preeclampsia. However the large NIH trial of calcium supplementation to over 4500 nulliparous women did not show a significant reduction in the incidence of preeclampsia.[54]

Prostaglandin imbalance and association with Renin- Angiotensin system:

An imbalance between vasodilatory and vasoconstrictor prostaglandins has been proposed as a cause of vasoconstriction and hypertension. The widespread dysfunction of the maternal vascular endothelium manifests as enhanced formation of factors such as endothelin, reactive oxygen species, and augmented vascular sensitivity to angiotensin II. Alternatively, the preeclampsia syndrome may also be evidenced as decreased formation of vasodilators such as nitric oxide and prostacyclin. Taken together, these alterations cause hypertension by impairing renal pressure natriuresis and increasing total peripheral resistance.

The hypertension occurring in preeclampsia is primarily due to vasospasm, with arterial constriction and relatively reduced intravascular volume compared to normal pregnancy. In addition, blood pressures in preeclampsia are labile, and the normal circadian blood pressure rhythms may be blunted or reversed. One study by Khalil et al in 2009 found increased arterial stiffness in women with preeclampsia, as well as in those with gestational hypertension, compared with normotensive controls; treatment with alpha methyldopa significantly improved the vascular stiffness in preeclampsia but did not normalize it.[55]

Recent study by R. A. Irani et al in 2008 demonstrate the presence of the angiotensin II type I receptor agonistic autoantibody (AT1-AA). This autoantibody can induce many key features of the disorder and upregulate molecules involved in the pathogenesis of preeclampsia.[56]

Insulin resistance:

It is well accepted that insulin resistance is a feature of pregnancy and that it peaks in the third trimester. Because this is the time that new onset hypertension is usually first manifest, the temporal relationship raises the possibility that hyperinsulinemia resulting from insulin resistance may play a pathogenic role in the development of hypertension in pregnancy, just as it has been proposed to do in essential hypertension. Similar mechanisms have been proposed including alteration of cell transport, increase in sympathetic nervous system tone, and alteration of sodium handling. Studies by Masden et al in 1973, Kaaja R et al in 1998 and Abundis EM et al in 1996, using oral glucose tolerance testing, have demonstrated an increase in insulin resistance in women with established new onset hypertension in pregnancy.[57,58,59]

To sum up, the two central pathophysiological themes of preeclampsia are placental trophoblast dysfunction and endothelial dysfunction within the maternal systemic vasculature. While placental dysfunction may not be the sole cause of preeclampsia, it may trigger, or significantly contribute to the vascular complications associated with preeclampsia.

PREDISPOSING FACTORS

The incidence of PIH is markedly influenced by parity. It more often affects nulliparous women. Because of the increasing incidence of chronic hypertension with advancing age, older women are at greater risk for superimposed preeclampsia. Thus, women at either end of reproductive age are considered to be more susceptible.

In a Case control study of 139 cases of preeclampsia by Eskenazi B et al in 1991, case patients were more likely than control patients to be nulliparas.[60]In another study by Campbell DM et al in 1985, the incidence of pre-eclampsia in a second pregnancy was less than that in a first pregnancy.[61]

It is related to race, ethnicity and thus to genetic predisposition. Walker in 2000 has shown increased susceptibility of African-American women to preeclampsia.[62]

Environmental factors are also likely to play a role as shown in a study by Palmer and associates in 1999 who reported that living at high altitude in Colorado increased the incidence of preeclampsia.[63]

Some investigators have concluded that socioeconomically advantaged women have a lesser incidence of preeclampsia. However, Lawlor and colleagues in 2005 did not observe this in an Aberdeen cohort of 3485 women.[64]

The relationship between maternal weight and the risk of preeclampsia is progressive. It increases from 4.3 percent for women with a body mass index less than 19.8 kg/m2 to 13.3 percent in those with a body mass index greater than 35 kg/m2.

In another study conducted by Dorothea Mostello et al in 2008, obese and overweight women had higher risks of recurrent preeclampsia as compared with women with normal BMI.[65]

History of pre-eclampsia in the previous pregnancy was also associated with increased risk as shown in the study by Eskenazi B et al in 1991.[60]

A history of pre-eclampsia was associated with 5 time's higher risk of pre-eclampsia in the second pregnancy as shown by Sohinee Bhattacharya et al in 2009.[66]

In women with twin gestations compared with those with singletons, the incidence of gestational hypertension (13 versus 6 percent) and the incidence of preeclampsia (13 versus 5 percent) are both significantly increased as demonstrated by Sibai and co-workers in 2000. The incidence is however unrelated to zygosity.

In a case-control study to assess the relation of cigarette smoking during pregnancy to the risk of preeclampsia and gestational hypertension, Marcoux S et al showed that compared with women who had never smoked; women who were smokers at the onset of pregnancy had a reduced risk of preeclampsia. The protective effect of smoking on preeclampsia was stronger for women who continued to smoke after 20 weeks of pregnancy. Nicotine inhibition of thromboxane A2 production might explain the decreased risk of pregnancy-induced hypertension among smokers. Despite these findings, the harmful consequences of smoking on pregnancy outcome outweigh its protective effect against pregnancy-induced hypertension.[67]

In a population-based nested case-control study Leena M et al in 2009 proved Blood group AB and factor V Leiden as risk factors for pre-eclampsia. High body mass index, diabetes, first pregnancy, and twin pregnancy increased the risk from 1.5-fold to 8.2-fold.[68]

C. J. Lee et al in a study of risk factors for pre-eclampsia in an Asian population in 2000 showed that women who had a history of pre-eclampsia, multiple gestation, a

prepregnancy BMI >24.2 kg/m2, were >34 years of age and were nulliparous were at increased risk of pre-eclampsia.[69]

Pre-eclampsia and eclampsia are also associated with higher levels of cord blood erythropoietin as shown by a study conducted by G Gupta et al in 2000.[70]

There is also a correlation with cholesterol levels and risk of PIH as demonstrated by a case-control study by Solomon G et al in 1999 comparing cholesterol, insulin, and glucose levels in the early third trimester of pregnancy among 31 women who developed pregnancy-induced hypertension with 31 women remaining normotensive through pregnancy. As compared with women remaining normotensive, women subsequently developing PIH had higher fasting cholesterol levels and higher fasting insulin levels. Thus higher fasting cholesterol and insulin levels in mid- to late pregnancy are associated with increased risk for PIH.[71]

Physiology of Vitamin D

There are two important compounds among the vitamin D groups in humans, which are vitamin D3 (also known as cholecalciferol) and vitamin D2 (ergocalciferol)..[72] Cholecalciferol and ergocalciferol are found in few types of foods, so sunlight exposure is the main source of vitamin D for humans, other than supplements. Solar ultraviolet B radiation (wavelength, 290 to 315nm) penetrates the skin and converts 7-dehydrocholesterol to previtamin D3, which is spontaneously isomerized into vitamin D3 (cholecalciferol).

Vitamin D from photosynthesis or food ingestion is metabolized in the liver to 25-hydroxyvitamin and subsequently hydroxylated in the renal proximal tubules by the enzyme 1α-hydroxylase to 1, 25-dihydroxy-vitamin D (1, 25 (OH) 2D, calcitriol), the biologically active form. 1, 25 (OH) 2D promotes intestinal calcium absorption.

When vitamin D deficiency decreases the absorption of dietary calcium and phosphorus, the level of parathyroid hormone (PTH) increases. Sufficient vitamin D stimulates calcium and phosphorus absorption by 30-40% and 80% respectively. Without vitamin D, no more than 10-15% of dietary calcium and approximately 60% of phosphorus are absorbed.[73]

Vitamin D-Binding proteins (DBP) are synthesized in hepatocytes and helps vitamin D to transport to target organs. Because DBP is the primary transporter of vitamin D and its metabolites, it has a role in maintaining the total levels of vitamin D for the organism and in regulating the amounts of free vitamin D available for specific tissues and cell types to utilize. DBP linked vitamin D is actively transported by megalin mediated endocytosis in the various target cells, and intracellular vitamin D binding proteins (IDBPs) help to regulate the intracellular metabolism of vitamin D thereafter.[74]

The renal production of 1,25 (OH)2D is tightly regulated by 1, 25-dihydroxyvitamin D itself, plasma PTH levels as a signal of calcium homeostasis, and fibroblast growth factor 23 (FGF 23) as a signal of phosphate status.[75] Free 1,25 (OH)2D can form a complex with vitamin D receptor, the VDR, and reduce transcription of CYP27B1 (1α-hydroxylase). PTH is a hormone secreted by the parathyroid glands which regulates serum calcium through its effects on bone, kidney, and the intestine. When the level of serum calcium decreases, the production of PTH in parathyroid gland increases and leads to calcium resorption from bone and the renal tubular fluid. In addition, PTH up-regulates 1α-hydroxylase enzyme, which converts inactive vitamin D into 1,25 (OH)2D60). FGF 23 is secreted by osteocytes in response to elevated

1, 25 (OH) 2D and increased plasma levels of phosphorous. FGF23 has three types of effect. First, FGF23 impairs sodiumphosphate cotransporters on the kidneys and small intestines, through internalization of the transporters by the cells and consequently, phosphate loss occurs. FGF23 also inhibits production of 1,25 (OH)2D and promotes breakdown of 1,25 (OH)2D2. Lastly, FGF 23 Inhibits production and secretion of parathyroid.[76]All three roles of FGF 23 contribute to decrease lowering the level of serum phosphate.

VITAMIN D

Vitamin D is known as a sunshine vitamin. It is produced in the skin by exposure to ultraviolet B radiation, and with a small portion from diet and/or supplements. The role of vitamin D in bone health has long been well established, with vitamin D deficiency being a causal factor in the development of rickets in children, osteomalacia in adults, and contributing to osteoporosis. Data are also suggestive of a potential role of vitamin D in other, non-bone related health conditions. Low vitamin D status has been associated with a wider range of adverse health outcomes, including cardiovascular disease, diabetes, cancer and psychiatric disorders. Vitamin D deficiency now has become a major public health concern because it is widespread over the world, even in countries with ample sunshine.[77]

During pregnancy, a woman maintains her vitamin D requirements to support her own health, but also needs the extra amount to support her fetus. Thus, achieving and maintaining adequate vitamin D is much more critical in pregnant women than in any other population.It is evident through various studies that vitamin D status during pregnancy is integral to maternal health, fetal development, and optimal neonatal outcomes as well as future health of the offspring. Regardless of the ethnicity and

region, an epidemic of vitamin D deficiency has been discovered over the world through a number of observational studies.[78]

Sources of Vitamin D

Vitamin D is a fat-soluble vitamin that includes both cholecalciferol (vitamin D3) and ergocalciferol (vitamin D2). 'Vitamin D' hereafter refers to both vitamin D3 and D2. Human beings obtain vitamin D through two ways: endogenous and exogenous sources.

For most people, vitamin D is largely endogenously derived through cutaneous synthesis of vitamin D3 following exposure of the skin to sunlight. Solar, ultraviolet B radiation (UVB, wavelength 290 to 315 nm), one of the components of sunlight, penetrates the skin and converts 7-dehydrochlesterol (7-DHC) (also called provitamin D) to previtamin D3, which is quickly converted to vitamin D3 thermally. Excessive exposure to sunlight does not cause vitamin D3 intoxication, because sunlight destroys any excessive previtamin D3 or vitamin D3 by converting it into inactive photoproducts. Additionally, vitamin D3 is fat-soluble and excess amounts can be taken up by adipocytes and stored in adipose tissue.[79]

It has been suggested by some vitamin D researchers, that approximately 5–30 minutes of sun exposure between 10 AM and 3 PM at least twice a week to the face, arms, legs, or back without sunscreen usually lead to sufficient vitamin D synthesis.[80]

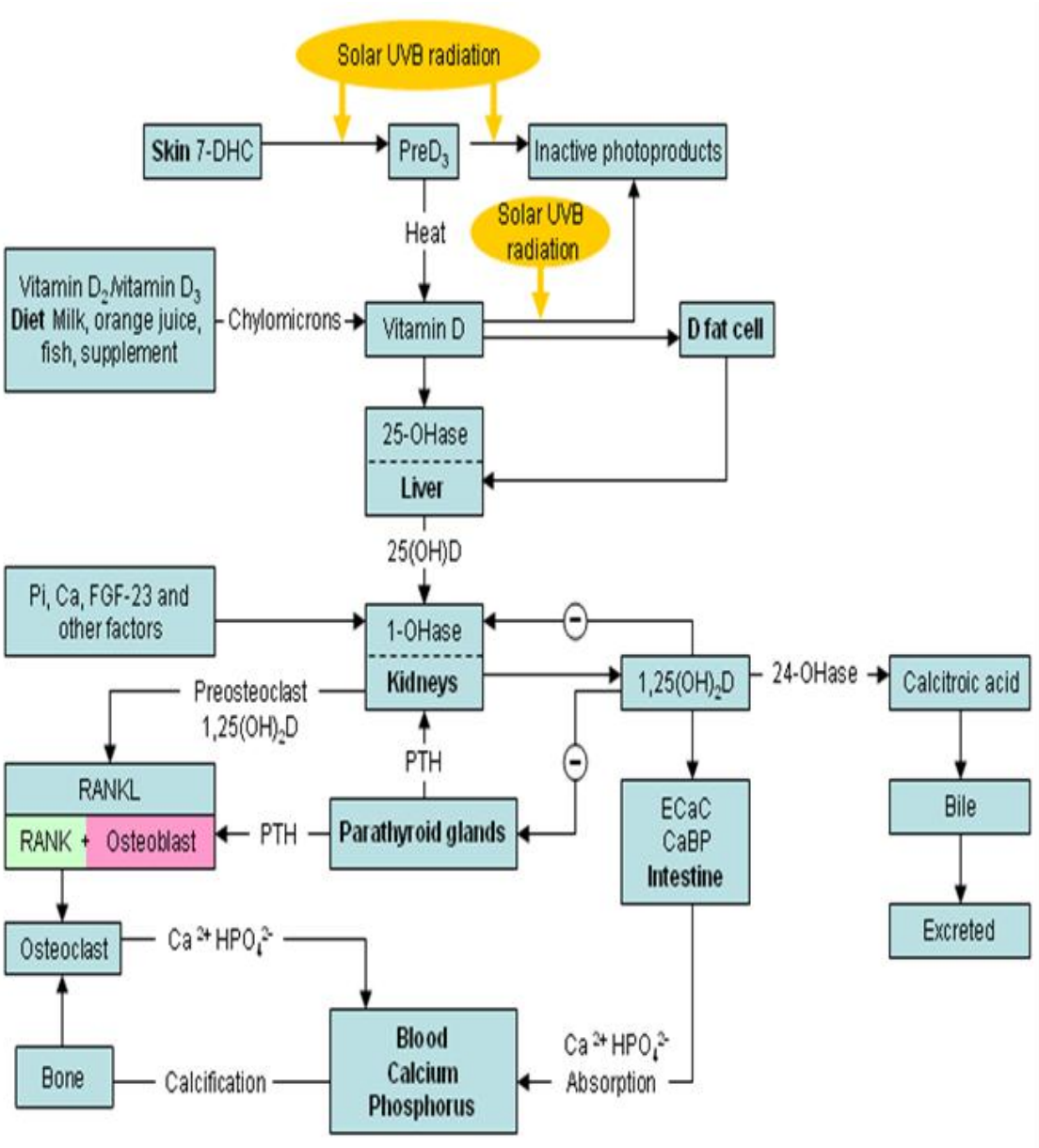


Figure 3 : Vitamin D synthesis and action

Exogenous sources of vitamin D include foods naturally rich in vitamin D, vitamin D fortified foods and vitamin D supplements. The dietary intake is more important when sun exposure is limited. Vitamin D3 is found naturally in only a few foods. These include oily fish, such as salmon, mackerel and herring, and fish liver oil due to majority fat concentrates in liver, with even smaller quantities available in egg yolks and meat. Plant sources of vitamin D are in the form of vitamin D2, which is produced through the ultraviolet irradiation of ergosterol from yeast and

mushrooms.[81]Several advanced nations have launched nationwide fortification programs to improve vitamin D status. Currently, vitamin D fortified foods are available in many countries. However, the fortification policies vary widely among countries. Vitamin D fortification and supplementation strategies implemented in the USA and Canada have significantly improved the vitamin D status in these nations. However, foods are rarely fortified with vitamin D in India. Two vitamin D fortification pilot studies in ostensibly healthy subjects were reported from India. These studies support the strategy of the fortification of foods in India for redressing malnutrition problems in India. They also suggest that to reach sufficiency, a daily intake of more than 1000 IU may be required to attain vitamin D sufficiency. Apart from these sources above, oral vitamin D supplements in different doses are widely available over-the-counter in most countries.[82] These probably offer the most effective, alternative way for those people who are unable to obtain adequate amounts of vitamin D from sun exposure or food sources.

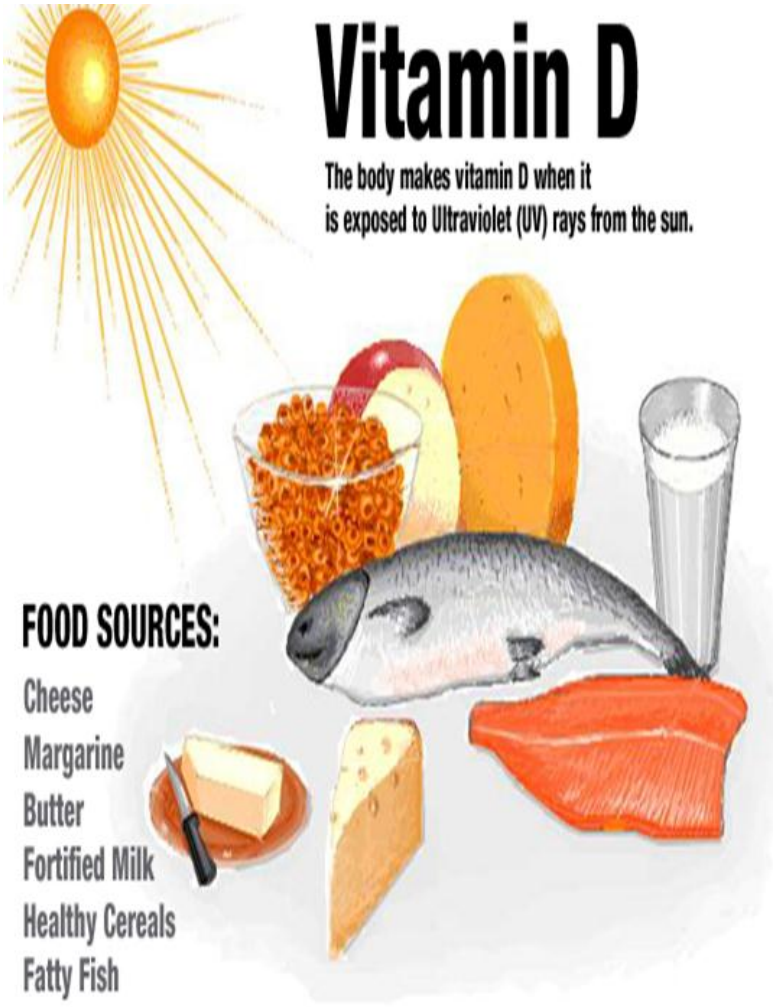


Figure 4 : Vitamin D food sources

Vitamin D, coming from actinic production or diet, enters the blood circulation where it is bound to the vitamin D binding protein (DBP), a major serum carrier protein for vitamin D and its metabolites with high affinity, which transports it to the liver and kidneys to undergo sequential hydroxylation.

The first step in the metabolic activation of vitamin D is hydroxylation of carbon 25 by vitamin D-25-hydroxylase, which converts vitamin D to 25-hydroxyvitamin D [25 (OH) D] in the liver. 25(OH) D is the major circulating form of vitamin D and the usual measure of vitamin D status for individuals.

