"ASSOCIATION OF VAGINAL pH ≥5 AT 13-20 WEEKS OF GESTATION WITH THE INCIDENCE OF PRETERM BIRTH: A PROSPECTIVE COHORT STUDY "

By

REG.NO: BJ011407

Dissertation

Submitted to the
KLE University, Belagavi, Karnataka

In Partial Fulfillment
of the requirements for the degree of

MASTER OF SURGERY
in
OBSTETRICS AND GYNAECOLOGY

DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY,
JAWAHARLAL NEHRU MEDICAL COLLEGE,
BELGAUM, KARNATAKA

APRIL – 2017

KLE UNIVERSITY, BELAGAVI,

KARNATAKA

Endorsement By The Head of the Department, Principal/Head Of The Institution

This is to certify that the dissertation entitled "**ASSOCIATION OF VAGINAL pH ≥ 5 AT 13-20 WEEKS OF GESTATION WITH THE INCIDENCE OF PRETERM BIRTH: A PROSPECTIVE COHORT STUDY**" is a bonafide research work done by **REG.NO: BJ011407.**

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LIST OF ABBREVIATIONS

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WHO	-	World Health Organization
LMP	-	Last Menstrual Period
MDG	-	Millennium Development Goal
pPROM	-	prelabor Premature Rupture Of Membranes
BV	-	Bacterial Vaginosis
PHC	-	Primary Health Center
BMI	-	Body Mass Index
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AV	-	Aerobic Vaginitis
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vICAM1	-	vascular Intercellular Adhesion Molecule 1
xMAP	-	Multiplex Analyte Profiling
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sIL6R	-	soluble Interleukin-6 Receptor Alpha
TNF-β	-	Tumour Necrosis Factor-Beta
CCL4	-	Macrophage Inflammatory Protein-1 Alpha
MMP9	-	Matrix Metalloproteinase-9
BDNF	-	Brain-Derived Neurotrophic Factor
GM-CSF2	-	Granulocyte-Monocyte-Colony Stimulating Factor
sTNFR1A	-	soluble Tumour Necrosis Factor Receptor I
fFN	-	fetal fibronectin
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ABSTRACT

Introduction

Preterm delivery remains the largest cause of neonatal mortality, accounting for 27% of the 3.1 million annual deaths worldwide. Maternal infection increases the incidence of and is responsible for 40% of spontaneous preterm deliveries making its prevention critical.Bacterial vaginosis is one of the major contributors to preterm birth with an incidence of 10-30% in all populations worldwide and 25% incidence in pregnant women.The most commonly utilized method of screening for Bacterial Vaginosis (BV) is the Amsel score. However, the procedure is difficult to reliably implement and unaffordable in rural, developing country primary health care centres. The vaginal pH acidity test is a simple, inexpensive procedure that can be easily administered in rural settings. The aim of this study is to assess whether vaginal pH $\geq$ 5 is associated with an increased incidence of preterm birth.

Methods

The study was a prospective cohort study nested within the ongoing community based randomized controlled trial assessing the efficacy of oral Clindamycin for reducing the incidence of preterm birth implemented in 12 Primary Health Center areas of Belgaum and Bagalkot districts of Karnataka during the period of July 2015 to August 2016. The trial screened permanent residents in the study area who presented for antenatal care (ANC) at 13-20 weeks of gestation for vaginal pH by obtaining a sample of fluid from the posterior fornix of the vagina with pH paper. The study population for prospective cohort study was 5148 pregnant women. Women with vaginal pH $\geq$ 5 and randomized to the placebo arm (n=809 women) formed the exposed group while 4,339 women with vaginal pH < 5 formed the unexposed group.

Results

The incidence of preterm birth in the exposed group was 15.3% compared to 11.8% in the un-exposed group with an absolute difference in the rate of preterm birth being 3.5%. i.e. 23% greater risk of preterm birth in women with vaginal pH $\geq$5. On performing logistic regression analysis, there was a statistically significant correlation with reference to cut-off of vaginal pH > 5 with increased risk of spontaneous preterm birth, in both early (OR -1.489, 95% CI 1.131-1.961, p value-0.005) and overall (OR-1.372, 95% CI 1.11-1.69 p value-0.003) preterm birth.

Conclusion

Vaginal pH ≥ 5.0 is significantly associated with increased risk of preterm birth and maybe considered as a good predictor of both early (< 34 weeks) and overall (<37weeks) preterm birth.

Keywords

Bacterial vaginosis, preterm birth, vaginal pH.

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Declaration by the Candidate

I hereby declare that this dissertation entitled "**ASSOCIATION OF VAGINAL pH ≥ 5 AT 13-20 WEEKS OF GESTATION WITH THE INCIDENCE OF PRETERM BIRTH: A PROSPECTIVE COHORT STUDY**" is a bonafide and genuine research work carried by me, under the guidance of **Dr. MRITYUNJAY.C.METGUD** **MD, FICOG**, Professor, Department of Obstetrics and Gynaecology, J. N. Medical College, Belagavi.

Date: **Dr. Swati Goudar**

Place: Belagavi

KLE UNIVERSITY, BELAGAVI,

KARNATAKA

Certificate by the Guide

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Place: Belagavi.

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Professor
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Declaration by the candidate

I hereby declare that the KLE University, Belagavi, Karnataka shall have the rights to preserve, use and disseminate this dissertation in print or electronic format for academic/ research purpose.

Date: **Dr. Swati Goudar**

Place:

Acknowledgement

With the conclusion of my dissertation, it's truly not complete without thanking the people involved in realization of this very important part of my postgraduation.

I thank my guide ***Dr. Mrityunjay C Metgud*** $_{MD,FICOG}$*, Professor of Obstetrics and Gynecology, for having immense patience in teaching, coaching and guiding on so many aspects of my learning experience here both academically and individually. His steady motivation is something I'll always cherish resulting in successful fruition of my thesis.*

I thank to ***Dr. M B Bellad*** $_{MD}$*, Professor and Head of Department of Obstetrics and Gynecology, whose brainchild is my dissertation for his keen interest, constant support and admonishment and ever available guidance.*

I truly feel blessed to be the daughter of ***Dr. S S Goudar*** $_{MD,MHPE}$*, Professor of Physiology and Research Coordinator, my First teacher who has stood by me every step of the way as the greatest coach for life, my telescope to the world of research, for sharing his vast knowledge on research to mould my small venture into it. You're the genius Sir who'll never cease to inspire me to achieve more and strive harder to be a better person every day.*

I sincerely thank ***Dr. Matthew Hoffman*** $_{MD}$*, Chair and Professor of Obstetrics and Gynecology, Christianacare, Delaware for his expert advice which were of tremendous help in building up my analysis and help my dissertation reach a logical conclusion.*

I am very grateful to ***Dr. M.K Swamy*** $_{MD}$*, Professor, Department of Obstetrics and Gynaecology, for his valuable support provided during my Post Graduate programme.*

I express my gratitude to ***Dr. Kamal Patil*** $_{MD}$*, Professor of Obstetrics and Gynecology, for teaching me the vital implication of discipline in life and for her perpetual encouragement to pursue higher ambitions.*

I thank ***Dr. Hema Dhumale*** $_{MD}$*, Professor of Obstetrics and Gynecology, for giving me the vision of the sturdy model, I would like to be in my future and for her unbroken support.*

I thank ***Dr. B.R Nilgar*** $_{MD}$*,* ***Dr. Anita Dalal*** $_{MD}$ *and* ***Dr.Yeshita Pujar*** $_{MD}$ *Professors of Obstetrics and Gynecology, for providing the opportunity to learn to develop as a teacher and be a better postgraduate every day.*

I express my heartfelt gratitude to all my beloved teachers ***Dr. Shridevi Metgud****,* ***Dr. Nikhil Vernekar****,* ***Dr. Neha Limbani****,* ***Dr. Mahadevi Savanur*** *and* ***Dr. Manjunath Hukkeri*** *for teaching us the core principles of our subject, guiding us on the apt management of cases, building confidence, boosting morale, where these invaluable experiences help shape into a confident skillful professional.*