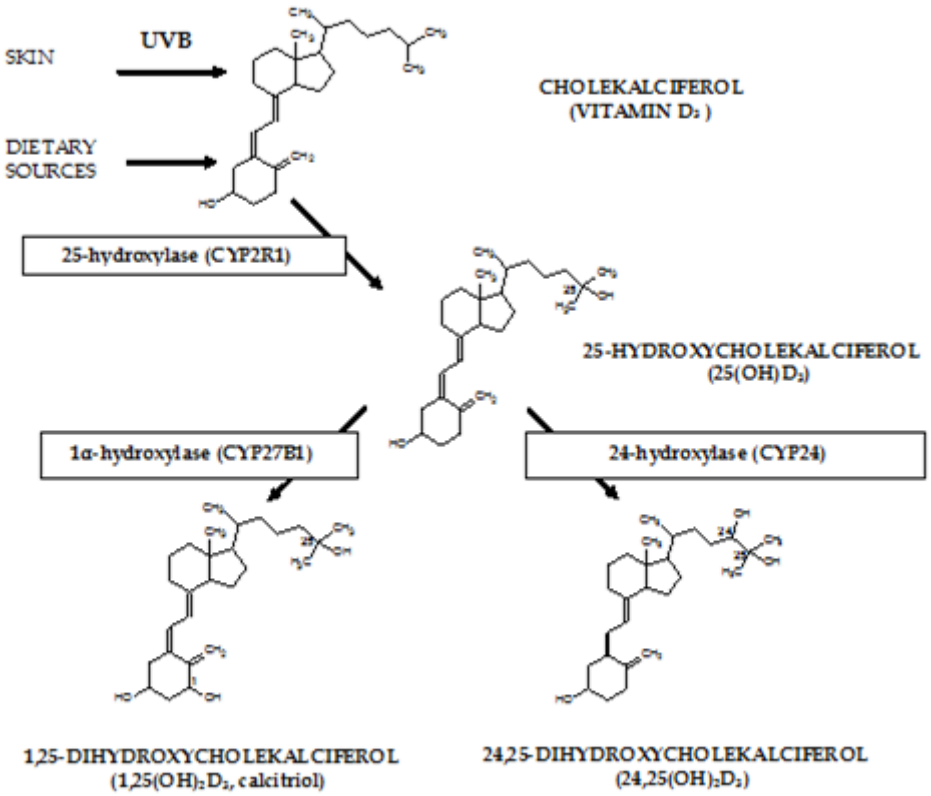


Figure 5 : Vitamin D pathway

The second hydroxylation is mediated by 25-hydroxyvitamin D-1-hydroxylase to convert it into the biologically active form, 1,25-dihydroxyvitamin D [1,25(OH)2D].This occurs mainly in the kidneys. Studies have found that 1 hydroxylation may also occur in many other extrarenal sites including the breasts, lungs, placenta, colon, osteoblasts and activated macrophages. This finding indicates an autocrine -paracrine role for 1,25 (OH)2D.(6) 25-hydroxyvitamin D-24-hydroxylase,which is a multicatalytic enzyme, catabolizes both 25(OH)D and 1,25(OH)2D to the water-soluble, biologically inactive, calcitroic acid, which is then excreted in urine.(70) Figure 5 depicts the vitamin D pathway.[83]

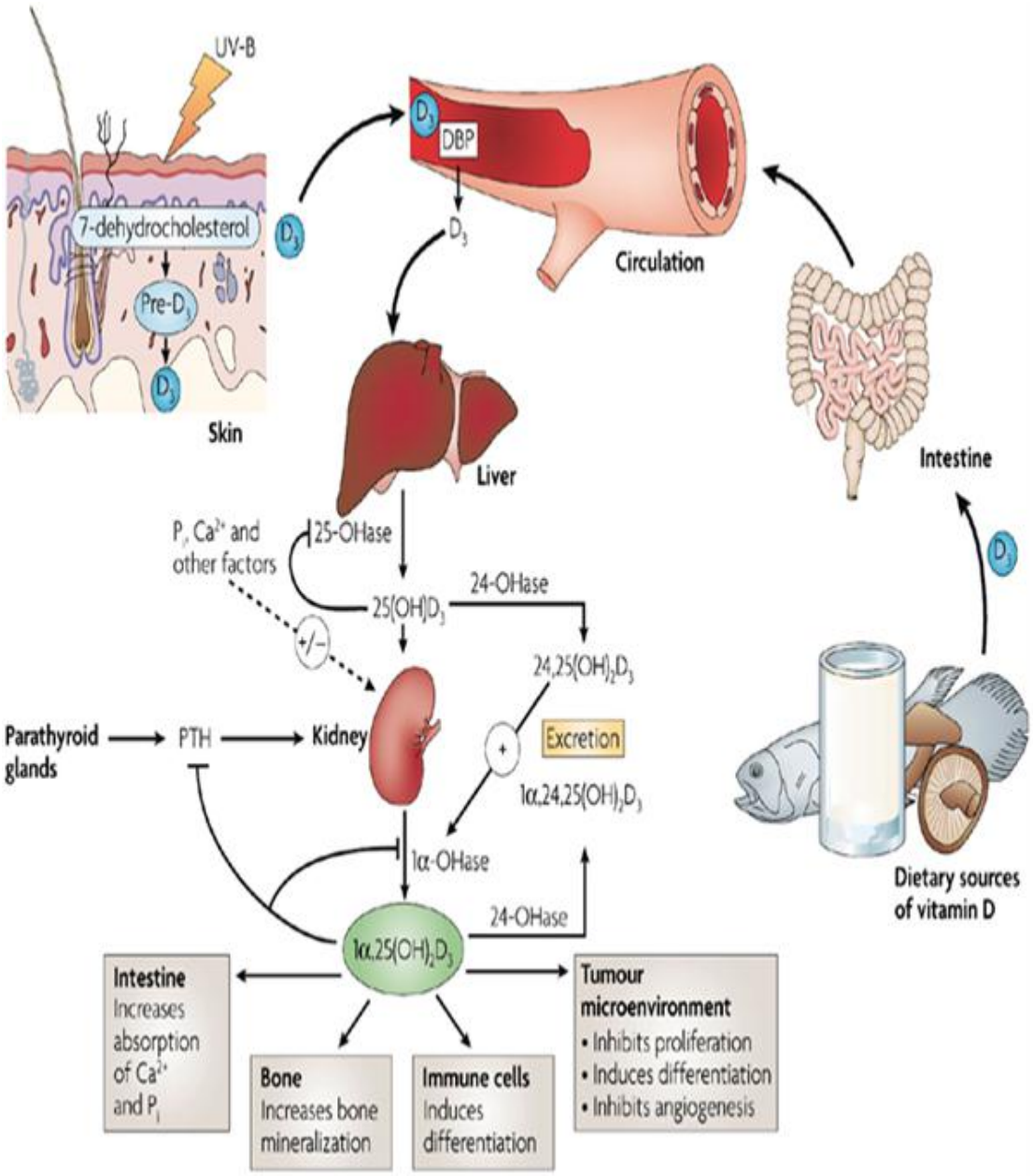


Figure 6 : Pathway of vitamin D synthesis and metabolism

Assessment of Vitamin D Status

Currently the measurement of the major circulating form of vitamin D, 25(OH)D, is the gold standard for determining individual vitamin D status. Serum 25(OH)D reflects vitamin D inputs both from cutaneous synthesis and dietary intake. Although 1,25 (OH)2D is the active form of vitamin D, it is not used for determining vitamin D status. The reasons are as follows:

- Firstly, the half-life of 25(OH)D is two to three weeks, much longer than that of 1,25(OH)2D, which has a half-life of only about four hours.
- Secondly, 1,25(OH)2D is usually normal or even elevated in patients with vitamin D deficiency.
- Thirdly, the concentration of 1,25(OH)2D is at picomolar levels, 100- to 1,000-fold less abundant than 25(OH)D in the blood circulation. It is more difficult to be detected, therefore, 1,25(OH)2D concentration does not reflect long-term vitamin D status.

Furthermore, low 25(OH)D has been linked with classic conditions of vitamin D deficiency, such as hypocalcemia and secondary hyperparathyroidism. Likewise, an increasing 25(OH) D level has been correlated with recovery from these conditions. Testing of serum 25(OH)D is most useful in patients who are at risk of vitamin D deficiency, including elderly patients, children with rickets and adults with osteoporosis. This measurement is also useful for purposes of planning or monitoring vitamin D therapy.

Classification of Vitamin D Status

Partly due to differences in 25(OH)D measurement techniques and the variability of vitamin D levels in the human body, there is a lack of consensus on the cut-off points that denote different vitamin D status categories.

In 2008, a review by vitamin D expert, Professor Michael Holick,(8) reported the following guidelines:

- Vitamin D deficiency: 25(OH)D< 50nmol/L (20ng/mL)
- Vitamin D insufficiency: 25(OH)D 51–74nmol/L(21–29ng/mL)
- Vitamin D sufficiency: 25(OH)D>75nmol/L (30ng/mL)
- Vitamin D toxicity: 25(OH)D> 375nmol/L(150ng/mL).

As the suppression of parathyroid hormone (PTH) is seen as beneficial for bone, serum 25(OH)D concentrations >75nmol/L are seen as desirable, as this is the concentration at which PTH approaches a minimum level and intestinal calciumabsorption is maximal. Notably, recent evidence suggests that the optimal concentration of 25(OH)D may be even higher, at least 80nmol/L, with regard to the potential role of vitamin D in non-bone health conditions.[84] The latest recommendations from the Institute of Medicine (IOM) of the United States of America advise that a serum 25(OH)D level of 50nmol/L(20ng/mL) would cover the requirements of 97.5% of the population, even under conditions of minimal sun exposure. Therefore, it supports < 50 nmol/L as vitamin D deficiency. However, this issue is still controversial.

To look at the other side of the coin, there are numerous studies which have thrown light on Vitamin D toxicity causing non-specific symptoms such as anorexia, weight loss, polyuria, and heart arrhythmias. More seriously, it can also raise blood levels of calcium which leads to vascular and tissue calcification, with subsequent damage to the heart, blood vessels, and kidneys. A serum 25(OH)D concentration consistently >500 nmol/L (>200 ng/mL) is considered to be potentially toxic .

Functions of Vitamin D

The main function is to regulate the plasma levels of calcium and phosphate with the help of biologically active form of vitamin D, Calcitriol (1, 25-DHCC). It acts at 3 different levels (intestine, kidney and bone) to maintain normal plasma calcium (9-11mg/dl). When it is formed in the kidneys or in extra-renal sites, is regulated by its binding efficiency with nuclear receptor (Vitamin D Receptor, VDR).

When these two combine together, resulting in a conformational change in the VDR, allowing it to bind with specific DNA sequences which is called as vitamin D

response elements (VDREs), that works on on target genes to activate or repress transcription of gene to engender biological actions of 1,25(OH)2D.

VDR is widely distributed throughout the different tissues of the human body, which indicates a wide range of biological functions of vitamin D.

The functions are categorized into two parts for general effects:

- Firstly, calcitriol helps in regulation of serum calcium and phosphate levels by actions at different sites involving intestine, bone, parathyroid and kidney.
- Secondly, Vitamin D has other roles in the body, including modulation of cell growth, neuromuscular and immune function, and reduction of inflammation. Many genes encoding proteins that regulate cell proliferation, differentiation, and apoptosis are modulated in part by vitamin D.

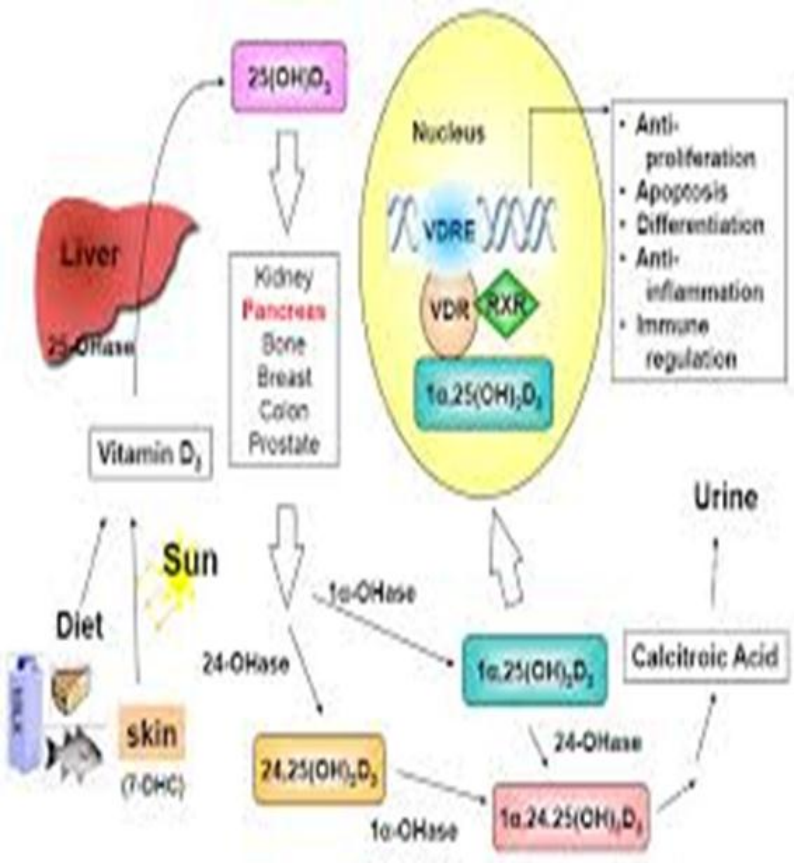


Figure 7 : Actions of Vitamin D

The major function of calcitriol on the intestine is to increase the efficiency of intestinal absorption of calcium and phosphate. Action on the bone is, calcitriol stimulates calcium uptake for deposition in the form of calcium phosphate. This is the reason why calcitriol is essential for bone formation. It works along with PTH hormone to increase the mobilization of calcium and phosphate from the bone which causes elevation in plasma calcium and phosphate levels. Action on the kidney is that calcitriol helps in minimizing the excretion of calcium and phosphate through kidney by reducing their excretion and enhancing reabsorption.

As a result, in vitamin D deficiency the serum calcium concentration may be normal, but bone mineralization is impaired. Severe vitamin D deficiency causes rickets in children and osteomalacia in adults and possibly contributes to osteoporosis.

There is some recent evidence suggesting that higher vitamin D status in the body is protective against prediabetes, metabolic syndrome and various cancers. Also adequate requirement of vitamin D is important for muscle performance and it may reduce the risk of falling in elderly people.[85]

The non-classic functions of vitamin D can be classified into 3 categories:-

1. Regulation of hormone secretion,
2. Regulation of cellular proliferation and differentiation, and
3. Modulation of immune function.

Therefore, it can be concluded that vitamin D insufficiency is linked with variety of diseases as it plays an important role in human health rather than bone health alone making researchers keep their enthusiasm in discovering the myriad of vitamin D.

Causes of Vitamin D Deficiency

There are a variety of factors attributing to decreased serum vitamin D levels in individuals ranging from living environment to lifestyle including physical characteristics.

Low ambient, ultraviolet radiation levels

At higher latitude, people are more likely to have a low vitamin D status. In winters, very small amount of vitamin D3 is produced in the skin because of reduced incidence of UV-rays with more latitude and also UVB photons need to pass through a greater distance of atmosphere. That is why, fewer photons reaches the earth. In winter at above 37° north latitude, the number of UVB photons reaching the earth's atmosphere is reduced to 80%. Clouds cover industrial pollution which reduces the amount of UVB irradiation reaching to the earth's surface.[86]

Limited sun exposure

Up to 95% of the body's vitamin D requirement comes from the synthesis in the epidermis on sun exposure. It is the. The time spent outdoors is an important factor in determining individual exposure to sunlight but due to lifestyle changesin the recent times, this has become difficult. Various factors that contributed to led people out of the sun are use of sunscreens, playing indoor games, excessive screen time (educational as well as non educational) and clothing. Additionally, solar radiation is also reduced by shades and window panesblocking the UV rays and hitting the skin by 60%.[87]This is the reason which mainly contributed to the higher prevalence of vitamin D deficiency in India and worldwide.

Inadequate vitamin D intake

Limited intake of foods rich in vitamin D such as milk products, fatty fish including liver oils which are excellent natural sources and deficiency of this can cause variety of diseases. Also no use of supplements may result in vitamin D deficiency. It was found that breastfed infants are at higher risk of developing vitamin D deficiency because human milk is not a good source of vitamin D even the content is lesser than infant formula. Therefore, even in a vitamin D-sufficient mother, the amount of vitamin D metabolites present in human milk cannot meet the required intake of vitamin D.

Physiological characteristics

Pigmentation reduces vitamin D production in the skin. Vitamin D deficiency can be secondary to a wide range of underlying causes, such as disorders of the gut, pancreas, liver and kidney, though most common reasons being insufficient sun exposure and nutritional deficiency. Vitamin D deficiency is common among elderly people because the capacity of the skin to synthesize the provitamin calcidiol (25-hydroxycholecalciferol) decreases with age.

Medication

There are various medications that reduce the vitamin D metabolism which are: anti-seizure drugs and glucocorticoids ultimately leading to vitamin D deficiency in patients.[88]

Socioeconomic status

Socioeconomic status plays an important role in the diet of a person. It determines the intake of a balanced diet along with adequate intake of all micronutrients and vitamins. Studies in different parts of the world have demonstrated correlation between the living condition and lifestyle and vitamin D status. Repeated unplanned and unspaced pregnancies in dietary deficient patients can aggravate Vit D deficiency in the mother and the foetus.

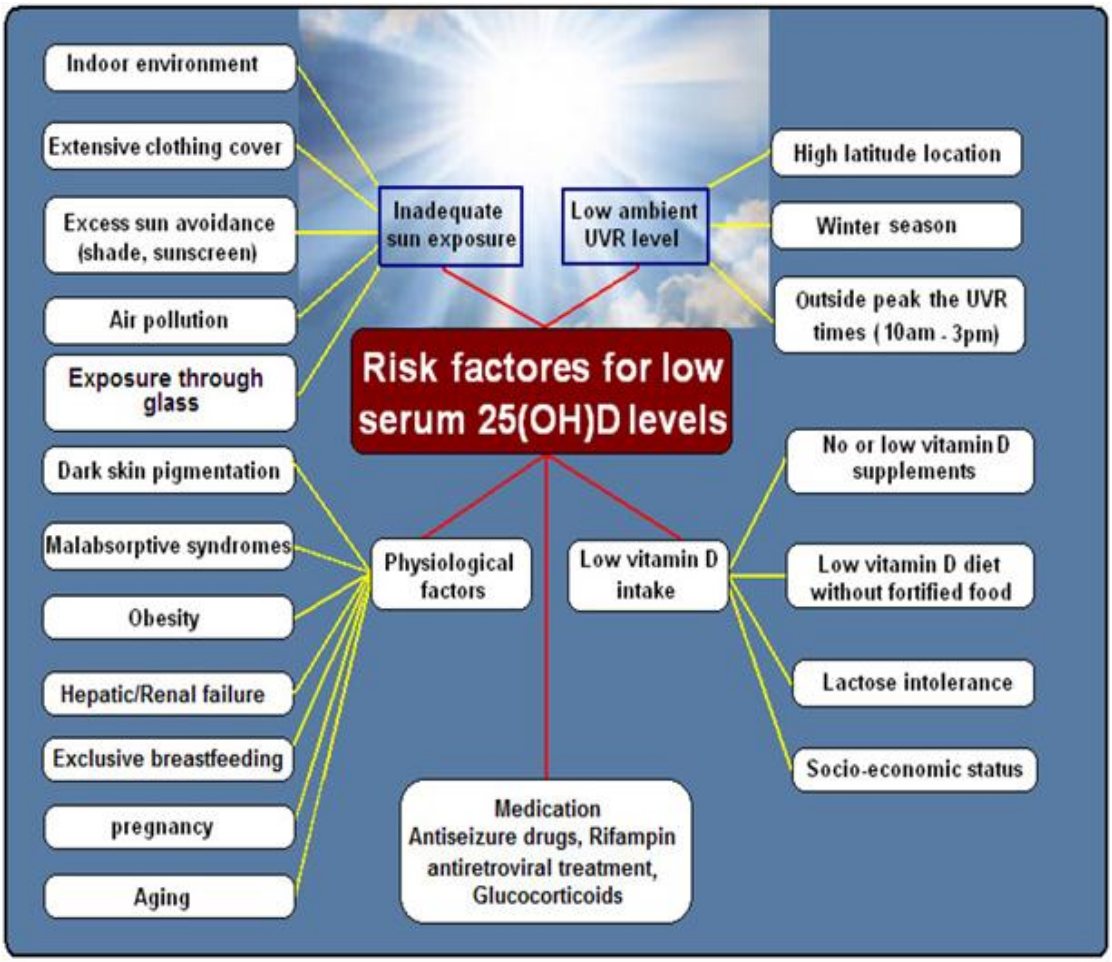


Figure 8 : Causes related to vitamin D deficiency.

Mechanisms of Vitamin D in Regulation of Blood Pressure

Vitamin D receptor (VDR) is an intracellular receptor which is present in thirty-six tissues which heterodimerize with the retinoid X receptor (RXR) and 10 tissues possess 1α-hydroxlyase besides the renal proximal tubule. This means that there is need for vitamin D in cells and various tissues during their biological processess. Various mechanisms have been suggested to be associated in the pathogenesis of hypertension.

An important factor responsible for the development of hypertension is the inadequate activation of renin-angiotensin-aldosterone system (RAAS). This is because, blockers of the RAAS, for example, inhibitors of renin, angiotensin-converting enzyme inhibitors, angiotensin II type 1 receptor antagonists, and mineralocorticoid receptor antagonist plays a major role in the treatment of hypertension. It was also reported that in animals and human studies, this vitamin D hormone was shown to regulate the RAAS at the clinical, pathophysiological and molecular levels.

The authors in some epidemiological studies proposed an inverse correlation between renin levels vitamin D and were published several years back.[89]Forman et al conducted a study recently to establish this relationship among 184 normotensive subjects.[90] The results of this study indicated that the there will be circulating angiotensin in individuals with suboptimal vitamin D levels along with blunted renal plasma flow responses to infused angiotensin II suggestive of activation of the RAAS in the lower plasma 25 (OH) D.

In a study done by Li et al. documented that without VDR there will be increased renin gene expression and plasma angiotensin II in vitamin D deficiency.[91] Consequently, there was raised blood pressure,cardiac hypertrophy and increased water intake in VDR null mice.

Similarly in another study, using mice showed similar phenotypes with VDR null mice lacking 1-α hydroxylase. This suppressive action of vitamin D on renin was independent of extracellular calcium or phosphorus.

One of its kind, Mechanistic study was performed related to theories but explains phenomenon by physical precess alone using the mouse Ren-1c gene promoter. The results indicated that 1, 25(OH) 2D3 binds to the VDR, and subsequently liganded VDR blocks formation of the cAMP-response element-binding protein complexes in the promoter region of the renin gene, resulting into to down-regulation of gene expression.

Recent studies suggested this concept of immunologic basis which is also mediated with the help of common hypertension.The degree of hypertension induced by angiotensin II or norepinephrine infusion was markedly blunted in the experimental studies using mice which lack T or B cells.It was also found that cytokines released from T cells and other inflammatory cells might be related with the development of prominent hypertension.

This was related to the fact that TNFα antagonist has been proven to be effective in reducing hypertension in the mice fed withfructose. It was also reported by various authors in some studies that interleukins such as IL-6, IL-17, and IL 10 were also involved in hypertension. Hence, it is has been confirmed with the help of many studies that in various diseases, vitamin D plays an important role in modulating innate and adaptive immune response.

Vitamin D works to regulate toll like receptor (TLR) signaling, which helps in reducing the levels of gene expression and protein release of proinflammatory

mediators, such as TNFα, IL-6, and MCP-180. It can be concluded from these perspectives, that anti-inflammatory effects of vitamin D could diminish the development of hypertension.

Sugden JA et al conducted a study evaluate whether vitamin D2 can improve or not the endothelial function in type 2 diabetes patients with low serum (OH)D level.[92] Since, endothelial cells were reported to consist VDRs, and supplements of vitamin D to improve endothelial functions in previous reports. The results indicated that a single dose supplement of 100,000U ergocalciferol (vitamin D2) significantly improved flow mediated vasodilation (FMD) of the brachial artery by 2.3% and reduced systolic blood pressure by 14mmHg in comparison to placebo.

In another study, asymptomatic people with vitamin D deficiency were found to have a lower FMD (flow mediated dilatation) measurement which was found to be significant but it was found to be increased after three month-replacement of vitamin D3. The results suggested that higher prevalence of vitamin D deficiency was due to loss of vitamin D binding proteins in the urine, among patients with chronic kidney disease (CKD).They also have ineffective vitamin D photosynthesis in the skin with low level of 1α-hydroxylase activity and elevated FGF23 levels.

With the help of various observational studies, author identified positive relationship between the level of 25(OH) D and flow mediated dilatation in CKD patients. In this aspect, author conducted an animal study and found that absence of vitamin D activities can cause low reduction in the expression of endothelial nitric oxide synthase leading to more arterial stiffness.[93]

The Effects of Vitamin D Supplement on Hypertension

Many interventional studies have been conducted till date but still the effect of vitamin D supplementation in reducing blood pressure in humans is still controversial. In a double-blind, placebo-controlled trial study conducted by Lind L et al in the late 1900's found that the reduction in diastolic blood pressure with treatment by 1 µg alphacalcidol, (a synthetic analogue of active vitamin D) for 6 months was obtained in the subjects with hypercalcemia (n=29) or primary hyperparathyroidism (n=33).[94, 95] However, the effect of alphacalcidol on hypertension is still not proved among subjects with impaired glucose tolerance (n=16).

A study was conducted by Krause R et al which reported that short term exposure to ultraviolet B had effects in reducing blood pressure in patients with untreated mild hypertension and with raised concentration of plasma 25(OH) D.[96]

In other studies, it was reported that among type 2 diabetes patients, even a single high dose of active vitamin D (100,000 IU) is helpful in improving systolic blood pressure where as the highest single dose of vitamin D3 (300,000 IU) among patients with type 2 diabetes did not lower down blood pressure. Hence, the daily supplementation of vitamin D3 (1,000-5,000 IU) has failed to prove the effect on blood pressure.

Likewise in a study by Sharb-Bidar S et al found that in 100 patients with type II diabetes, the combination of vitamin D3 plus calcium supplementation had an effect on SBP and DBP [97]

Women's Health Initiative Calcium/Vitamin D conducted a randomized double blind trial in 36,282 postmenopausal women for 8 weeks showed that vitamin D3 plus

calcium improved blood pressure in elderly women. However, vitamin D3 (400 IU) plus calcium (1,000mg) daily supplementation was not effectivein reducing blood pressure when followed for 7 years.

This was reported from various studies possible that vitamin D could lower BP in some specific race/ethnic groups therefore, skin color is a factor of circulating levels of 25(OH) D. This is the reason why African-Americans are known to have significantly higher levels of hypertension than whites.

In a study conduted by Forman JP et al evaluated that for 3 months SBP was reduced after the supplementation of vitamin D3 when compared to placebo. Interestingly, more individuals were noted with SBP lowering with less vitamin D levels (<20 nM/mL).

In a study done by Scragg R et al. found that in healthy adults who were predominantly whites had no effects of high dosage of vitamin D3 on blood pressure control for 18 months.[98] However, due to smaller sample size (n≤100) conducted for shorter duration among non-white populations failed to prove the effect of vitamin D on BP.

It was estimated from the recent report that several meta-analysis have been conducted in normotensive or hypertensive individuals (429 participants) to know the effect of vitamin D supplementation on hypertension. One such was conducted by Wu including four double-blind randomized controlled trials (RCTs) for oral vitamin D supplementation. It was found that vitamin D significantly decreased SBP by 2.44mmHg but was not useful in DBP. They also recommended that the change of blood pressure was not affected by the dosage of vitamin D asupplementation, study length, in subgroup anlysis. However, some of the reports of meta-analyses reported that vitamin D supplementation was not beneficial for blood pressure control.

In a study conducted by Kunter SK et al in which he reported about 16 RCTs of oral vitamin D (vitamin D2 or D3) and concluded that there was no significant effect of vitamin D in reducing blood pressure.[99]. With the help of subgroup analysis it was found that significant reduction in DBP was recorded among the participants with cardiometabolic disease.[100]

A study done by Beveridge KA et al in which he included 46 RCTs (4,541 participants) in the trial-level meta-analysis and individual data from 27 RCTs (3,092 participants) which used active or inactive forms of vitamin D or vitamin D analogues for more than 4 weeks. The results indicated that there was no effect of vitamin D in the treatment of BP which was observed with the help of trials and data collected individually.

In this meta-analysis, Qi D et al analyzed total 917 individuals from eight RCTs using active vitamin D for more than 3 months.[101]The results indicated only minor reduction in the blood pressure.

Hence, the use of vitamin D or its analogues in treatment of individual patient for hypertension to lower BP is not marked. The results of RCTs and meta-analysis do not support the use as a population-level intervention to lower BP. These discrepancies might be due to heterogeneity of patient baseline characteristics, differences in sample size and follow-up periods, and different vitamin D dosages. Instead many experimental and epidemiologic studies have showed the roles of vitamin D in controlling BP in various ways. Further research related to RCTs are required to confirm the real effect of vitamin D on reduction in blood pressure and define the optimum dose, dosing interval, and type of vitamin D to administer.

VITAMIN D IN PREGNANCY

Deficiency of vitamin D is a major health problem especially during pregnancy. It not only increases the chances for risk of infantile rickets but may also result in poor fetal growth and development. Additionally, the lack of vitamin D may also predispose to gestational diabetes mellitus and preeclampsia. Hence there was a need for this review of the changing metabolism of vitamin D during pregnancy and possible health consequences of insufficiency of maternal vitamin D.

Adaptations of Vitamin D Metabolism in Pregnancy

Adequate intake of vitamin D is essential for the growth and development of maternal and fetal health as the body goes through several changes in order to optimize fetal growth. Vitamin D is known to produce insulin secretion by pancreatic beta cells thereby affecting levels of glucose. The deficiency of vitamin D in early pregnancy increases the risk for gestational diabetes in later stages of pregnancy. At the third trimester, fetal calcium deposition has been shown to peak at 350 mg/day and at the end of pregnancy about 25 to 30 grams of calcium is transferred to the foetus.[102]

There are three possible ways that increased calcium requirements can be met:

1. Maternal intestinal absorption of calcium is increased .
2. Reduction in maternal renal excretion of calcium.
3. Higher resorption of calciumfrom the maternal skeleton.

Regardless of the reduction in excretion of renal calcium, there was less concentration of maternal serum calciumin the pregnancy, reaching the bottom level at mid-gestation making ionized calcium normal as a result there is expansion in plasma volume with a fall in serum calcium and serum albumin levels.

The concentration of 1, 25(OH) 2Dis increased throughout pregnancy to adapt for the changes that takes place in serum calcium so that there is more efficiency of

absorption of intestinal calcium. Several studies, reported thatduring the first trimester of a normal pregnancy, maternal serum concentrations of 1, 25(OH) 2D is higher.

During the second trimester, the levels were 50 to 100% more in the state of non-pregnancy and during the third trimester, it was found to be 100% providing sufficient calcium to the foetus during pregnancy.The intake of 1, 25(OH) 2D may arise from several other origin as with the help of immunohistochemistry, the analysis of 1α-hydroxylase expression have investigated the presence of the enzyme in both placental and decidual tissue.

In a crosssectional study done by Maghbooli et al on 741 pregnant women it was evaluated that a total prevalence of deficiency of vitamin D (< 25 nmol/l) was found to be 70.6%.[103] However,during pregnancy, the results are not suggestive of change in plasma 25(OH)D levels.

Also a longitudinal study conducted by Holmes et al found that plasma 25(OH)D concentrations were significantly reduced in pregnant women, when compared with non- pregnant women, at 20 weeks ($P<0.0001$) and 35 weeks of gestation ($P<0.0001$).[104]However, no studies have been conducted in this regard to prove these findings correct.

Prevalence of Vitamin D Deficiency in Pregnancy

Globally, among pregnant women, higher prevalence indeficiency of vitamin D was noted. The range varied from 5% to 84% (this huge difference in percentage may be due to use of different cut-off points). Thus, lack of vitamin D in pregnancy is epidemic and is the most under-diagnosed and under-treated nutritional deficiency.

In a study done by Bodnar et al found that insufficiency of vitamin D was common among both white and black pregnant women, even when mothers gave compliance with intake of prenatal vitamin D(109). At the time ofdelivery, it was found that lack and insufficiency of vitamin D took place in 29.2% and 54.1% of black women in comparison to 55% and 42.1% of white women, respectively.

In a study conducted by Wang et al investigated 77 pregnant women and reported that the mean level of 25(OH) D was 36.0±19.7nmol/L. About 57.1% women showed deficiency of vitamin D (25(OH) D< 37.5nmol/L) followed by insuffiency of 97.4% (25(OH) D 37.5-80nmol/L).[105]

Similarly, in an another study conducted by Sachan et al reported that, the mean maternal serum 25(OH)D was only 35±22.5nmol/L leading to increased prevalence rate (84%) of vitamin D deficiency(56).A study conducted by Sahu et al alsoshowed that approximately74% of pregnant women had vitamin D deficiency.[106]In a study, it was reported by Kazemi et aI that higher prevalence of vitamin D deficiency in pregnant women has been noted in a differentcountries[107] and same findings were also reported by Javaid et al [108] Weiler et al [109] and Cavalier et al.[110]

Effects of Vitamin D Deficiency in Pregnancy on maternal health

In pregnancy, reduce maternal vitamin D status has been found to be related with variety of deleterious outcomes in pregnancy (Grundmann& von Versen-Höynck, 2011.[111]

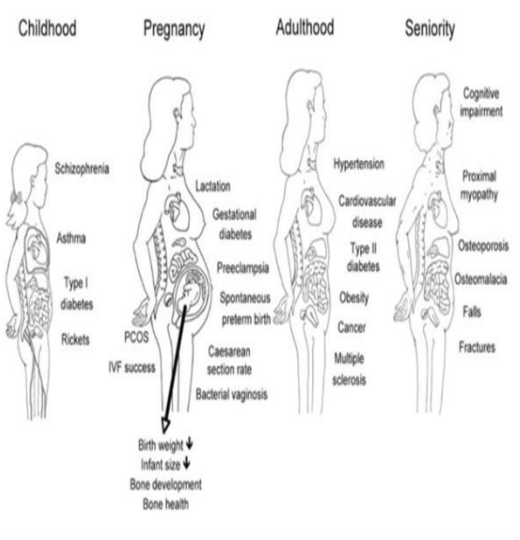


FIGURE 9 - Possible implications of vitamin D deficiency in pregnancy (Grundmann& von Versen-Höynck, 2011).[112]

During pregnancy, deficiency of vitamin D has detrimental effects on maternal bone health with an increased risk of preeclampsia associated with insulin resistance followed by gestational diabetes mellitus including primary caesarean section and bacterial vaginosis.

Aghajafari et al conducted a systematic review and meta-analysis in 2013, to assess an association between insufficiency of 25-OHD and adverse pregnancy outcomes including birth variables. The results reported that insufficient serum levels of 25-OHD were linked with gestational diabetes (pooled odds ratio 1.49, 95% confidence interval 1.18 to 1.89), pre-eclampsia (1.79, 1.25 to 2.58).

Preeclampsia

There are various mechanisms related to the pathophysiology of preeclampsia due to which vitamin D is supposed to have both direct and indirect effects on immune dysfunction, hypertension and inflammation.In a nested case control study conducted by Bodnar et al reported that in early pregnancy,there exists a significant relation between 25(OH)D concentrations and subsequent preeclampsia.[15] The results of the

study indicated that pregnant women have five-fold increased risk of preeclampsia with 25(OH)D levels <37.5ng/mL.

In another prospective study, conducted by Haugen et al investigated thatthere was 27% reduced risk of preeclampsia for pregnant women taking 10 to 15µg/d (400 to 600IU per day) of vitamin D when compared with women taking no supplements (OR=0.73, 95%CI: 0.58-0.92).[113] It is hypothesized that lower calcium levels, perhaps through mediation of vitamin D, ultimately lead to the formation of preeclampsia. More studies related to interventional trial with larger sample size arerequired to further prove whether vitamin D supplementation can decrease or not the incidence of these complications in pregnancy.

Gestational diabetes mellitus (GDM)

The deficiency of vitamin D may result from impaired glucose metabolism. Various Studies explained the role of vitamin D in maintaining normal glucose homeostasis.There is no sufficient data regarding the development of gestational diabetes (GDM) and vitamin D.From various studies, it is known that 1, 25(OH) 2D stimulates secretion of insulin as one of its non-classic functions. Many authors also reported an inverse association between the risk of GDM and higher fasting glucose levels with maternal plasma 25(OH) D concentrations. In a study done by Maghbooli et al found that the prevalence in GDM patients of severe vitamin D deficiency was higher in comparison to normoglycemic pregnancies (< 12.5).

A nested, case-control study was done by Zhang et al which included 953 pregnant women and recorded that at 16 weeks of gestation,maternal plasma 25(OH)D concentrations, at an average of, were significantly low in women who subsequently developed GDM in comparison to controls.[114] The authors also found that among

non-hispanic and white subjects each 12.5nmol per litre decrease in 25(OH)D concentrations was related to a 1.29-fold increase in GDM risk, (OR=1.29, 95% CI: 1.05-1.60).

Soheilykhah et al conducted a matched case-control study which included 54 women with GDM and 11 women with normoglycemic controls. It was reported by authors that at 24–28 weeks of gestation,maternal 25(OH)D concentrations were significantly less in women with GDM.[115] The results indicated that 83% of women with GDM had 25(OH)D levels <50 nmol/L when compared with 71% of controls.

In a study done by Hossein-Nezhad et al estimated that about 29% of women had 25(OH) D levels <15 nmol/L. The prevalence of GDM in this subgroup was more when compared to women with 25(OH) D levels ≥35 nmol/L.This difference was found to be statistically significantwhereas other studiesdo not detect a statistically significant association between 25(OH) D level and GDM.

A study conducted by Farrant et al investigated 559 pregnant women and found no relation between GDM and second trimester 25(OH)D levels.[116] Also Makgoba et al assessed 90 cases of GDM and 158 controls and reported no link between blood samples in first trimester and subsequent development of GDM.

Primary caesarean section and bacterial vaginosis

Various unexpected maternal outcomes may be associated with the intake of low vitamin D status as well. Recently, Merewood et al conducted a studyto investigate that there was an inverse association between serum 25(OH) D levels and the risk of having a primary caesarean section.[117] The results indicated that women with 25(OH)D< 37.5nmol/L were almost four times prone to have a caesarean section than those with 25(OH)D ≥ 37.5nmol/L (adjusted OR=3.84; 95% CI: 1.71-8.62).

This could be explained by poor functioning of muscles which results from the deficiency of vitamin D. (127) Another study conducted by Bodnar et al found that the maternal deficiency of vitamin D was associated with bacterial vaginosis because of the actions of 1,25(OH)2D on the immune system in the first trimester of pregnancy.[118]As the status of vitamin D improved ($P < 0.001$) the prevalence of bacterial vaginosis also get reduced. Nearly 57% of the women had bacterial vaginosis with a serum 25(OH) D concentration of< 20 nmol/L compared with 23% of women with a serum 25(OH)D concentration > 80nmol/L.

Bone health

It has been demonstrated from various studies that pregnancy and lactation have been related to reduction in bone density. During whole pregnancy approximately 2% to 5% of maternal bone loss was seen.There are various cases of pregnancy-associated osteoporosis have been reported till date but no study has directly investigated whether deficiency of vitamin D affects maternal bone health during pregnancy. Only one study showed that pregnant women had greater bone loss in winters than those in summers, which evaluated a possible vitamin D effect.

Offspring Health Consequences of Maternal Vitamin D Deficiency in Pregnancy

Maternal Vitamin D deficiency is common and is highly influenced by variables such as ethnicity, sun exposure and vitamin D supplementation. It was found that relationship exists between cord and maternal blood concentrations of 25(OH) D from various evidences.During pregnancy, sufficient vitamin D status is indispensable because it meets requirements of maternal responses to the calcium demands of the foetus and neonate, as the intake of vitamin D to them is highlydependent on the mothers.