I am grateful to ***Mr. Amit Revankar MBA****, my resourceful Data manager for having spent countless hours building up my vast database and attempting to analyse it with me,* ***Mr.Chandrashekhar****,* ***Mr.Killedar****,* ***Mr.Raju*** *,****Mr.Sanjay*** *,****Mr.Keval,*** ***Mr.Prashant*** *and all the cherished research unit members for their everlasting help and support.*

I will always fondly remember the priceless, infinite memories I have shared with Seetha, Swarnika, Naveen my pillars of strength, Neha my other half for life, SurAbhi my most fun sweet chirpy labour-room mate, my five darling junior sweethearts Sanket, Pranita, Anusha, Ritika and Arpita, my unforgettable rock-solid seniors Steffi, Manoj and Satwik, Prarthana and all my postgraduates colleagues all with whom I've spent days and nights training shouting learning and teaching making

this otherwise arduous journey into a beautiful meaningful transition into consultancy.

I humbly acknowledge the substantial role of my study subjects who have enabled this important undertaking in research reach a notable conclusion.

I thank the Principal of J. N. Medical College, ***Dr (Mrs.) Niranjana S Mahantshetti*** $_{MD,}$ *for her kind permission to allow me to undertake this study.*

This endeavor has been possible for me with the inspiration and blessings of my parents and my sister who have always been there for me.

I am thankful to ***Mr. Anand*** *and* ***Mr. Arun, Library Xerox Centre*** *for formatting, printing and binding of my dissertation.*

Lastly and most importantly I pay my respects to The Almighty for making this venture possible to see the light of the day.

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INTRODUCTION

The World Health Organization (WHO) defines preterm birth as any birth before 37 completed weeks of gestation, or fewer than 259 days since the first day of last menstrual period (LMP).Preterm birth can be further sub classified on the basis of gestational age: extremely preterm (<28 weeks), very preterm (28-32 weeks), and moderate or late preterm (32-<37 completed weeks of gestation).

More than 1 in 10 babies are born preterm. Over 1 million children die each year due to complications of preterm birth and most of the survivors face a lifetime of disability, including learning disabilities and visual and hearing problems.

Prematurity is the leading cause of early neonatal deaths and now the second leading cause of death after pneumonia in children under the age of 5. Global progress in child survival and health to 2015 and beyond cannot be achieved without addressing preterm birth.

New WHO estimates of global rates of preterm births, indicate that of the 135 million live births worldwide in 2010, about 15 million babies were born too early, representing a preterm birth rate of 11.1%. Over 60% of preterm births occurred in sub-Saharan Africa and South Asia where 9.1 million births (12.8%) annually are estimated to be preterm.[1]

Over 60% of preterm births occur in Africa and South Asia. The 10 countries with the highest numbers include Brazil, the United States, India and Nigeria, demonstrating that preterm birth is truly a global problem. In the poorest countries, on

average, 12% of babies are born too soon compared with 9% in higher-income countries. Within countries, poorer families are at higher risk.[2]

Urgent action is needed to address the estimated 15 million babies born too soon which is essential in order to progress on the Millennium Development Goal (MDG) for child survival by 2015 and beyond, since 40% of under-five deaths are in newborns, and it will also give added value to maternal health that is MDG 5 investments.[1]

Preterm birth is a syndrome with a variety of causes which can be classified into two broad subtypes:

1. Spontaneous preterm birth (spontaneous onset of labor or following prelabor premature rupture of membranes (pPROM)) and
2. Provider-initiated preterm birth (defined as induction of labor or elective caesarian birth before 37 completed weeks of gestation for maternal or fetal indications (both "urgent" or "discretionary"), or other non-medical reasons.[3]

The number and causes of provider-initiated preterm birth are more variable. Globally, the highest burden countries have very low levels due to lower coverage of pregnancy monitoring and low cesarean birth rates (less than 5% in most African countries). However, in a recent study in the United States, more than half of all provider-initiated preterm births at 34 to 36 weeks gestation were carried out in absence of a strong medical indication. Unintended preterm birth also can occur with the elective delivery of a baby thought to be term due to errors in gestational age assessment.