Vitamin D has many outcomes before the birth of foetus, as well as soon after birth, that includes congenital rickets, hypocalcemia, with impaired neurocognitive development, risk of infantile autism, language difficulties, Inflammatory and immune disorders. Besides skeletal formation, it is also involved in a wide range of physiological processeswhich may impair maternal, or offspring health, or both.[119]

Association of Vitamin D levels and hypertension

Mainly, there are 2 compounds of vitamin D in humans i.e. Vitamin D3 (cholecalciferol) and Vitamin D2 (ergocalciferol). Ultraviolet-B radiation penetrates in the skin and leads to the conversion of 7-dehydocholesterol to pre-vitamin D3, which then undergoes isomerisation to form vitamin D3 (cholecalciferol). It then undergoes hydroxylation (by the action of 25-hydroxyvitamin-D-hydroxylase) to form 25-hydroxyvitamin D (25(OH) D) in the liver which then gets converted to 1, 25-dihydroxyvitamin D3, the biological active form (1,25 (OH2)D3) in the kidneys, blood vessels, and heart promoting absorption of calcium.[120]

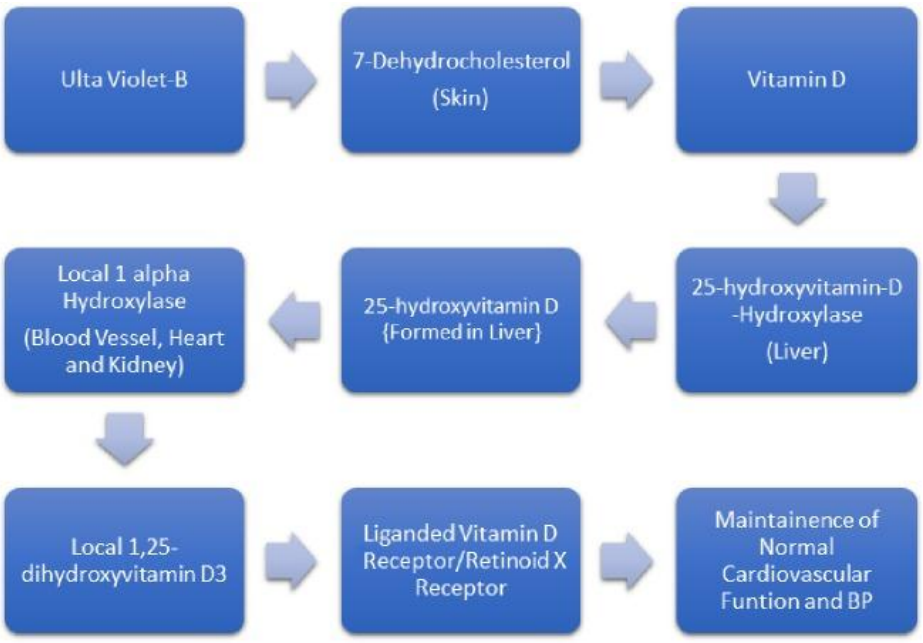


Figure 10 : Role of Vitamin D in Maintaining Blood Pressure[120]

In most of the tissues and organs, VDR (vitamin D receptor) is present including heart, endothelium, vascular smooth muscle, and T cells which possess 1 alpha-hydoxylase. It is a nuclear receptor to which vitamin D3 binds with higher affinity and specificity. This VDR is phosphorylated when it gets bounds with VD3, leading to a conformational change upon the surface. Now, VDR gets activated that interacts with the retinoid X receptor (RXR) creating a heterodimer which binds in the region of gene promoter to Vitamin D-responsive elements.

The connection with corepressors or coactivator complexes activates the VDR/RXR which regulates transcription of genes for protein synthesis that modulates different functions of VD3 (e.g., musculoskeletal health, calcium homeostasis, and normal BP and cardiovascular function).

With this, we can establish that the role of Vitamin D in a cell, tissue, or organ is based on the production of an adequate measure of VD3 and expression levels of VDR/RXR and co-receptor proteins, including cell-specific transcriptional elements to manage genes that encode proteins changing signaling of vitamin D.

Clinical studies

A study was conducted by Kota, et alto evaluate whether systolic, diastolic blood pressure, and mean arterial pressure is increased among subjects with deficiency of Vitamin D and found that Vitamin D deficiency is linked with regulation of renin-angiotensin-aldosterone system (RAAS).[121] The review of such kind investigated that individuals with higher amounts of vitamin D had decreased blood pressure and a low risk of developing hypertension.

A study was conducted in 2013 which included subjects with hypertension. The author observed that for every increased (10 ng/ml)inlevels of vitamin D, there was

less risk of developing hypertension was estimated. The general population with the most noticeable levels of vitamin D had a 30% less danger of developing hypertension when compared with the general population having lower levels. In any case, main part of the reviews were taken fromthe United States, concluding that authors cannot know without a doubt if the outcomes would be the same in different countries.[12]

Very few studies have been conducted till date to evaluate the impact of Vitamin D on blood pressure in individuals suffering from hypertension. One such author conducted a meta-analysis and indicated that Vitamin D supplementation can lower down systolic blood pressure.[123]

Jorde et al. in 2010 conducted a study to exhibit a connection between low serum Vitamin D levels and hypertension. But the results indicated that Vitamin D prescription does not have the capacity to prevent hypertension in the future is still not confirmed.[124]

A group of scientists conducted a study to check the effect of taking supplements of Vitamin D to prevent from the risk of hypertension in African-Americans in 2013 who had normal blood pressure. They studied 250 subjects and provided them with 1,000 IU per day, 2,000 IU per day, and 4,000 IU per day, or a placebo for 3 months. The results indicated that for every rise in supplements of Vitamin D levels in the body, systolic blood pressure was reduced whereas the diastolic pressure remained the same.

An experiment conducted in 2012 in Denmark investigated the effects of Vitamin D supplements in reducing blood pressure in subjects with hypertension. It was evaluated that subjects who had 3,000 IU per day of Vitamin D and a placebo pill for 20 weeks had lower blood pressure than the subjects under the placebo pill. The

author also studied different types of blood pressure and came to know that subjetcs in the Vitamin D group who had low levels of Vitamin D at the beginning of the study had major reduction in the blood pressure and concluded that vitamin D is found to be be more effective in reducing the blood pressure.[125]

Carrara, et al. in 2013 reviewed the role of Vitamin D supplementation in regulation of the BP in the body among Italian population.The study group consists of hypertensives subjects to which 25,000 IU per week of Vitamin Dwas givenfor a total of eight weeks. The author found out that vitamin D levels were increased throughout the study and BPwas highly reduced. The study concluded that Vitamin D may help to reduce the risk of hypertension.[126]

Vimaleswaran et al. conducted a study to assess whether 25(OH) D levels are significantly related with blood pressure and HTN risk.To Meta-analyse 146,581 participantsdifferent variants of genes that affect 25(OH) D synthesis or substrate availability was used. They recorded that 10% increment in each genetically instrumented 25(OH) D concentration was related to decrease in systolic BP (-0.37 mmHg, P = 0.052) and diastolic BP (-0.29 mmHg, P = 0.01), with an 8.1% reduction in the odds of HTN (P = 0.002). The results of this study conducted later, further established that increased 25(OH) D concentrations might reduce the risk for HTN.

Caro, et al. concluded that serum 25(OH) Vitamin D levels do not have any significant statistical association with blood pressure.[127] In 2008, an experiment used data from a large experiment that assigned women to receive either 1-gram per day of calcium, plus 400 IU per day of Vitamin D, or a placebo pill. They observed that there was no significant difference in blood pressure changes between both the groups.

A study was conducted by Lee et al to evaluate the difference between the levels of serum Vitamin D and parathyroid hormone (PTH). They found significant connection

in relation to hypertension among individuals of Chinese.[128]Also in a cross-sectional review of 251 individuals (aged 40 or more years old) by Kashi, et al., it was concluded that there was no association between hypertension and serum 25(OH) Vitamin D, calcium, and PTH levels.[129]

In a review conducted by Sanijder et al found that in old aged individuals various unknown elements might have an impact on the relation between Vitamin D and hypertension. They also reviewed that effect of Vitamin D on blood pressure might be indirectly based onparathyroid hormone performance.[130]

In a nutshell, author summarized thathypertension is one of the long-term medical disease, which can have impact on cardiovascular system which may ultimately lead to death. This study evaluated whether serum Vitamin D concentration is responsible for causing hypertension or not. As the opposing consequences lay down various reviews on the role of Vitamin D in prevention and treatment of the development of hypertension one of which was that Vitamin D levels in the body regulates the blood pressure indirectly. It needs further exploration with the help of studies in order to prove the association. It is recommended that physicians should alwaysexamine levels of Vitamin D in order to control the increasing incidence of hypertension.

Umar Net al[131] in 2016 conducted a cross-sectional study which compared serum 25-hydroxy vitamin D level in women having preeclamptic and normotensive pregnancies. The authors found there was deficiency of Vitamin D in 95% of preeclamptic and normotensive pregnant women. The difference of vitamin D level between the two groups was not found significant. Although there was an inverse correlation betweenserum vitamin D and systolic blood pressure and arterial pressure in preeclamptic group, but this was not statistically significant.

The authors concluded that Vitamin D deficiency does not seem to be affected by the state of preeclamptic and normotensive pregnancy.The correlation of systolic blood pressure and arterial pressure and vitamin D needs to be explored further by increasingthe sample size.

Sahu M et al[132] in 2017 conducted a prospective comparative study which evaluated the serum vitamin-D levels in normal pregnant females and pre-eclampsia or eclampsia individuals in the third trimester admitted for termination or in labour and to assess the neonatal outcome and neonatal serum calcium levels of babiesborn to mother in both the groups. The authors found most pregnant females had vitamin D deficiency pointing towards universal prevalence. Only 10% had suboptimal to optimal level of vitamin D while 90% had vitamin deficiency.

The hypertensive group had lower meanserum vitamin D level (9.06±5.20 ng/ml) as compared to normotensive group (13.67±7.24 ng/ml). Neonatal outcome was poorer in the hypertensive group. Neonates born to hypertensive mothers had lower mean calcium level (8.30±1.46mg/dl) in comparison to those born to normotensive mothers (8.82±0.918mg/dl).

The authors revealed that there exists a consistent involvement of hypertensive disorders with deficiency of maternal serum vitamin Dand neonatal morbidity.

Sonuga AAet al[133] in 2017 conducted a case-control study to assess serum levelsof 25-hydroxy Vitamin D (25(OH) D3) among normotensive pregnant women and preeclamptic women. The results of the study indicated that preclamptic women had significantly lower levels of VitaminD at 20 weeks (24.5±4.6 vs 36.59±5.1), 30 weeks (23.8±3.9 vs 34.14±3.7), and postpartum (21.7±5.5 vs 32.62±3.2) when compared to the control group. The authors concluded that Vitamin D insufficiency might be

related to preeclampsia and recommended that diet rich in Vitamin D and its supplementationcan help to lower the risk of preeclampsia and can also show better results for improved pregnancy outcomes in preeclampsia.

O'Callaghan KMet al[134] in 2018 conducted an interventional and observational study. Evidences from dietary studies on vitamin D were suggestive of the fact that hypertensive disorders in pregnancyare associated with lowmaternal vitamin D status and increased risk of hypertensive disorders. The results indicated towards anincreased risk of gestational hypertensive disorders at 25(OH) D concentrations <50 nmol/L.

The possibilitythat a fairly narrow target range for circulating 25(OH) D for achievement of clinically-relevant improvements requires further exploration. The author concluded that hypertension alone deteroriate intrauterine growth and assessment of the relationship between status of vitamin D statu and all types of hypertension in pregnancy is a suitable area for clinical research in future and should get priority in randomised trials.

MATERIAL AND METHODS

A hospital based cross sectional prospective comparative study was conducted to compare maternal Vitamin D and calcium levels of term hypertensive and normotensive pregnant women. 120 pregnant women were divided into following groups of 60 pregnant women each:

Study Group: 60 term hypertensive pregnant women in labour defined as BP>140/90 mmHg

Control Group: 60 term normotensive pregnant women in labour defined as BP<140/90 mmHg

Place of study: Department of Department of Obstetrics and Gynaecology, antenatal care in BLDE (Deemed to be University) Shri B. M. Patil Medical College, Hospital & Research Centre, Vijayapur.

Field of study: Tertiary health care center.

Study duration: 2 years (October 2016 to October 2018).

Study population: Pregnant women were divided into following groups of 60 pregnant women each:

Study Group: 60 term hypertensive pregnant women in labour defined as BP>140/90 mmHg

Control Group: 60 term normotensive pregnant women in labour defined as BP<140/90 mmHg

Study design: A hospital based cross sectional prospective comparative study.

SOURCE OF DATA:

Term pregnant women with singleton pregnancy attending outpatient department and admitted for antenatal care in BLDE (Deemed to be University) Shri B. M. Patil Medical College, Hospital & Research Centre, Vijayapur.

Sample size: 120 patients

Sample size was calculated using the formula:

$$n = \frac{(Z_{\alpha} + Z_{\beta})^2 2SD^2}{MD^2}$$

Where,

n=sample size

Z= Z Statistic at a level of significance

SD= Anticipated Standard deviation

MD= Anticipated mean difference

With anticipated mean difference of Vitamin D between two study groups as 6.2, anticipated standard deviation as 8, 90% power and level of significance as 1.1, the minimum sample size per group is 60. Hence sample size of 120 patients was selected for the study.

INCLUSION CRITERIA:

- Singleton pregnancy at term.
- All hypertensive pregnant patients (BP >140/90 mmHg) at term.

EXCLUSION CRITERIA:

- Women diagnosed with vitamin D Deficiency before the 3rd trimester.
- Multiple pregnancy.
- Polyhydraminos.
- Medical diseases like Diabetes Mellitus, Hypothyroidism.
- Pregnant patients at term having anaemia i.e., Haemoglobin < 7gm /dl.

METHODOLOGY

On enrolment, brief history and clinical examination was done.

In both the enrolled groups blood samples for vitamin D and serum calcium were drawn and sent to the laboratory and subsequently their levels were evaluated in cord blood also.

Vitamin D deficiency is defined as 25(0H) D levels below 15ng/ml.

Severe vitamin D deficiency is defined as below 10ng/ml.

Maternal calcium deficiency is defined as blood calcium levels below 8.5 mg/dl.

Cord blood vitamin D deficiency is defined as below 10 ng/dl.

Cord blood calcium levels deficiency is defined as below 8 mg/dl.

Additional factors such as BMI, age, mode of delivery, fetal outcome were also studied in both the groups.

All the pregnant mothers who agreed to participate in this study gave their informed consent prior to their inclusion in the study. On admission, patient demographic profile, complete history was recorded, and comprehensive clinical examination was done. In all the patients, blood samples for routine examination along with LFT, RFT, random blood sugar, serum electrolytes, serum uric acid, serum vitamin D and serum

calcium were drawn, and serum levels of these biochemical parameters were determined according to standard laboratory procedures. Serum vitamin D quantification was done by chemiluminescent assay. Those with diabetes mellitus, anemia, renal failure, hypothyroidism, multifetal gestation and immunosuppressive disorders were excluded from the study.

All women were followed up until delivery and early postpartum period. Neonatal outcome in terms of maturity, complications and serum vitamin D and serum calcium was assessed.

STATISTICAL ANALYSIS

Quantitative data is presented with the help of Mean and Standard deviation. Comparison among the study groups is done with the help of unpaired t test as per results of normality test. Qualitative data is presented with the help of frequency and percentage table. Association among the study groups is assessed with the help of Fisher test, student 't' test and Chi-Square test. 'p' value less than 0.05 is taken as significant**.**

Pearson's chi-squared test

$$\mathrm{X}^2 = \sum_{i=1}^{n} \frac{(O_i - E_i)^2}{E_i}$$

Where X2 = Pearson's cumulative test statistic.

Oi = an observed frequency;

Ei = an expected frequency, asserted by the null hypothesis;

n = the number of cells in the table.

Results were graphically represented where deemed necessary.

Appropriate statistical software, including but not restricted to MS Excel, SPSS ver. 20 will be used for statistical analysis. Graphical representation will be done in MS Excel 2010.

OBSERVATIONS AND RESULTS

A hospital based cross sectional prospective comparative study was conducted to compare maternal Vitamin Dand calcium levels of term hypertensive and normotensive pregnant women.120 pregnant women were divided into following groups of 60 pregnant women each:

Study Group: 60 term hypertensive pregnant women in labour defined as BP>140/90 mmHg

Control Group: 60 term normotensive pregnant women in labour defined as BP<140/90 mmHg

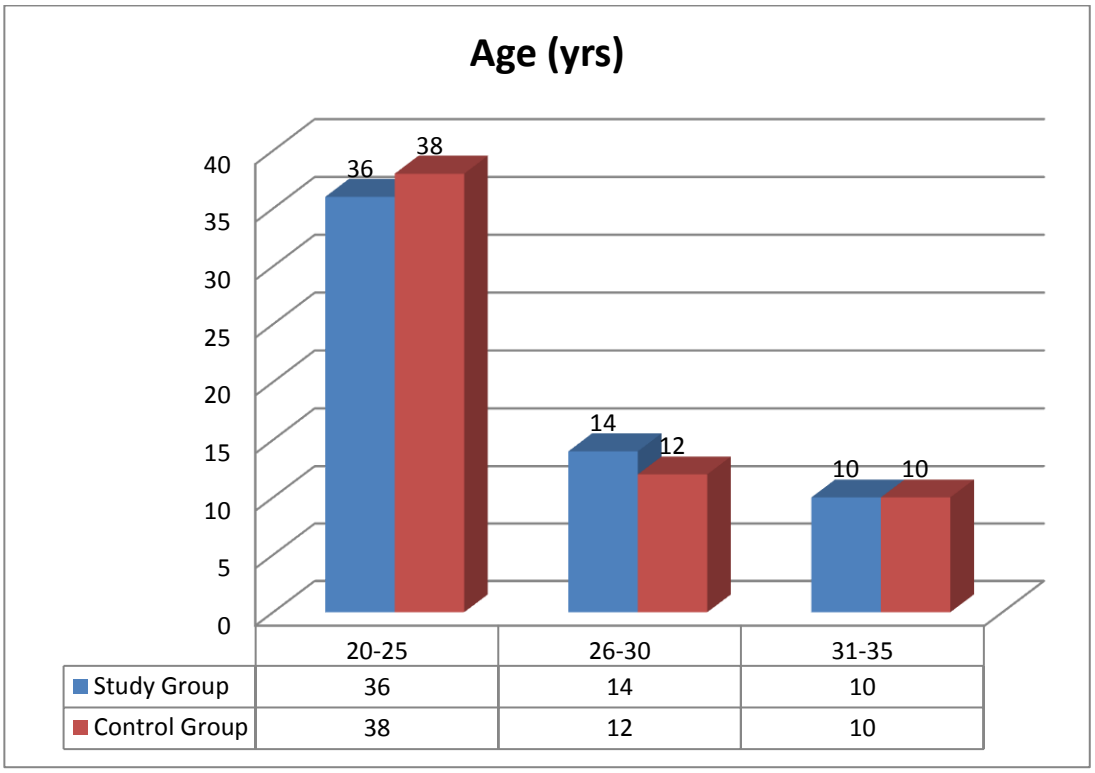
Distribution of patients according to Maternal Age

Majority of the patients (60%) in Study Group were in the age group of 20-25 years followed by 23.3% in the age group of 26-30 years and 16.7% in the age group of 31-35 years. The mean age of patients in Study Group was 25.93 ± 3.99 years.

Majority of the patients (63.3%) in Control Group were in the age group of 20-25 years followed by 20% in the age group of 26-30 years and 16.7% in the age group of 31-35 years. The mean age of patients in Control Group was 25.23 ± 4.67years. There was no significant association between the groups as per Student t-test ($p>0.05$).

Table 2: Distribution of patients according to Maternal Age

Age (years)	Study Group		Control Group		p Value
	N	%	N	%	
20-25	36	60%	38	63.3%	
26-30	14	23.3%	12	20%	
31-35	10	16.7%	10	16.7%	0.901
Total	60	100%	60	100%	
Mean age	25.93 ± 3.99		25.23 ± 4.67		



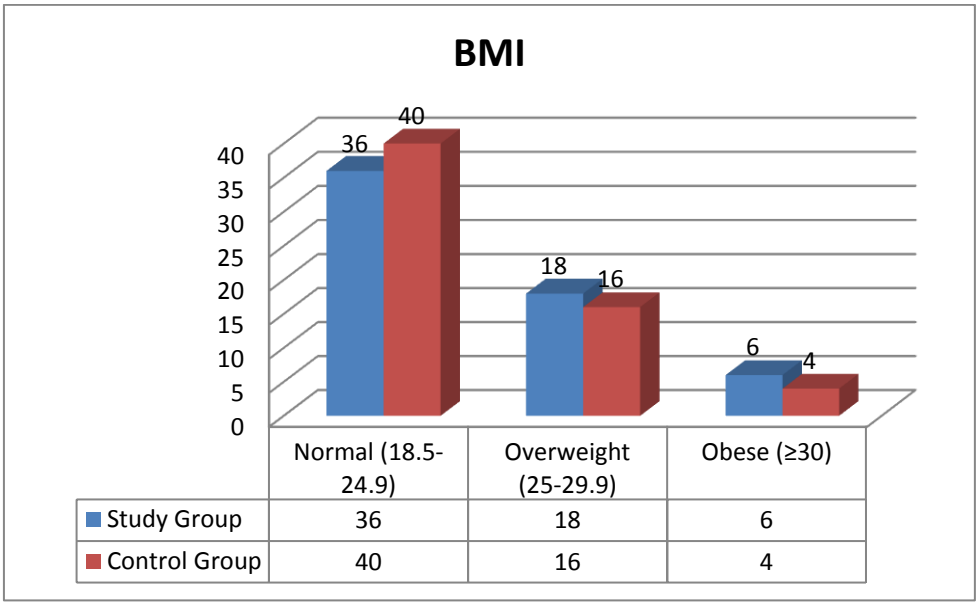
Graph 1: Distribution of patients according to Maternal Age

Distribution of patients according to BMI

60% patients in Study Grouphad BMI in the normal range while 30% and 10% patients were overweight and obese respectively. 66.7% patients in Control Grouphad BMI in the normal range while 20.7% and 19.3% patients were overweight and obese respectively. The mean BMI of patients in Study Group and Control Group was 25.25 ± 2.96 kg/m^2 and 24.08 ± 2.97 kg/m^2 respectively. There was no significant difference between the groups as per Student t-test (p>0.05).

Table 3: Distribution of patients according to BMI

BMI	Study Group		Control Group		p Value
	N	%	N	%	
Normal (18.5-24.9)	36	60%	40	66.7%	0.695
Overweight (25-29.9)	18	30%	16	20.7%	
Obese (≥30)	6	10%	4	19.3%	
Total	60	100%	60	100%	
Mean ± SD	25.25 ± 2.96		24.08 ± 2.97		



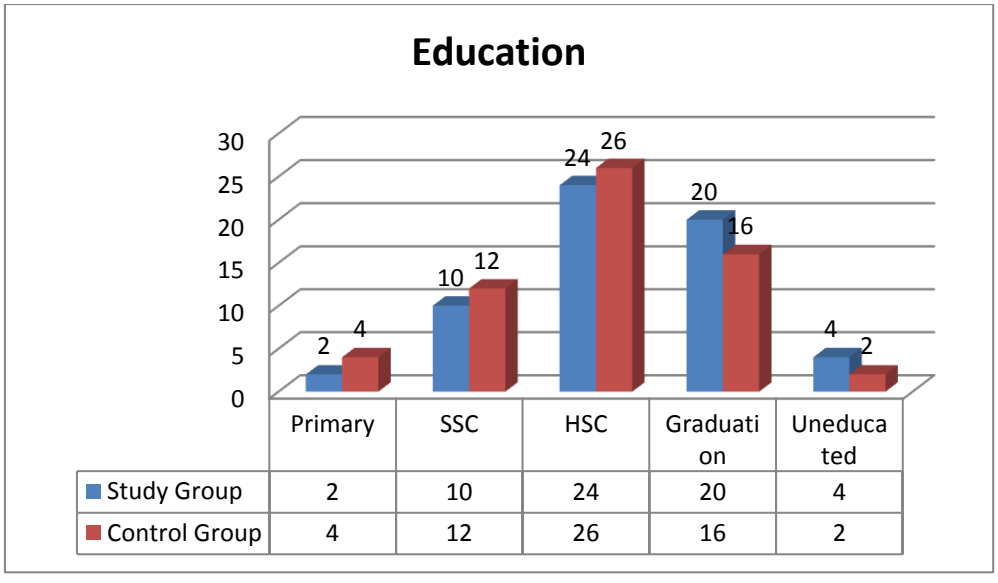
Graph 2: Distribution of patients according to BMI

Distribution of patients according to Education

3.3% patients in Study Group were educated upto primary level while 16.7% and 40% patients studied till SSC and HSC level respectively. 33.3% patients were graduates while 6.7% patients had no education. 6.7% patients in Control Group were educated upto primary level while 20% and 43.3% patients studied till SSC and HSC level respectively. 26.7% patients were graduates while 3.3% patients had no education. There was no significant difference between the groups as per Chi-Square test ($p>0.05$).

Table 4: Distribution of patients according to Education

Education	Study Group		Control Group		p Value
	N	%	N	%	
Primary	2	3.3%	4	6.7%	0.728
SSC	10	16.7%	12	20%	
HSC	24	40%	26	43.3%	
Graduation	20	33.3%	16	26.7%	
Uneducated	4	6.7%	2	3.3%	
Total	60	100%	60	100%	



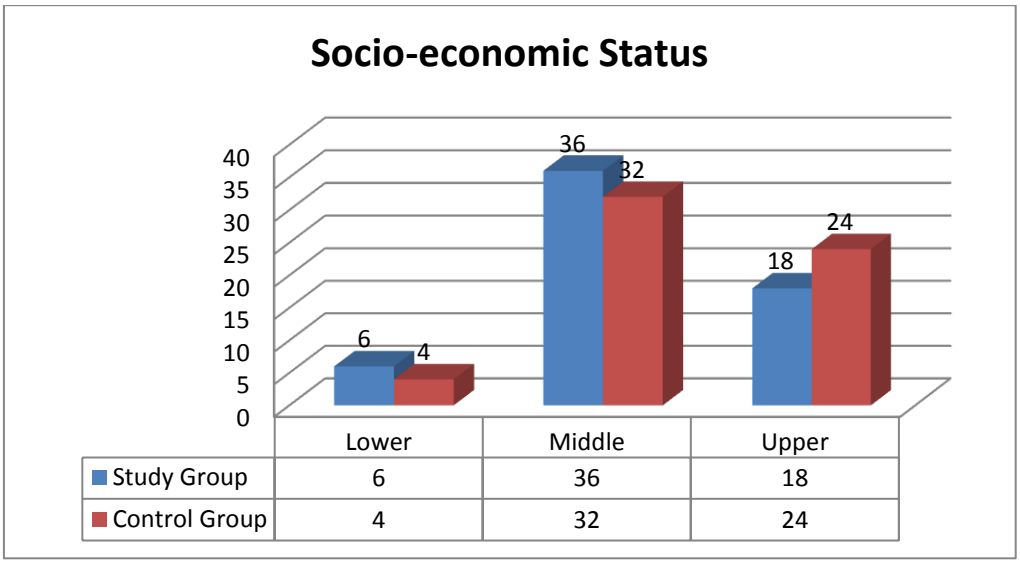
Graph 3: Distribution of patients according to Education

Distribution of patients according to Socio-economic Status

Majority of patients in both groups were from middle class (60% and 53.3% respectively) followed by upper class (30% and 40% respectively) and lower class (10% and 6.7% respectively). There was no significant difference between the groups as per Chi-Square test (p>0.05).

Table 5: Distribution of patients according to Socio-economic Status

Socio-economic Status	Study Group		Control Group		p Value
	N	%	N	%	
Lower	6	10%	4	6.7%	0.474
Middle	36	60%	32	53.3%	
Upper	18	30%	24	40%	
Total	60	100%	60	100%	



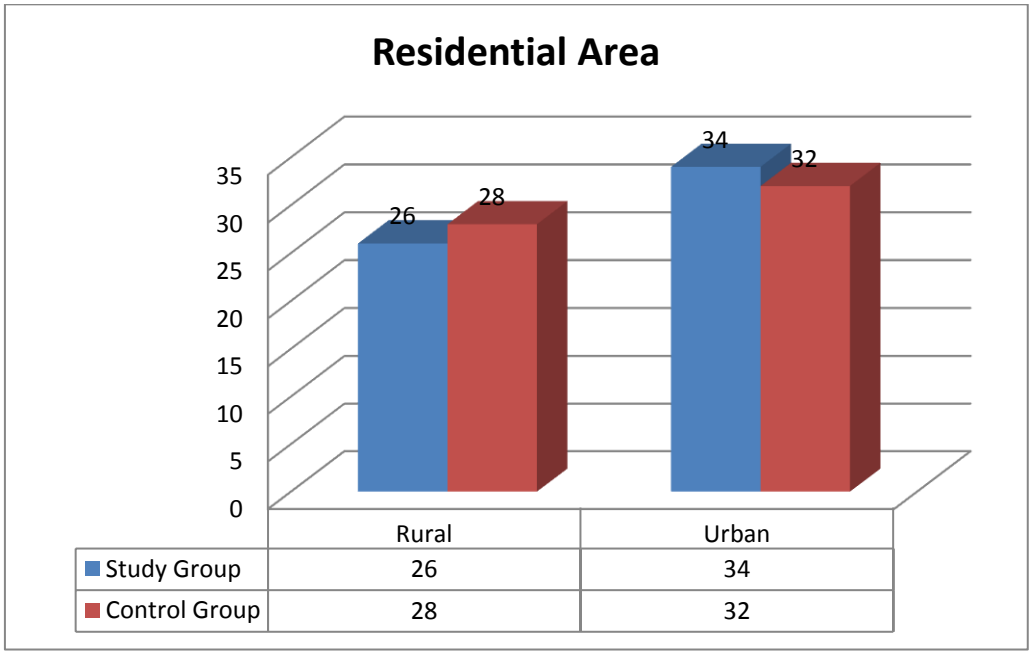
Graph 4: Distribution of patients according to Socio-economic Status

Distribution of patients according to Residential Area

56.7% patients in Study Groupresided in urban areas while 43.3% patients were from rural areas. 53.3% patients in Control Groupresided in urban areas while 46.7% patients were from rural areas. There was no significant difference between the groups as per Chi-Square test (p>0.05).

Table 6: Distribution of patients according to Residential Area

Residential Area	Study Group		Control Group		p Value
	N	%	N	%	
Rural	26	43.3%	28	46.7%	
Urban	34	56.7%	32	53.3%	0.714
Total	60	100%	60	100%	



Graph 5: Distribution of patients according to Residential Area

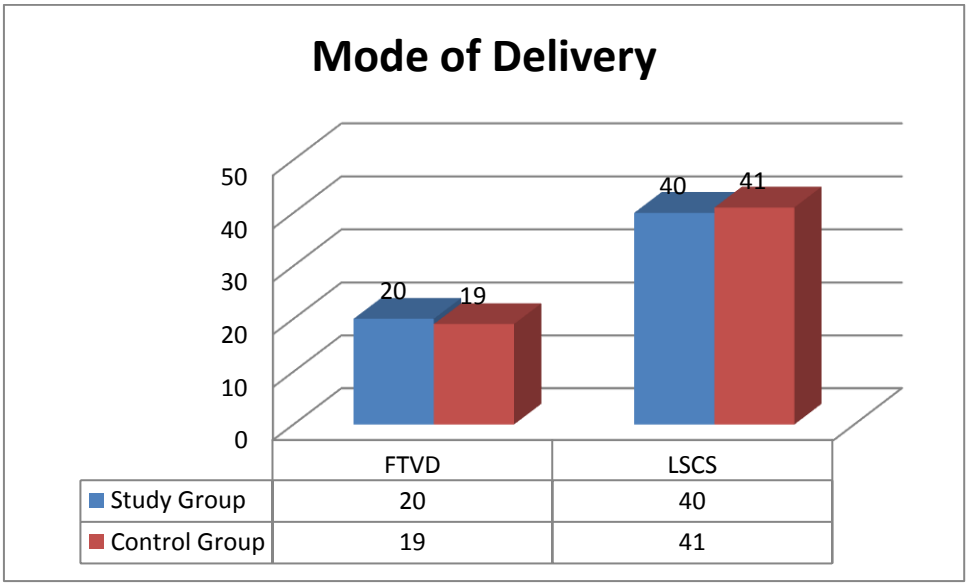
Distribution of patients according to Mode of Delivery

20 (23.3%) patients in Study Grouphad Full term vaginal delivery (FTVD) while 40 (66.7%) patients had Lower Segment Cesarean Section (LSCS) delivery. 19 (31.7%) patients in Control Grouphad Full term vaginal delivery (FTVD) while 41 (68.3%) patients had Lower Segment Cesarean Section (LSCS) delivery.There was no significant difference between the groups as per Chi-Square test (p>0.05).

Table 7: Distribution of patients according to Mode of Delivery

Mode of Delivery	Study Group		Control Group		p Value
	N	%	N	%	
FTVD	20	23.3%	19	31.7%	0.881
LSCS	40	66.7%	41	68.3%	
Total	60	100%	60	100%	

FTVD – Full term vaginal delivery; LSCS - Lower segmentcesarean section



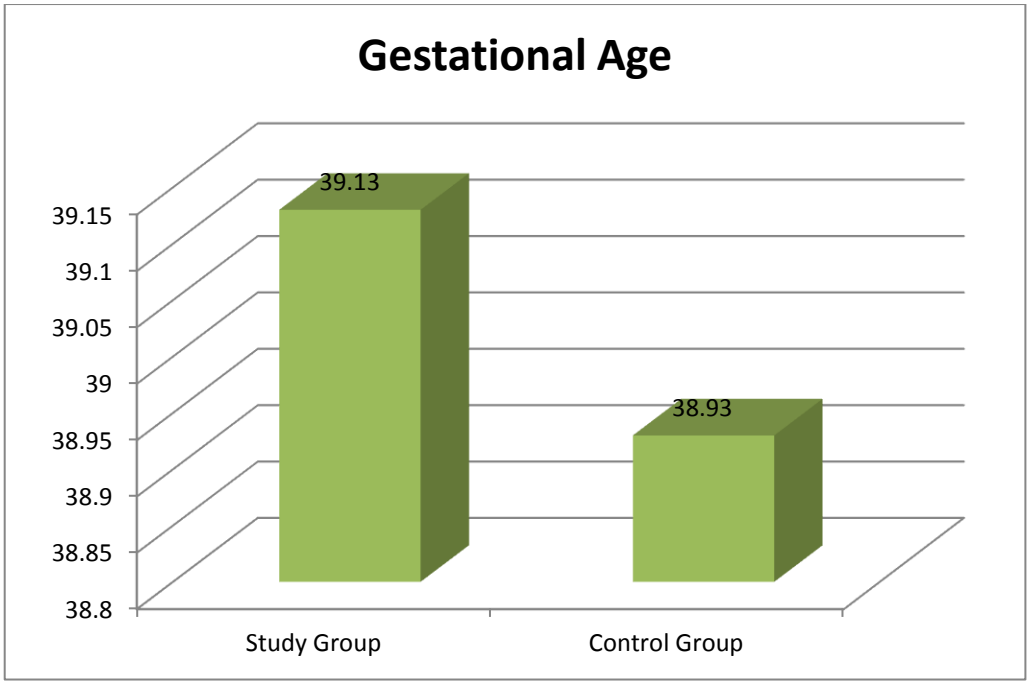
Graph 6: Distribution of patients according to Mode of Delivery

Comparison of Mean Gestational Age between groups

The mean gestational age between groups was comparable (39.13 ± 1.40 vs. 38.93 ± 1.80weeks). The difference was statistically not significant as per Student t-test ($p>0.05$).

Table 8 : Comparison of Mean Gestational Age between groups

Parameter	Study Group		Control Group		p Value
	Mean	SD	Mean	SD	
Gestational Age	39.13	1.40	38.93	1.80	0.498



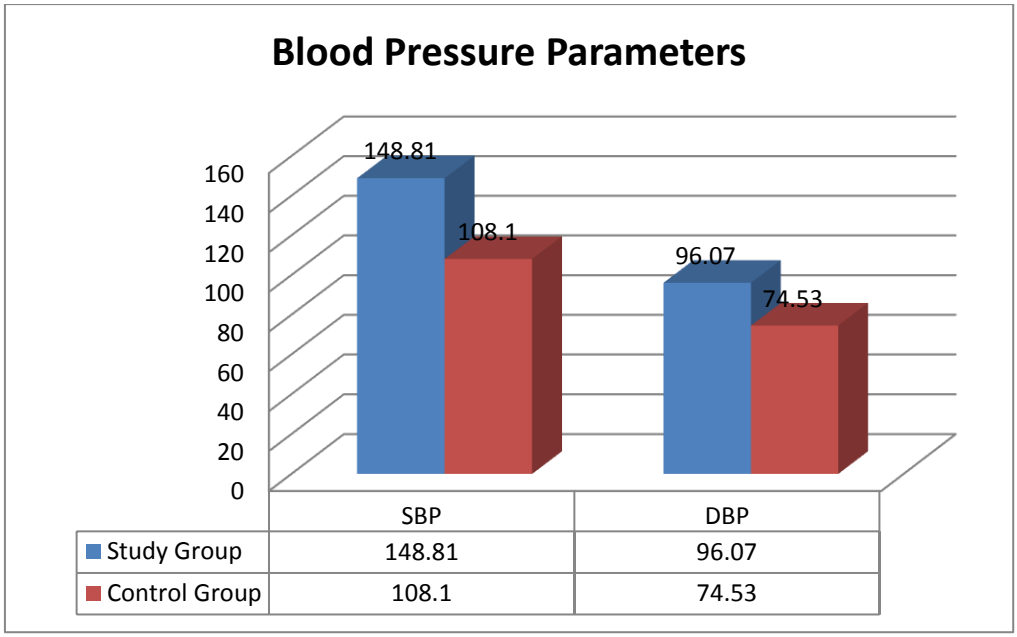
Graph7 : Comparison of Mean Gestational Age between groups

Comparison of Blood Pressure Parameters between groups

The systolic blood pressure (SBP) (148.81 ±6.54 vs. 108.10 ±7.18 mmHg) and diastolic blood pressure (DBP) (96.07 ±6.48 vs. 74.53 ± 8.96 mmHg) of patients were significantly higher in Study Group as per Student t-test (**p<0.05**).