Current screening tests for the prediction of spontaneous preterm labour can be divided into three general categories:

- Risk factor assessment
- Cervical length measurement
- Biochemical markers[4]

Spontaneous preterm birth is a multi-factorial process, resulting from the interplay of factors causing the uterus to change from quiescence to active contractions and to birth before 37 completed weeks of gestation. The precursors to spontaneous preterm birth vary by gestational age and social and environmental factors, but the cause of spontaneous preterm labor remains unidentified in up to half of all cases. Maternal history of preterm birth is a strong risk factor and most likely driven by the interaction of genetic, epigenetic and environmental risk factors.[5-7]

The syndrome of altered vaginal flora has been consistently linked to an approximately 2-fold increase in the risk of spontaneous preterm birth. However, it is still unclear whether Bacterial Vaginosis (BV) itself is directly related to preterm birth in a cause-effect manner. If BV is directly related, it likely occurs through a mechanism that results in microbial colonization leading to inflammation of the upper genital tract. The link between upper genital tract microbial infection and spontaneous preterm birth appears stronger than the association of BV with preterm labour.[8,9]

But from a clinical perspective there is other substantial evidence to denote BV as a marker of lower genital tract marker (silent) for upper genital tract infection.[10]

The sensitivity of BV to identify women ultimately destined to deliver preterm is approximately 30%.[11]

The most commonly utilized method of screening for BV is the Amsel score, identifying BV by the presence of 3 of 4 criteria, vaginal pH >4.5, abnormal discharge, clue cells, and positive whiff test.[9] However, the procedure is difficult to reliably implement and unaffordable in rural, developing country Primary Health care Centers (PHC). The vaginal pH acidity test is a simple, inexpensive procedure that can be easily administered in rural settings.[12]

With this background, the aim of this study is to highlight measurement of vaginal pH and correlation of pH being ≥ 5 with the incidence of preterm birth as a cost-effective screening tool to identify BV in resource limited settings for timely administration of appropriate treatment for the prevention of preterm birth, hence reducing the burden of prematurity. This study also helps to investigate any other association of underlying maternal and fetal conditions with BV cumulatively resulting in preterm birth.

OBJECTIVES

The objectives of the present study are:

Primary Objective:

To determine the association between vaginal pH ≥ 5 at 13-20 weeks of gestation and the incidence of preterm birth.

Secondary Objectives:

1. To perform a sensitivity analysis to determine if there is a cut-off for the pH beyond which the preterm birth rate increases or if there is a dose effect (e.g. the higher the pH the greater the rate of preterm birth)

2. To assess association between vaginal pH ≥ 5 at 13-20 weeks of gestation and stillbirth rate, perinatal mortality and neonatal mortality. Sex of the baby, birth weight, place and mode of delivery, delivery attendant.

3. To assess the association between covariates such as maternal age and education, gravidity, parity, BMI, haemoglobin and prior preterm delivery for multiparous women on the incidence of preterm birth.

REVIEW OF LITERATURE

Incidence

The WHO defines preterm birth as any birth before 37 completed weeks of gestation, or fewer than 259 days since the first day of LMP. Preterm birth can be further sub classified on the basis of gestational age: extremely preterm (<28 weeks), very preterm (28-32 weeks), and moderate or late preterm (32-<37 completed weeks of gestation).[1]

Preterm labor is defined as the occurrence of regular uterine contractions (≥4 contractions in 20 minutes or ≥8 contractions in one hour) and cervical changes (effacement ≥80% and dilatation ≥1 cm) in women with intact fetal membranes and gestational age 20-37 weeks.[13]

These subdivisions are important since decreasing gestational age is associated with increasing mortality, disability, intensity of neonatal care required and cumulative increase in costs.[14]