Table 9 : Comparison of Blood Pressure Parameters between groups

Blood Pressure Parameters	Study Group		Control Group		p Value
	Mean	SD	Mean	SD	
SBP	148.81	6.54	108.10	7.18	**<0.001**
DBP	96.07	6.48	74.53	8.96	**<0.001**



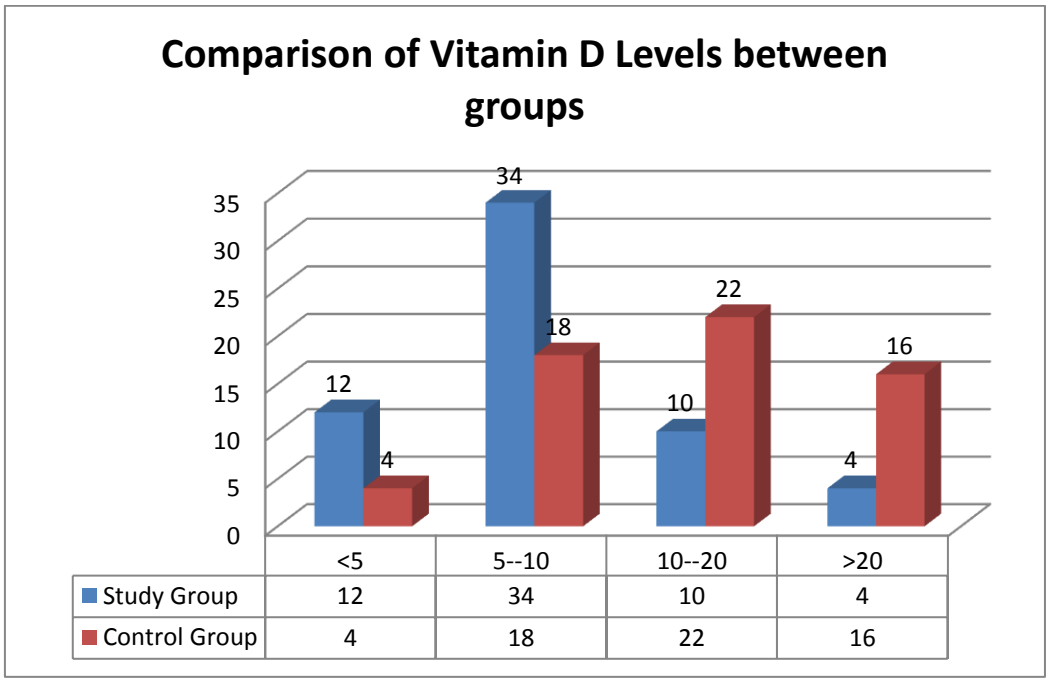
Graph8 : Comparison of Blood Pressure Parameters between groups

Comparison of Vitamin D Levels between groups

12 (20%) patients in Study Group had very severe vitamin D deficiency (<5 ng/ml) as compared to 4 (6.7%) patients in Control Group while 34 (56.7%) patients in Study Group had severe vitamin D deficiency as compared to 18 (30%) patients in Control Group. Mild deficiency of vitamin D was observed in 10 (16.6%) and 22 (36.7%) patients respectively The mean Vitamin D level was significantly lower in Study Group as compared to Control Group (8.73 ± 5.28 vs. 14.20 ±7.82ng/ml) (**$p<0.05$**).

Table 10 : Comparison of Vitamin D Levels between groups

Vitamin D (ng/ml)	Study Group		Control Group		p Value
	N	%	N	%	
<5	12	20%	4	6.7%	
5-10	34	56.7%	18	30%	
10-20	10	16.6%	22	36.7%	<0.001
>20	4	6.7%	16	26.6%	
Total	60	100%	60	100%	
Mean ± SD	8.73 ± 5.28		14.20 ± 7.82		



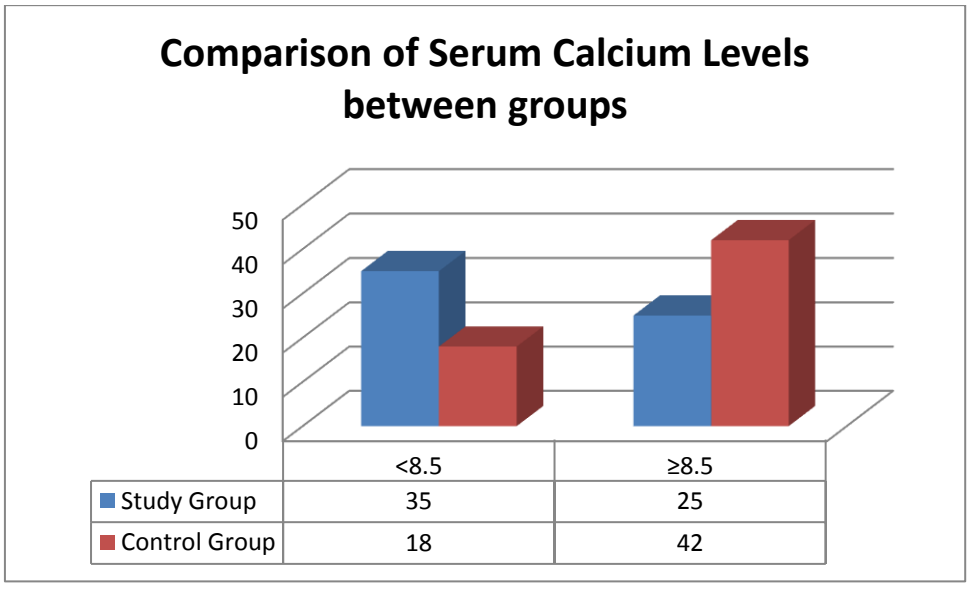
Graph 9 : Comparison of Vitamin D Levels between groups

Comparison of Serum Calcium Levels between groups

35 (58.3%) patients in Study Group had calcium deficiency (<8.5 mg/dl) while 25 (41.7%) patients had normal calcium levels. 18 (30%) patients in Control Group had calcium deficiency (<8.5 mg/dl) while 42 (70%) patients had normal calcium levels. The mean serum calcium levels was significantly lower in Study Group as compared to Control Group (8.05 ± 1.44 vs. 9.66± 1.70mg/dl) (**p<0.05**).

Table 11 : Comparison of Serum Calcium Levels between groups

Serum Calcium (mg/dl)	Study Group		Control Group		p Value
	N	%	N	%	
<8.5	35	58.3%	18	30%	**0.002**
≥8.5	25	41.7%	42	70%	
Total	60	100%	60	100%	
Mean ± SD	8.05 ± 1.44		9.66 ± 1.70		



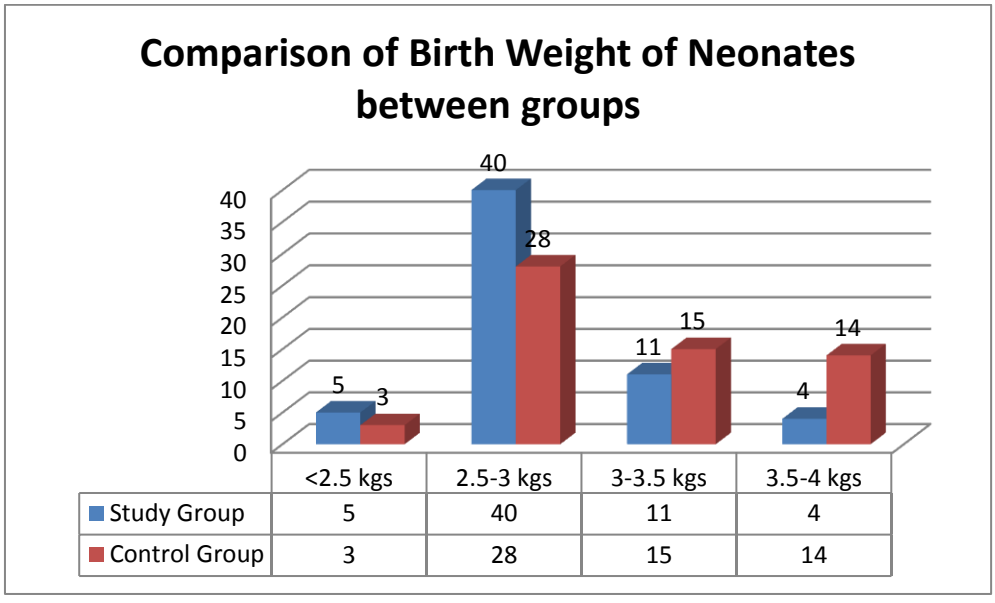
Graph 10 : Comparison of Serum Calcium Levels between groups

Comparison of Birth Weight of Neonates between groups

The mean birth weight of neonates was 2.86 ± 0.36 kgs and 3.11 ± 0.54 kgs in Study Group and Control Group respectively. There was statistically significant difference between the two groups as per Student t- test (**p<0.05**).

Table 12 : Comparison of Birth Weight of Neonates between groups

Birth Weight	Study Group		Control Group		p Value
	Mean	SD	Mean	SD	
<2.5 kgs	5	8.3%	3	5%	**0.032**
2.5-3 kgs	40	66.7%	28	46.7%	
3-3.5 kgs	11	18.3%	15	25%	
3.5-4 kgs	4	6.7%	14	23.3%	
Total	60	100%	60	100%	
Mean ± SD	2.86 ± 0.36		3.11 ± 0.54		



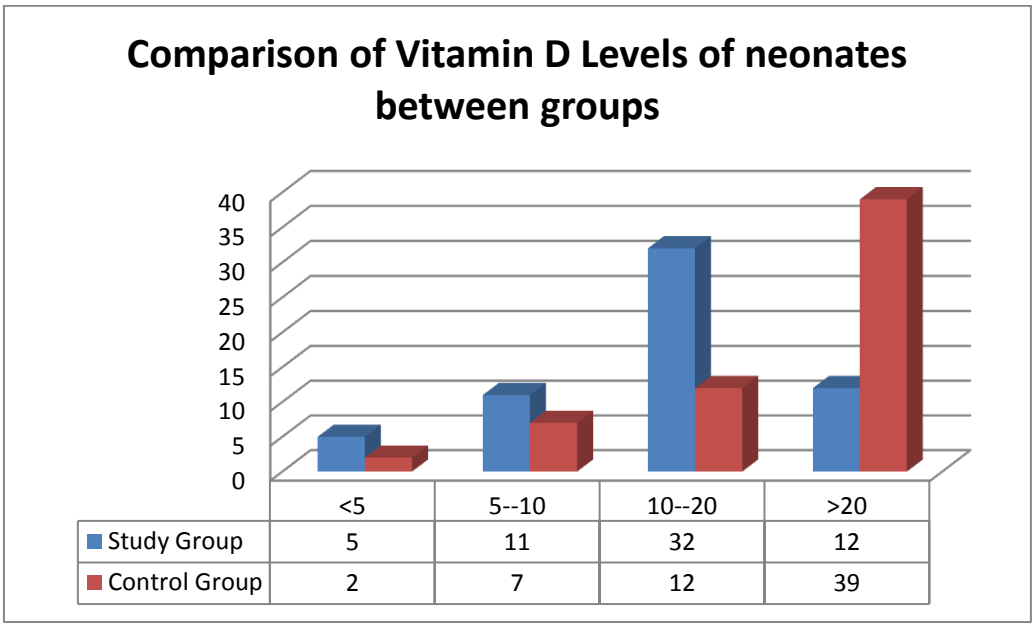
Graph 11 : Comparison of Birth Weight of Neonates between groups

Comparison of Vitamin D Levels of neonates between groups

5 (8.3%) neonates in Study Grouphad very severe vitamin D deficiency (<5 ng/ml) as compared to 2 (3.3%) neonates in Control Group while 11 (18.4%) neonates in Study Group had severe vitamin D deficiency as compared to 7 (11.7%) neonates in Control Group. Mild deficiency of vitamin D was observed in 32 (53.3%) and 12 (20%) neonates respectively The mean Vitamin D level of neonates was significantly lower in Study Group as compared to Control Group (13.67 ± 6.12 ng/ml vs. 23.65 ± 10.42ng/ml) (**$p<0.05$**).

Table 13 : Comparison of Vitamin D Levels of neonates between groups

Vitamin D (ng/ml)	Study Group		Control Group		p Value
	N	%	N	%	
<5	5	8.3%	2	3.3%	<0.001
5-10	11	18.4%	7	11.7%	
10-20	32	53.3%	12	20%	
>20	12	20%	39	65%	
Total	60	100%	60	100%	
Mean ± SD	13.67 ± 6.12		23.65 ± 10.42		



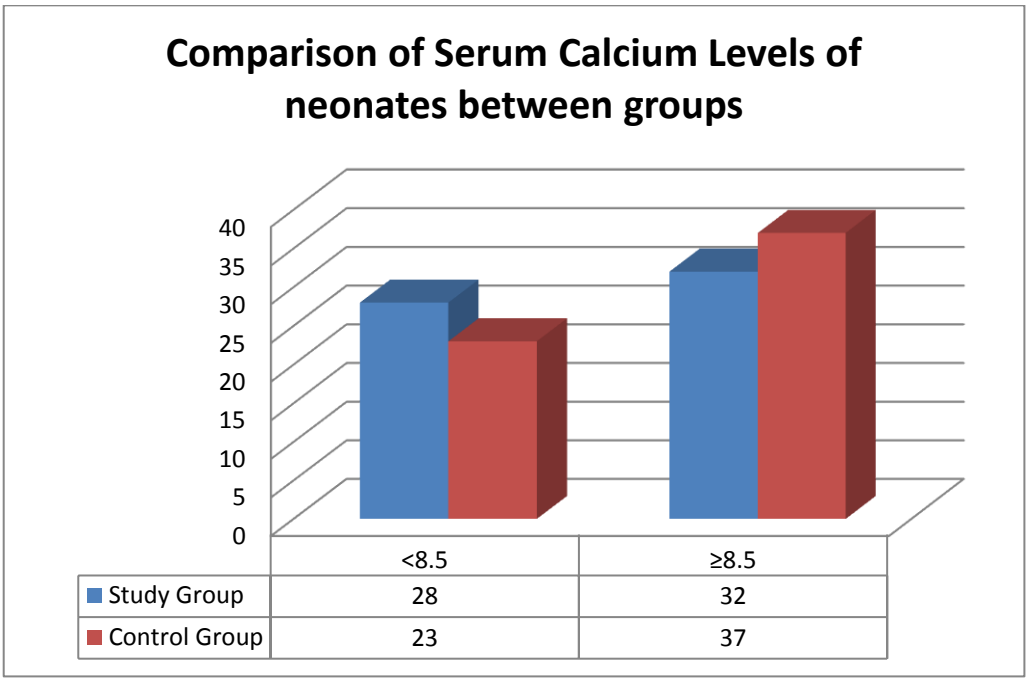
Graph 12 : Comparison of Vitamin D Levels of neonates between groups

Comparison of Serum Calcium Levels of neonates between groups

28 (46.7%) neonates in Study Group had calcium deficiency (<8.5 mg/dl) while 32 (53.3%) neonates had normal calcium levels. 23 (38.3%) neonates in Control Group had calcium deficiency (<8.5 mg/dl) while 37 (61.7%) neonates had normal calcium levels. The mean serum calcium levels of neonates was significantly lower in Study Group as compared to Control Group (8.31 ± 1.48 mg/dl vs. 9.26 ± 1.67mg/dl) (**$p<0.05$**).

Table 14 : Comparison of Serum Calcium Levels of neonates between groups

Serum Calcium (mg/dl)	Study Group		Control Group		p Value
	N	%	N	%	
<8.5	28	46.7%	23	38.3%	**<0.001**
≥8.5	32	53.3%	37	61.7%	
Total	60	100%	60	100%	
Mean ± SD	8.31 ± 1.48		9.26 ± 1.67		



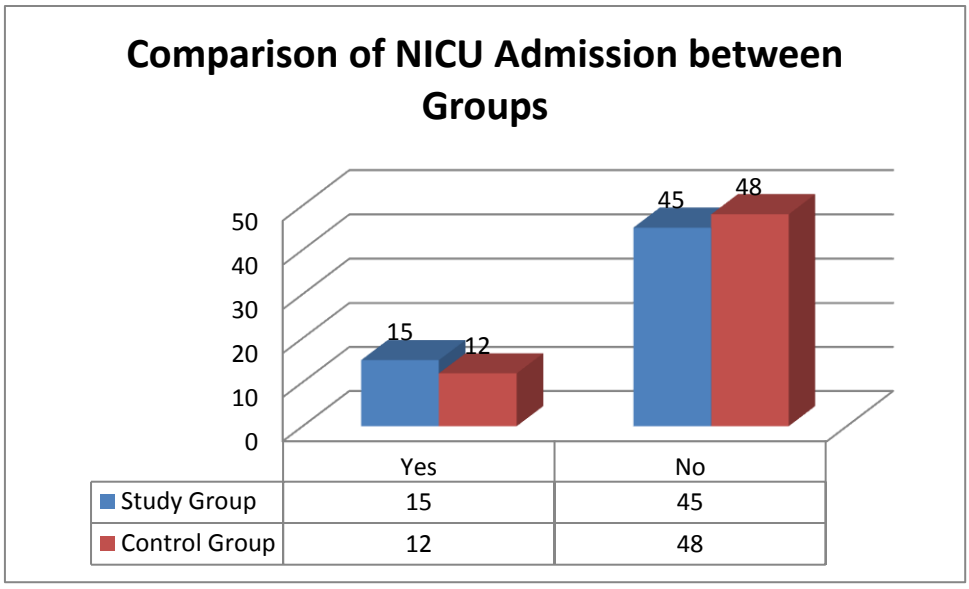
Graph 13 : Comparison of Serum Calcium Levels of neonates between groups

Comparison of NICU Admission between Groups

The number of NICU admission was lower in Control Group as compared to Study Group (25% vs. 20%), however the difference was statistically not significant as per Chi-Square test (p>0.05).

Table 15 : Comparison of NICU Admission between Groups

NICU Admission	Study Group		Control Group		p Value
	N	%	N	%	
Yes	15	25%	12	20%	0.512
No	45	75%	48	80%	
Total	60	100%	60	100%	



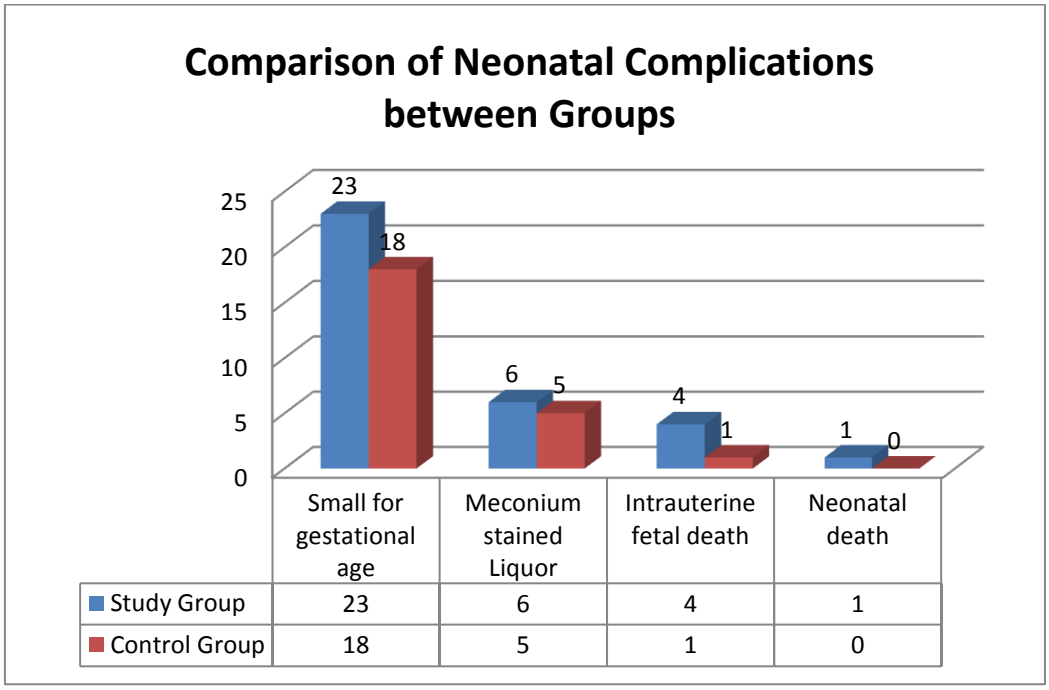
Graph 14 : Comparison of NICU Admission between Groups

Comparison of Neonatal Complications between Groups

In Study Group, 23 (38.3%) neonates were small for gestational age while Meconium stained Liquor and Intrauterine fetal death occurred in 6 (10%) and 4 (6.7%) neonates respectively. 1 (1.7%) neonate died during neonatal period. In Control Group, 18 (30%) neonates were small for gestational age while Meconium stained Liquor and Intrauterine fetal death occurred in 5 (8.3%) and 1 (1.7%) neonates respectively. There was no significant difference between the groups as per Chi-Square test ($p>0.05$).