The global average preterm birth rate in 2010 was 11.1%. Preterm delivery is the largest cause of neonatal mortality rate (NMR), accounting for 27% of the 3.1 million deaths worldwide over and is the second largest direct cause of child deaths in children younger than 5 years old on and an associated increase of 5.3 million preterm births from 2005 to 2010.[15,16]

More than 60 % of preterm births were in the south Asia and sub-Saharan Africa, where 52 % of the global live births occur. Rates are highest on average for low-income countries (11.8%), followed by lower middle-income countries (11.3%)

and lowest for upper middle- and high-income countries (9.4% and 9.3%). However, relatively high preterm birth rates are seen in many individual high-income countries where they contribute substantially to neonatal mortality and morbidity.[2]

The 10 countries with the highest numbers of estimated preterm births are India, China, Nigeria, Pakistan, Indonesia, United States, Bangladesh, the Philippines, Democratic Republic of the Congo and Brazil. These 10 countries account for 60% of all preterm births worldwide.[1]

Annually, India contributes to the greatest number of neonatal, stillbirths and maternal deaths in the world accounting for. In the past decade, the Government of India has promoted delivery at the PHC and referral centers to improve maternal and neonatal health care.[1,15,17-19]

Compounding the issue, the risk of preterm birth is highest in developing countries where an estimated 12% of births are preterm compared to 5-7% in developed countries. Of the near 13 million preterm births worldwide in 2005, 11 million were in Africa and Asia.[2]

The economic costs for a preterm baby is high and in terms of neonatal intensive care and ongoing health-care and educational needs and added social costs, with many families experiencing the sudden loss of a preterm baby and prolonged stressful hospital stay.

In addition to its contribution to NMR, preterm birth has lifelong side effects on neurodevelopmental outcomes such as increased risk of cerebral palsy, impaired learning, visual disorders and incidence of chronic disorders in adulthood.

Preterm labour is responsible of up to 50% of pediatric neurodevelopmental disorders. Infants born prematurely are likewise at increased risk for a variety of long term medical complications such as respiratory, gastrointestinal, cardiovascular, and metabolic disorders.[19]

MDG 4 calls for a reduction in the under-5 mortality rate by two-thirds between 1990 and 2015 and MDG 5 for a reduction in the maternal mortality ratio by three-quarters during the same period. Child survival programs have primarily focused on important causes of death after the first 4 weeks of life such as pneumonia, diarrhea, malaria and vaccine-preventable conditions, resulting in a decline in under-5 mortality rates.

While important, the concomitant lack of attention to important causes of neonatal mortality like preterm birth (the single largest cause of neonatal mortality, contributing to 29% of neonatal deaths) has resulted in neonatal deaths becoming an increasing proportion of under-5 deaths (from 37% in 1990 to 40% in 2010), and demonstrating a slower rate of decline than that for under-5 deaths.

Since prematurity contributes significantly to child mortality, Born Too Soon presents a new goal for the reduction of deaths due to complications of preterm birth.

- For countries with a current neonatal mortality rate level of more than or equal to 5 per 1,000 live births, the goal is to reduce the mortality due to preterm birth by 50% between 2010 and 2025.
- For countries with a current neonatal mortality rate level of less than 5 per 1,000 live births, the goal is to eliminate remaining preventable preterm

deaths, focusing on equitable care for all and quality of care to minimize long-term impairment.