Table 16 : Comparison of Neonatal Complications between Groups

Neonatal Complications	Study Group		Control Group		p Value
	N	%	N	%	
Small for gestational age	23	38.3%	18	30%	0.702
Meconium stained Liquor	6	10%	5	8.3%	
Intrauterinefetal death	4	6.7%	1	1.7%	
Neonatal death	1	1.7%	0	-	



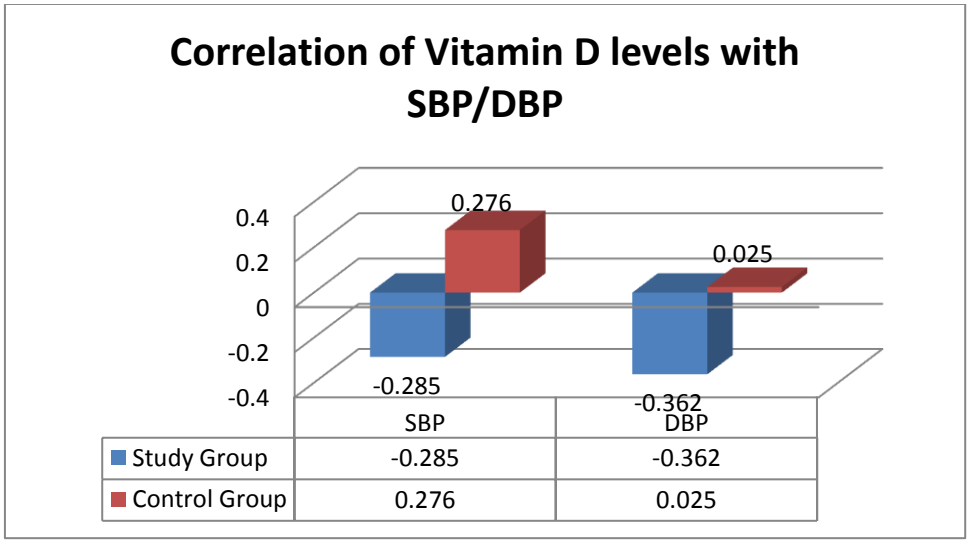
Graph 15 : Comparison of Neonatal Complications between Groups

Correlation of Vitamin D levels with Systolic Blood Pressure (SBP)/Diastolic Blood Pressure (DBP)

A significant negative correlation was observed between Vitamin D and Systolic Blood Pressure (SBP)/Diastolic Blood Pressure (DBP) in Study Group while a positive correlation was observed between Vitamin D and SBP/DBP in Control Group but this correlation was not significant.

Table 17 : Correlation of Vitamin D levels with Systolic Blood Pressure (SBP)/ Diastolic Blood Pressure (DBP)

Parameter	Study Group		Control Group	
	Correlation	p Value	Correlation	p Value
SBP	-0.285	**<0.05**	0.276	>0.05
DBP	-0.362	**<0.05**	0.025	>0.05



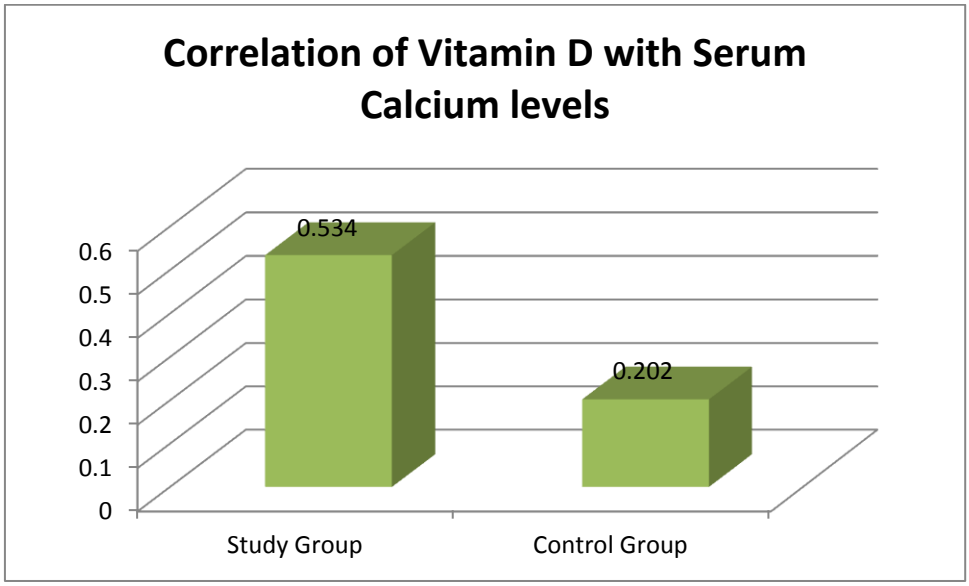
Graph 16 : Correlation of Vitamin D levels with Systolic Blood Pressure (SBP)/Diastolic Blood Pressure (DBP)

Correlation of Vitamin D with Serum Calcium levels

A significant positive correlation was observed between Vitamin D and Serum Calcium in Study Group. A positive correlation was observed between Vitamin D and Serum Calcium in Control Group but this correlation was not significant (p>0.05).

Table 18 : Correlation of Vitamin D with Serum Calcium levels

Parameter	Study Group		Control Group	
	Correlation	p Value	Correlation	p Value
Calcium	0.534	**<0.05**	0.202	>0.05



Graph 17 : Correlation of Vitamin D with Serum Calcium levels

DISCUSSION

A hospital based cross sectional prospective comparative study was conducted to compare maternal Vitamin D and calcium levels of term hypertensive and normotensive pregnant women. 120 pregnant women were divided into following groups of 60 pregnant women each:

Study Group: 60 term hypertensive pregnant women in labour defined as BP>140/90 mmHg

Control Group: 60 term normotensive pregnant women in labour defined as BP<140/90 mmHg.

Vitamin D is a pro hormone derivative from cholesterol. It has two origins: Endogenous and exogenous. During endogenous synthesis, 7-dehydrocholesterol in the deep layers of the skin under the effect of ultraviolet UVB (290-315 nm) forms previtamin D which undergoes thermal isomerization to form cholecalciferol. Then in the liver it is hydroxylated to 25-hydroxy derivative, calcidiol. This is released into circulation bound to a vitamin D binding globulin, which is the main storage form. In kidney, the calcidiol undergoes either 1-hydroxylation to yield active metabolite, 1,25-dihydroxy-vitamin D (calcitriol), or 24-hydroxylation to yield a probably inactive metabolite.

Calcitriol exerts the hormonal action via binding to nuclear vitamin D receptors, which are present throughout the body, including pregnancy-specific tissues.It helps in implantation and placental function because of angiogenic, immunomodulatory, and anti-inflammatory effects. The major role of vitamin D is in the synthesis and regulation of genes in the early developmental phase of the placenta.[135, 136] Placental

abnormalities are seen with preeclampsia and they takes place before the remodeling of the vascular structures.[137, 138]In the development of immunological tolerance in pregnancy along with the presence of a sufficient level of vitamin D, immunomodulatory properties of vitamin D have been reported to play a key role in the prevention and management of PE[139]. It has been found that low vitamin D levels are related to increase IL-6 concentrations with the help of stress induced kinase, p38 inactivation, and inhibition of inflammatory cytokines of alpha TNF.[140,141] Also, vitamin D maintains maternal calcium homeostatsis for foetal bone development and is a potent endocrine biosynthesis for the regulation of the renin-angiotensin system. The placental growth factor (PIGF)is significantly decreasedwith an increased risk for preeclampsia and eclampsia.

Vitamin D insufficiency has been associated with several adverse health outcomes, including pregnancy outcomes, and is increasingly recognized as a public health concern. It is responsible for approximately 50,000 maternal deaths yearly worldwide, 25% of all cases of fetal growth restriction, and 15% of preterm births in developed countries[143].

Observational data suggestted a link between low 25-hydroxyvitamin D (25(OH) D) levels - the best measure of vitamin D status in humans - and an increased risk of adverse pregnancy outcomes such as gestational diabetes, pregnancy induced hypertension and fetal growth restriction[144].

Preeclampsia, a complex, dynamic pregnancy disorder, is associated with increased maternal and fetal morbidity and mortality especially in developing countries. Although the precise factors that may cause preeclampsia are unknown, the most accepted mediator of this disorder is the remodeling of uteroplacental arteries and is

considered as woman's immunological intolerance to the semiallogenic fetus and placenta. Ultimately, abnormal placentation leads to widespread vasospasm and endothelial injury in multiple organs[145].

In the present study, majority of the patients (60%) in Study Group were in the age group of 20-25 years followed by 23.3% in the age group of 26-30 years and 16.7% in the age group of 31-35 years. The mean age of patients in Study Group was 25.93 ± 3.99 years. Majority of the patients (63.3%) in Control Group were in the age group of 20-25 years followed by 20% in the age group of 26-30 years and 16.7% in the age group of 31-35 years. The mean age of patients in Control Group was 25.23 ± 4.67years. There was no significant association between the groups as per Student t-test ($p>0.05$).

60% patients in Study Group had BMI in the normal range while 30% and 10% patients were overweight and obese respectively. 66.7% patients in Control Group had BMI in the normal range while 20.7% and 19.3% patients were overweight and obese respectively. The mean BMI of patients in Study Group and Control Group was 25.25 ± 2.96 kg/m^2 and 24.08 ± 2.97 kg/m^2 respectively. There was no significant difference between the groups as per Student t-test ($p>0.05$).

3.3% patients in Study Group were educated upto primary level while 16.7% and 40% patients studied till SSC and HSC level respectively. 33.3% patients were graduates while 6.7% patients had no education. 6.7% patients in Control Group were educated upto primary level while 20% and 43.3% patients studied till SSC and HSC level respectively. 26.7% patients were graduates while 3.3% patients had no education. There was no significant difference between the groups as per Chi-Square test ($p>0.05$).

In our study, majority of patients in both groups were from middle class (60% and 53.3% respectively) followed by upper class (30% and 40% respectively) and lower class (10% and 6.7% respectively). There was no significant difference between the groups as per Chi-Square test ($p>0.05$).

56.7% patients in Study Group resided in urban areas while 43.3% patients were from rural areas. 53.3% patients in Control Group resided in urban areas while 46.7% patients were from rural areas. There was no significant difference between the groups as per Chi-Square test ($p>0.05$). This is similar to the studies of Dhillon MK et al[146], Umar N et al[131], Sahu M et al[132] and Aghade SM et al[147].

Dhillon MK et al[146] hospital based observational comparative study investigating the serum vitamin D and calcium levels in eclampsia, preeclampsia and healthy pregnant women and assessing its role in the etiology of PE and eclampsia observed statistically no significant differences were found in age, gravidity, parity, weight and height among the demographic data between the groups.

Umar N et al[131] cross-sectional analytical study comparing serum 25-hydroxy vitamin D level between preeclamptic and normotensive pregnancies found average age of both groups was 25 years. Average BMI was 22 kg/m2. Parity and gravid status of both groups were also matched. All women were housewives with occasional exposure to sun, and of low socioeconomic status.

Sahu M et al[132] prospective comparative study evaluating the serum vitamin-D levels in normal pregnant females and pre-eclampsia or eclampsia individuals in the third trimester and assessing the neonatal outcome found no statistically significant differences in age ($p=0.792$) and habituation between the two groups. The level of education ($p=0.015$) and socioeconomic status ($p=0.023$) was significantly lower in

the preeclamptic and eclamptic group compared to the normotensive pregnant women. The preeclampsia and eclampsia group had either irregular or no ANC ($p<0.001$).

Aghade SM et al[147] case–control study assessing total serum calcium concentration in preeclampsia and the association between preeclampsia and calcium level found mean age, gestational age, and BMI did not show any significant difference between the two groups.

It was observed in our study that 20 (23.3%) patients in Study Group had Full term vaginal delivery (FTVD) while 40 (66.7%) patients had Lower Segment Cesarean Section (LSCS) delivery. 19 (31.7%) patients in Control Group had Full term vaginal delivery (FTVD) while 41 (68.3%) patients had Lower Segment Cesarean Section (LSCS) delivery.There was no significant difference between the groups as per Chi-Square test ($p>0.05$).

Bakacak M et al[148] study assessing vitamin D levels in eclampsia, preeclampsia and healthy pregnant women and the role of vitamin D deficiency in the etiology of preeclampsia (PE) reported cesarean section was statistically significantly higher in the eclamptic and preeclamptic patient groups compared to healthy pregnant women ($P<0.001$).

It was observed in the present study that the mean gestational age between groups was comparable (39.13 ± 1.40 vs. 38.93 ± 1.80 weeks). The difference was statistically not significant as per Student t-test ($p>0.05$).

Umar N et al[131] cross-sectional analytical study comparing serum 25-hydroxy vitamin D level between preeclamptic and normotensive pregnancies reported gestational age in both groups was between 21 - 37 weeks. Average age of both groups was 25 years.

In our study, the systolic blood pressure (SBP) (148.81 ± 6.54 vs. 108.10 ± 7.18 mmHg) and diastolic blood pressure (DBP) (96.07 ± 6.48 vs. 74.53 ± 8.96 mmHg) of patients were significantly higher in Study Group as per Student t-test (**p<0.05**). This is comparable to the studies of Dhillon MK et al[146], Umar N et al[131], Aghade SM et al[147] and Bakacak M et al[148].

Dhillon MK et al[146] hospital based observational comparative study investigating the serum vitamin D and calcium levels in eclampsia, preeclampsia and healthy pregnant women and assessing its role in the etiology of PE and eclampsia reported systolic and diastolic blood pressures were significantly higher in the eclamptic and pre eclamptic patient groups compared to the healthy pregnant women (P<0.001 and P<0.001, respectively).

Umar N et al[131] cross-sectional analytical study comparing serum 25-hydroxy vitamin D level between preeclamptic and normotensive pregnancies reported preeclamptic group showed higher systolic and diastolic blood pressure and arterial pressure. Normotensive group showed significantly lower systolic and diastolic blood pressure and arterial pressure. The difference between two groups was obviously significant.

Aghade SM et al[147] case–control study assessing total serum calcium concentration in preeclampsia and the association between preeclampsia and calcium level observed systolic and diastolic blood pressures were significantly increased ($p < 0.05$) in preeclamptic patients compared with normal pregnant women.

Bakacak M et al[148] study assessing vitamin D levels in healthy pregnant women with eclampsia and preeclampsia investigated the role of vitamin D deficiency in causing preeclampsia (PE). The author found that systolic and diastolic blood pressures were significantly higher in the eclamptic and preeclamptic groupwhen compared to the healthy pregnant women (P<0.001 and P<0.001, respectively).

In the present study, 12 (20%) patients in Study Grouphad very severe vitamin D deficiency (<5 ng/ml) as compared to 4 (6.7%) patients in Control Group while 34 (56.7%) patients in Study Group had severe vitamin D deficiency as compared to 18 (30%) patients in Control Group. Mild deficiency of vitamin D was observed in 10 (16.6%) and 22 (36.7%) patients respectively The mean Vitamin D level was significantly lower in Study Group as compared to Control Group (8.73 ± 5.28 vs. 14.20 ±7.82ng/ml) (**p<0.05**). This is concordant to the studies of Dhillon MK et al[146], Umar N et al[131], Sahu M et al[132], Bakacak M et al[148], Sadin B et al[149], Sonuga AA et al[133] and O'Callaghan KM et al[134].

Dhillon MK et al[146]hospital based observational comparative study investigating the serum vitamin D and calcium levels in eclampsia, preeclampsia and healthy pregnant women and assessing its role in the etiology of PE and eclampsia reported mean values of serum 25-hydroxyvitamin D levels in study group and control group were 9.83 ± 6.11 ng/ml and 14.92± 3.35 ng/ml respectively (Normal Value of vitamin D =30-74 ng/ml). The decrease in Vitamin D levels in study group as compared to control group showed statistically significant association (p value= 0.04).

Umar N et al[131] cross-sectional analytical study comparing serum 25-hydroxy vitamin D level between preeclamptic and normotensive pregnancies reported vitamin D levels were lower in both groups and did not differ significantly.

Sahu M et al[132] prospective comparative study evaluating the serum vitamin-D levels in normal pregnant females and pre-eclampsia or eclampsia individuals in the third trimester and assessing the neonatal outcome reported level of serum vitamin D was significantly lower in the preeclamptic and eclamptic group. 75% of the patients in the hypertensive group with either preeclampsia or eclampsia were found to have very severe deficiency (<5 ng/ml) as compared to 25% of those in the healthy normotensive group. Among the 20 pregnant mothers (out of 200), with suboptimal to optimal (>20 ng/ml) serum vitamin D, only 6 (30%) were from the hypertensive group and rest 14 (70%) were from normotensive group. The mean serum vitamin D level was 9.06±5.20 ng/ml in diseased group compared to 13.67±7.24ng/ml in healthy pregnant group which was statistically significant.

Bakacak M et al[148] study assessing vitamin D levels in eclampsia, preeclampsia and healthy pregnant women and the role of vitamin D deficiency in the etiology of preeclampsia (PE) reported when the vitamin D levels were compared among the three groups, it was found to be 23.7 ± 5.93, 18.5 ± 5.47 and 19.3 ± 4.31 ng/ml in healthy pregnant women, eclamptic women and preeclamptic women, respectively. Vitamin D levels were found to be significantly lower in the eclamptic and preeclamptic groups compared to healthy pregnant women (P<0.001). No statistically significant differences were found between the eclamptic and preeclamptic patient groups.

Sadin B et al[149] observational case-control study determining vitamin D status, in preeclamptic women and healthy pregnant controls reported 60% of the preeclamptic women were vitamin D-deficient, as reflected by the number of persons with serum 25(OH)D levels below 10 ng/ml and 40% were vitamin D insufficient, as reflected by the number of persons with serum 25(OH)D levels between 10 ng/ml to 30 ng/ml. In

control group 10% and 90% were vitamin D deficient and vitamin D insufficient, respectively.

Sonuga AA et al[133]case-Control Study assessing serum levels of 25-hydroxy Vitamin D (25(OH)D3) in normotensive pregnant women and preeclamptic women reported preclamptic women had significantly lower levels (P<.05) of Vitamin D at 20 weeks (24.5±4.6 vs 36.59±5.1), 30 weeks (23.8±3.9 vs 34.14±3.7), and postpartum (21.7±5.5 vs 32.62±3.2) when compared to control group.

O'Callaghan KM et al[134]conducted an observational, interventional study on vitamin D to find the association between low maternal status of vitamin D and increased risk of hypertensive disorders. They reported that increased risk of gestational hypertensive disorders at 25(OH)D concentrations <50 nmol/L, caution should be exercised with dosing in trials, given the lack of data on long-term safety. The results concluded that hypertension alone limits intrauterine growth evaluatingthe relationship between vitamin D status and all terms of hypertension in pregnancy is a relevant area for clinical research.

35 (58.3%) patients in Study Group of our study had calcium deficiency (<8.5 mg/dl) while 25 (41.7%) patients had normal calcium levels. 18 (30%) patients in Control Group had calcium deficiency (<8.5 mg/dl) while 42 (70%) patients had normal calcium levels. The mean serum calcium levels was significantly lower in Study Group as compared to Control Group (8.05 ± 1.44 vs. 9.66 ± 1.70 mg/dl) (**p<0.05**).These findings were consistent with the studies ofDhillon MK et al[146] and Aghade SM et al[147].

Dhillon MK et al[146]hospital based observational comparative study investigating the serum vitamin D and calcium levels in eclampsia, preeclampsia and healthy pregnant

women and assessing its role in the etiology of PE and eclampsia reported mean values of serum calcium in study group and control group were 8.76±1.12 mg% and 9.48±0.93mg% respectively (Normal value of Calcium =8.5 to 10 mg%). The decrease in calcium levels among study group in comparison to control group showed statistically significant association ($p<0.01$).

Aghade SM et al[147] case–control study assessing total serum calcium concentration in preeclampsia and the association between preeclampsia and calcium level reported preeclamptic patients had significantly decreased serum calcium levels compared with the healthy controls ($p < 0.05$). Also, serum calcium level was much lower in severe preeclampsia than in mild preeclampsia.

It was observed in our study that the mean birth weight of neonates was 2.86 ± 0.36 kgs and 3.11 ± 0.54 kgs in Study Group and Control Group respectively. There was statistically significant difference between the two groups as per Student t- test (**$p<0.05$**).

Bakacak M et al[148] study assessing vitamin D levels in eclampsia, preeclampsia and healthy pregnant women and the role of vitamin D deficiency in the etiology of preeclampsia (PE) observed a week of pregnancy and birth weight were significantly lower in the eclamptic and preeclamptic patient groups compared to the healthy pregnant women.

In our study, 5 (8.3%) neonates in Study Group had very severe vitamin D deficiency (<5 ng/ml) as compared to 2 (3.3%) neonates in Control Group while 11 (18.4%) neonates in Study Group had severe vitamin D deficiency as compared to 7 (11.7%) neonates in Control Group. Mild deficiency of vitamin D was observed in 32 (53.3%) and 12 (20%) neonates respectively The mean Vitamin D level of neonates was

significantly lower in Study Group as compared to Control Group (13.67 ± 6.12 ng/ml vs. 23.65 ± 10.42ng/ml) (**p<0.05**).

28 (46.7%) neonates in Study Group had calcium deficiency (<8.5 mg/dl) while 32 (53.3%) neonates had normal calcium levels. 23 (38.3%) neonates in Control Group had calcium deficiency (<8.5 mg/dl) while 37 (61.7%) neonates had normal calcium levels. The mean serum calcium levels of neonates was significantly lower in Study Group as compared to Control Group (8.31 ± 1.48 mg/dl vs. 9.26 ± 1.67mg/dl) (**p<0.05**).

Sahu M et al[132] prospective comparative study evaluating the serum vitamin-D levels in normal pregnant females and pre-eclampsia or eclampsia individuals in the third trimester and assessing the neonatal outcome reported the mean neonatal serum calcium in the hypertensive group was 8.30±1.46 mg/dl as compared to 8.82±0.92 mg/dl in the healthy pregnant group which was statistically significant. The calcium deficient state may be because of the preterm and low birth weight babies. Neonatal serum calcium deficiency was more prevalent in the babies born to pregnant mothers with severe (5-10 ng/ml) and very severe (<5 ng/ml) serum vitamin D deficiency.Out of the 81 newborn babies with calcium deficiency (<8 mg/dl), 48 (59.3%) were from hypertensive (preeclampsia or eclampsia) group and 33 (40.7%) were from normotensive group.

It was observed in the present study that the number of NICU admission was lower in Control Group as compared to Study Group (25% vs. 20%), however the difference was statistically not significant as per Chi-Square test (p>0.05).

Sahu M et al[132] prospective comparative study evaluating the serum vitamin-D levels in normal pregnant females and pre-eclampsia or eclampsia individuals in the third

trimester and assessing the neonatal outcome reported mean birth weight of the new-born in the hypertensive group (2.22±0.42 kg) was lower as compared to normotensive group (2.51±0.42 kg).

In Study Group of the present study, 23 (38.3%) neonates were small for gestational age while Meconium stained Liquor and Intrauterine fetal death occurred in 6 (10%) and 4 (6.7%) neonates respectively. 1 (1.7%) neonate died during neonatal period. In Control Group, 18 (30%) neonates were small for gestational age while Meconium stained Liquor and Intrauterine fetal death occurred in 5 (8.3%) and 1 (1.7%) neonates respectively. There was no significant difference between the groups as per Chi-Square test ($p>0.05$).

Sahu M et al[132] prospective comparative study evaluating the serum vitamin-D levels in normal pregnant females and pre-eclampsia or eclampsia individuals in the third trimester and assessing the neonatal outcome reported preeclampsia and eclampsia group most babies had preterm birth and almost 62 out of 100 required SNCU admission due to prematurity or other neonatal complications like growth restriction, respiratory distress, meconium aspiration syndrome or hypoxic ischemic encephalopathy. The difference in the two groups in terms of neonatal NICU admission was statistically significant.

In the present study a significant negative correlation was observed between Vitamin D and Systolic Blood Pressure (SBP)/Diastolic Blood Pressure (DBP) in Study Group while a positive correlation was observed between Vitamin D and SBP/DBP in Control Group but this correlation was not significant. Similar observations were noted in the studies of Umar N et al[131] and Sadin B et al[149].

Umar N et al[131]cross-sectional analytical study comparing serum 25-hydroxy vitamin D level between preeclamptic and normotensive pregnancies reported in the preeclamptic group, an inverse correlation was observed between serum vitamin D and blood pressure (systolic BP rs = - 0.10, p=0.60; AP rs = -0.12, p = 0.51).

Sadin B et al[149] observational case-control study determining vitamin D status, in preeclamptic women and healthy pregnant controls reported mean serum concentration of 25(OH)D of preeclamptic women were significantly lower than that of healthy controls (P=0.002). Mean values of SBP and DBP were significantly higher in the preeclamptic subjects com-pared to the control.

In our study, a significant positive correlation was observed between Vitamin D and Serum Calcium in Study Group. A positive correlation was observed between Vitamin D and Serum Calcium in Control Group but this correlation was not significant (p>0.05). This is consistent with the studies of Sahu M et al[132] and Aghade SM et al[147].

Sahu M et al[132] prospective comparative study reported correlation between maternal serum vitamin D and neonatal serum calcium level showed a significant positive correlation with r = 0.652 and p <0.001. As the serum vitamin D level increases the serum calcium level also increases linearly and this increase was statistically significant.

Aghade SM et al[147] case–control study assessing total serum calcium concentration in preeclampsia and the association between preeclampsia and calcium level reported a significant negative correlation between serum calcium and systolic/diastolic blood pressure.

SUMMARY

A hospital based cross sectional prospective comparative study was conducted to compare maternal Vitamin D and calcium levels of term hypertensive and normotensive pregnant women. 120 pregnant women were divided into following groups of 30 pregnant women each:

- **Study Group:** 60 term hypertensive pregnant women in labour defined as BP>140/90 mmHg
- **Control Group:** 60 term normotensive pregnant women in labour defined as BP<140/90 mmHg

The following observations were noted:

1. Majority of the patients (60%) in Study Group were in the age group of 20-25 years followed by 23.3% in the age group of 26-30 years and 16.7% in the age group of 31-35 years. The mean age of patients in Study Group was 25.93 ± 3.99 years.

2. Majority of the patients (63.3%) in Control Group were in the age group of 20-25 years followed by 20% in the age group of 26-30 years and 16.7% in the age group of 31-35 years. The mean age of patients in Control Group was 25.23 ± 4.67years. There was no significant association between the groups as per Student t-test (p>0.05).

3. 60% patients in Study Group had BMI in the normal range while 30% and 10% patients were overweight and obese respectively. 66.7% patients in Control Group had BMI in the normal range while 20.7% and 19.3% patients were overweight and obese respectively. The mean BMI of patients in Study Group and Control Group was 25.25 ± 2.96 kg/m^2 and 24.08 ± 2.97 kg/m^2 respectively. There was no significant difference between the groups as per Student t-test ($p>0.05$).

4. 3.3% patients in Study Group were educated upto primary level while 16.7% and 40% patients studied till SSC and HSC level respectively. 33.3% patients were graduates while 6.7% patients had no education. 6.7% patients in Control Group were educated upto primary level while 20% and 43.3% patients studied till SSC and HSC level respectively. 26.7% patients were graduates while 3.3% patients had no education. There was no significant difference between the groups as per Chi-Square test ($p>0.05$).

5. Majority of patients in both groups were from middle class (60% and 53.3% respectively) followed by upper class (30% and 40% respectively) and lower class (10% and 6.7% respectively). There was no significant difference between the groups as per Chi-Square test ($p>0.05$).

6. 56.7% patients in Study Group resided in urban areas while 43.3% patients were from rural areas. 53.3% patients in Control Group resided in urban areas while 46.7% patients were from rural areas. There was no significant difference between the groups as per Chi-Square test ($p>0.05$).

7. 20 (23.3%) patients in Study Group had Full term vaginal delivery (FTVD) while 40 (66.7%) patients had Lower Segment Cesarean Section (LSCS) delivery. 19 (31.7%) patients in Control Group had Full term vaginal delivery (FTVD) while 41 (68.3%) patients had Lower Segment Cesarean Section (LSCS) delivery. There was no significant difference between the groups as per Chi-Square test ($p>0.05$).

8. The mean gestational age between groups was comparable (39.13 ± 1.40 vs. 38.93 ± 1.80 weeks). The difference was statistically not significant as per Student t-test ($p>0.05$).

9. The systolic blood pressure (SBP) (148.81 ± 6.54 vs. 108.10 ± 7.18 mmHg) and diastolic blood pressure (DBP) (96.07 ± 6.48 vs. 74.53 ± 8.96 mmHg) of patients were significantly higher in Study Group as per Student t-test (**$p<0.05$**).

10. 12 (20%) patients in Study Group had very severe deficiency vitamin D (<5 ng/ml) as compared to 4 (6.7%) patients in Control Group while 34 (56.7%) patients in Study Group had severe vitamin D deficiency as compared to 18 (30%) patients in Control Group. Mild deficiency of vitamin D was observed in 10 (16.6%) and 22 (36.7%) patients respectively. The mean Vitamin D level was significantly lower in Study Group as compared to Control Group (8.73 ± 5.28 vs. 14.20 ± 7.82 ng/ml) (**$p<0.05$**).

11. 35 (58.3%) patients in Study Group had calcium deficiency (<8.5 mg/dl) while 25 (41.7%) patients had normal calcium levels. 18 (30%) patients in Control

Group had calcium deficiency (<8.5 mg/dl) while 42 (70%) patients had normal calcium levels. The mean serum calcium levels was significantly lower in Study Group as compared to Control Group (8.05 ± 1.44 vs. 9.66 ± 1.70 mg/dl) (**p<0.05**).

12. The mean birth weight of neonates was 2.86 ± 0.36 kgs and 3.11 ± 0.54 kgs in Study Group and Control Group respectively. There was statistically significant difference between the two groups as per Student t- test (**p<0.05**).

13. 5 (8.3%) neonates in Study Group had very severe deficiency of vitamin D (<5 ng/ml) as compared to 2 (3.3%) neonates in Control Group while 11 (18.4%) neonates in Study Group had severe deficiency of vitamin D as compared to 7 (11.7%) neonates in Control Group. Mild deficiency of vitamin D was observed in 32 (53.3%) and 12 (20%) neonates respectively The mean Vitamin D level of neonates was significantly lower in Study Group as compared to Control Group (13.67 ± 6.12 ng/ml vs. 23.65 ± 10.42 ng/ml) (**p<0.05**).

14. 28 (46.7%) neonates in Study Group had calcium deficiency (<8.5 mg/dl) while 32 (53.3%) neonates had normal calcium levels. 23 (38.3%) neonates in Control Group had calcium deficiency (<8.5 mg/dl) while 37 (61.7%) neonates had normal calcium levels. The mean serum calcium levels of neonates was significantly lower in Study Group as compared to Control Group (8.31 ± 1.48 mg/dl vs. 9.26 ± 1.67 mg/dl) (**p<0.05**).

15. The number of NICU admission was lower in Control Group as compared to Study Group (25% vs. 20%), however the difference was statistically not significant as per Chi-Square test (p>0.05).

16. In Study Group, 23 (38.3%) neonates were small for gestational age while Meconium stained Liquor and Intrauterine fetal death occurred in 6 (10%) and 4 (6.7%) neonates respectively. 1 (1.7%) neonate died during neonatal period. In Control Group, 18 (30%) neonates were small for gestational age while Meconium stained Liquor and Intrauterine fetal death occurred in 5 (8.3%) and 1 (1.7%) neonates respectively. There was no significant difference between the groups as per Chi-Square test ($p>0.05$).

17. A significant negative correlation was observed between Vitamin D and Systolic Blood Pressure (SBP)/Diastolic Blood Pressure (DBP) in Study Group while a positive correlation was observed between Vitamin D and SBP/DBP in Control Group but this correlation was not significant.

18. A significant positive correlation was observed between Vitamin D and Serum Calcium in Study Group. A positive correlation was observed between Vitamin D and Serum Calcium in Control Group but this correlation was not significant ($p>0.05$).

CONCLUSION

From the present study, it was concluded that 25 Hydroxy Vitamin D and calcium levels were lowered in patients of pregnancy induced hypertension as compared to normotensive pregnant women. Early detection of deficiencies of these parameters (both prior to conception or in early period of pregnancy and in females having previous history of PIH) may be helpful in preventing occurrence of PIH. So, this study conveys the message that predictive and prognostic value of these parameters may be helpful in the early diagnosis of PIH. All these endeavors may contribute to alleviate maternal morbidity and preterm birth outcomes.

Proper guidance to pregnant women (both having previous history of PIH or in early period of pregnancy) regarding dietary modifications by including Vitamin D and calcium rich food sources, may be beneficial in preventing PIH occurrence. Moreover, it may decrease burden on health care set up in developing countries where medical facilities can be channelized to tackle other major health concerns among populations... Further studies among larger and different population are required to add to the existing matter.

Vitamin D plays an important role in the pathological process of preeclampsia. When there is higher risk of developing vitamin D deficiency, vitamin D supports to reduce the risk of hypertensive pregnancies in such populations. It is also helpful for those pregnant patients with a history of preeclampsia by reducing the incidence of preeclampsia and eclampsia in the current pregnancy.

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ANNEXUERS

ETHICAL CLERENCE CERTIFICATE

Institutional Ethical Committee
BIJAPUR-586 103.
OUTWARD
No. 61/16
Date. 06/10/16
B.L.D.E. U's Shri B.M. Patil Medical College.

B.L.D.E. UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103
INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 04-10-2016 *at* 02pm *to scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis has been accorded Ethical Clearance.*

Title "Comparative study of vitamin D levels in term hypertensive and Normotensive pregnant women"

Name of P.G. student Dr Anveeka Kumar.
Dept of OBG.

Name of Guide/Co-investigator Dr V.R. Gobbur
Prof of OBG.

DR.TEJASWINI. VALLABHA
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

Following documents were placed before E.C. for Scrutinization
1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

INFORMED CONSENT FORM

TITLE OF THE PROJECT	**:**	COMPARATIVE STUDY OF VITAMIN D LEVELS IN TERM HYPERTENSIVE AND NORMOTENSIVE WOMEN .
PRINCIPAL INVESTIGATOR	**:**	DR ANVESHA KUMAR POST GRADUATE, DEPARTMENT OF OBSTETRICS and GYNECOLOGY, B.L.D.E. (Deemed to be University) SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH, CENTER VIJAYPUR – 586103, KARNATAKA.
PG GUIDE	**:**	DR.(PROF).V.R.GOBBUR $_{MD}$ PROFESSOR, M.D. DEPARTMENT OF OBSTETRICS AND GYNECOLOGY. B.L.D.E. (Deemed to be University) SHRI B.M. PATIL MEDICAL COLLEGE HOSPITAL AND REASEARCH CENTER, VIJAYPUR– 586103, KARNATAKA.

PURPOSE OF RESEARCH:

I have been informed that this will be a correlational prospective comparative study of vitamin D levels between term hypertensive and normotensive pregnant women visiting BLDE (Deemed to be University) Shri B.M. Patil Medical College Hospital & Research Centre, Vijayapur, and Karnataka.

I have been explained about the reason for doing this study and selecting me/my ward as a subject for this study. I have also been given free choice for either being included or not in the study.

PROCEDURE:

I/my ward have been explained that, I/my ward will be subjected to general physical examination and investigations in pregnant women with hypertensive delivery risk factors visiting the hospital.

I/my ward will be followed up with certain routine blood and urine investigations, until I/my ward will be discharged.

RISKS AND DISCOMFORTS:

I/my ward understand that I/my ward would not have any discomfort with my study. I/my ward understand that necessary measures will be taken to reduce any kind of complications as and when they arise.

BENEFITS:

I/my ward understand that my participation in this study will help to compare the vitamin D levels in term hypertensive and normotensive women pregnant women.

The rationale behind this study is to highlight that vitamin D screening & supplementation is important in pregnancy to prevent adverse complications like preeclampsia and eclampsia in term hypertensive and normotensive women.

CONFIDENTIALITY:

I/my ward understand that medical information produced by this study will become a part of this Hospital records and will be subjected to the confidentiality and privacy regulation of BLDE (Deemed to be University) Shri B.M. Patil Medical College Hospital and Research Centre, Vijayapur, Karnataka.Information of a sensitive, personal nature will not be a part of the medical records, but will be stored in the investigator's research file and identified only by a code number. The code key connecting name to numbers will be kept in a separate secure location.

If the data are used for publication in the medical literature or for teaching purpose, no names will be used and other identifiers such as photographs and audio or video tapes will be used only with my special written permission. I understand that I may see the photograph and videotapes and hear audiotapes before giving this permission.

REQUEST FOR MORE INFORMATION:

I understand that I may askmore questions about the study at any time. Dr. Anvesha Kumar is available to answer my questions or concerns. I/my ward understand that I will be informed of any significant new findings discovered during the course of this study, which might influence my continued participation.

If during this study, or later, I wish to discuss my participation in or concerns regarding this study with a person not directly involved, I am aware that the social worker of the hospital is available to talk with me and that a copy of this consent form will be given to me for careful reading.

REFUSAL OR WITHDRAWL OF PARTICIPATION:

I/my ward understand that my participation is voluntary and I may refuse to participate or may withdraw consent and discontinue participation in the study at any time without prejudice to my present or future care at this hospital.

I/my ward also understand that Dr. Anvesha kumar will terminate my participation in this study at any time after he has explained the reasons for doing so and has helped arrange for my continued care by my own physician or therapist, if this is appropriate.

STUDY SUBJECT CONSENT STATEMENT:

I/my ward confirm that Dr.Anvesha kumar has explained to me the purpose of this research, the study procedure that I will undergo and the possible discomforts and benefits that I may experience, in my own language.

I/my ward have been explained all the above in detail in my own language and I understand the same. Therefore I agree to give my consent to participate as a subject in this research project.

_____ _____

(Participant) Date

_____ _____

(Witness to above signature) Date

ANNEXURE IX

CASE SHEET PROFORMA

Name : DATE :

Age/Sex :

I.P.NO : Ph No. :

Occupation : Case no :

DOA : DOD :

Address :

Chief complaints :

History of presenting complaints:

ANTENATAL HISTORY

I. Trimester:

II. Trimester:

III. Trimester:

Obstetric history

- Married life :
- Obstetric score :

Menstrual history

- Past menstrual cycle :
- LMP :
- EDD : POG:

Past history :

Family history :

Personal history :

GENERAL PHYSICAL EXAMNIATION:

Build and nourishment:

P.R :

Height : B.P :

Weight : BMI:

R.R :

Temp :

Breast :

Thyroid :

Spine :

Pallor/ icterus/ cyanosis/clubbing/oedema /lymphadenopathy.

SYSTEMIC EXAMINATION:

CVS :

RS :

PER ABDOMEN:

Uterine height : Presentation :

FHS : Uterine contraction :

PER SPECULUM EXAMINATION:

(IF REQUIRED)

PER VAGINAL EXAMINATION:

(IF REQUIRED)

Effacement :

Os Dilatation:

INVESTIGATIONS / INTERVENTIONS:

MATERNAL SERUM VITAMIN D LEVEL:

MATERNAL SERUM CALCIUM:

CORD BLOOD LEVEL OF: Serum CALCIUM:

Serum VITAMIN D

CBC :

Blood grouping and Rh typing :

RBS :

Urine Examination

- Urine Routine :
- Urine microscopy :

HIV &Hbs Ag :

Ultrasound examination:

- Date :
- Period of gestation :

5. PIH profile:

LFT – Serum bilirubin (total) :

Serum bilirubin (unconjugated) :

Serum protein:

Serum albumin:

Serum A/G ratio:

SGOT:

SGPT:

ALP:

SERUM CREATININE:

SERUM UREA

SERUM URIC ACID:

aPPt (Only in hypertensive) :

INR (Only in hypertensive):

PT (Only in hypertensive):

FOLLOW UP

Antenatal events :

Delivery date :

Gestational Age :

Type of delivery : FTVD/ Preterm delivery /LSCS

Foetal outcome :

Birth Weight :

Live and healthy/ stillborn /AGA/IUGR/Asphyxia

NICU Admission :

Others :

REMARKS

KEY TO MASTER CHART

OP/IP NO	: OUTPATIENT /INPATIENT NUMBER
SBP	: SYSTOLIC BLOOD PRESSURE
DBP	: DIASTOLIC BLOOD PRESSURE
BMI	: BODY MASS INDEX
NICU	: NEONATAL INTENSIVE CARE UNIT
SSC	: SECONDARY SCHOOL CERTIFICATE
HSC	: HIGHER SECONDARY SCHOOL CERTIFICATE
LSCS	: LOWER SEGMENT CEASEREAN SECTION
FTVD	: FULL TERM VAGINAL DELIVERY
SGA	: SMALL FOR GESTATIONAL AGE