After the publication of this report, a technical expert group will be convened to establish a goal for reduction of preterm birth rate by 2025, for announcement on World Prematurity Day 2012.[1]

Etiology

Distinguishing spontaneous and provider-initiated preterm birth is of importance to programs aiming to reduce preterm birth. For spontaneous preterm births, the underlying causes need to be understood and addressed, while in the case of provider-initiated preterm births both the underlying conditions (e.g. preeclampsia) and obstetric policies and practices require assessment and to be addressed.[1]

Evidence suggests that spontaneous preterm labour and delivery are a heterogeneous condition with many triggers or precipitating factors including maternal genital tract haemorrhage, cervical dysfunction, idiopathic uterine contractions, infection, malnutrition, multifetal pregnancy, and spontaneous rupture of the fetal membranes.[20]

Four distinct mechanisms for the pathogenesis of preterm labour have been described and include premature activation of the fetal hypothalamic pituitary axis, mechanical stretch, inflammation/ matrix remodelling, and placental abruption.[21,22]

Maternal infection increases the incidence of and is responsible for 40% of spontaneous preterm deliveries making its prevention critical.[7] Historically, clinical

efforts have focused on treating preterm labor with tocolytic medications to prolong pregnancy, however, these efforts have not improved latency or outcomes.[23,24]

With emerging evidence that the process of preterm birth begins in early pregnancy, efforts have begun to concentrate on earlier prevention.[25,26]The risk of adverse outcome is greater the earlier the genital tract is abnormally colonized, and antibiotic treatment of gestational infection may have its strongest effect at ~20 weeks gestation. Laboratory, animal and clinical evidence consistently find ascending lower genital tract infections are linked to subclinical chorioamnionitis that causes preterm birth. The ensuing production of prostaglandins initiates a process of cervical softening and uterine contractions or weakens the integrity of the amniotic membranes that ultimately leads to preterm birth.[27]

Women with abnormal vaginal flora, including BV, experience higher rates of preterm delivery. The pathogenesis of preterm birth may begin early in gestation and thus treatment of gestational infection may have its strongest effect when implemented in the first three to five months of pregnancy.[24]

Bacterial vaginosis (BV) is a syndrome characterized by alterations in the vaginal bacterial flora from the normal state in which *Lactobacillus* species are predominant to a pattern in which a variety of other bacteria, including *Gardnerellavaginalis*, anaerobes, and *Mycoplasma hominis*, predominate. These changes in flora contribute to an increased vaginal fluid pH, increased levels of organic acids and volatile amines in vaginal fluid, and symptomatic vaginal discharge.[28]

Bacterial vaginosis is the most common cause of abnormal vaginal discharge in adult women. The prevalence of BV ranged between 10-30% in different populations all over the world.[29]

84% of women with BV reported no symptoms

18.8% of women with no prior sexual history can still be affected by BV

25% of asymptomatic pregnant women have BV.[30]

In our hospital the prevalence of BV in pregnant was reported to be 40%.[13]

The prevalence of BV increases based on lifetime number of sexual partners.

Lactobacillus is normal flora in the vagina that produces an acidic medium via the hydrogen peroxide (H2O2), which transforms the glycogen present in the normal vaginal epithelium to lactic acid hence maintain the acidic medium of the vagina to 4.5. This acidic medium produced by the lactobacillus suppresses the growth of other microorganisms.[29]

Under normal conditions of low pH, Lactobacilli are able to produce hydrogen peroxide (H2O2) which is toxic to bacteria in two ways: firstly by producing toxic hydroxic radicals and secondly by combining heavy pool of chlorine ions in the vagina to produce chlorinium ions. In situations of increased alkalinity, such as bleeding in pregnancy, sexual intercourse or vaginal douching, or in conditions where antibiotics are used or where there is a change in endocrine status, lactobacilli at high pH are less efficient at producing H2O2 which permits the overgrowth of other organisms.[31]

Organisms associated with BV, such as Mobiluncus species and other anaerobes are able to produce the ketoacid succinate, which is responsible for blunting the chemotactic response and reducing the killing ability of polymorphic nuclear leucocytes(PMNLs). This results in a vicious circle of increasing numbers of organisms without an inflammatory response. Thus, there is a large concentration of potentially pathogenic organisms with no obvious cellular host response.[31]

'Full BV' is defined as a predominant granular microflora with uncountable bacteria all over the slide, and more than 20% of epithelial cells covered with bacteria (clue cells). Mixed areas with streaks of BV-like flora or sporadic clue cells combined with other types of microflora (normal-appearing microflora, flora with small bacilli or aerobic coccoid flora) were classified as 'partial BV'. These BV streaks are characterised by small, uncountable bacteria that overlie one another so that they cannot be distinguished or counted individually, and appear to be identical to the streaks seen in full BV. In partial BV, however, these streaks occur on the same slides as aerobic vaginitis (AV) flora or normal flora.

Aerobic vaginitis

Aerobic vaginitis is a condition in which disturbed microflora with an absence of lactobacilli is not overwhelmed by anaerobic bacteria, as in typical BV, but rather contains a significant number of aerobic facultative pathogenic flora from the bowel. In moderate and severe cases, an elevated host immune reaction can be demonstrated by an increased number of leucocytes, which sometimes have a 'toxic' appearance. As this condition produces large amounts of pro-inflammatory cytokines, it was suggested that it can cause preterm labour by initiating the prostaglandin cascade. In full BV, we and others found unexpectedly low levels of interleukin IL-8 and a

striking absence of leucocytes, in spite of the presence of moderate amounts of IL-1b.8. It can therefore be postulated that BV is a condition in which there is relative immune suppression in response to bacterial overgrowth, whereas in AV there is rather a sepsis-like local overreaction of the immune response.

The diagnosis of AVF should be refined and a distinction made between abnormal anaerobic-type (granular) flora (BV flora) and aerobic microflora (short bacilli or cocci; AV flora), and that the inflammatory response in the vaginal fluid should be reappraised, as aerobic microflora and the finding of increased vaginal leucocytosis correlate with greater concentrations of pro-inflammatory cytokines present in the vagina and with enzymatic activity leading to preterm contractions and intrauterine infection. An increasing number of studies show an higher risk of preterm rupture of the membranes and preterm birth when an the number of leucocytes were found to be elevated in the vaginal fluid, especially when counted in proportion to the epithelial cells.

Aerobic vaginitis corresponds to another type of disturbed microflora, in which the lactobacilli are replaced by aerobic facultative pathogens (intestinal microorganisms, such as Escherichia coli, enterococci, Staphylococcus spp. And group B streptococci), vaginal leucocytosis and parabasal cells. Sexually transmitted infections with organisms, such as Chlamydia trachomatis, Neisseria gonorrhoeae and Trichomonas vaginalis have to be excluded. The clinical picture of severe AV often includes a red, inflamed vaginal mucosa, a yellowish sticky discharge, a high pH above 6 and an odour that is unpleasant but not like fishy odour.[4]

Many diseases like PTB are due to a combination of genetic susceptibility and environmental exposure. A woman may have the environmental exposure (BV), but if

she does not have the genetic susceptibility (gene polymorphism) to mount a damaging inflammatory response then little harm may occur. Conversely, a woman may possess the gene polymorphism to mount a damaging inflammatory response, but if she does not have environmental exposure (BV) then damage may not occur. However, when both susceptibility and exposure are present, the risk of an adverse outcome will be increased, and this is referred to as the gene–environmental interaction.[31]

Abnormal vaginal microflora or infection leads to adherence, invasion, and host inflammatory response. That response may be appropriate resulting in tissue repair and healing. Alternatively, the response may be exaggerated (hyper-response) resulting in tissue damage from increased production and release of proinflammatory cytokines. Conversely, the response may be inadequate (hyporesponse) leading to overwhelming infection. Both a hyper-response and a hypo-response may result in mortality and morbidity due to tissue damage.

If antibiotics are used late in this process, it may not be possible to prevent irreversible tissue damage, morbidity, and mortality. In contrast, if antibiotics are used early, before tissue damage occurs, this damage might be prevented. Accordingly, the earlier the gestational age at which clindamycin is administered to women with objective evidence of risk of infection-related preterm birth, the more likely it is to be able to demonstrate a reduction in the rate of preterm birth.[33]

Therapeutic obstetric care, such as cervical cerclage, bed rest, corticosteroid therapy and progesterone have improved fetal outcomes in developed countries.[34] However, in developing countries like India, with more neonatal deaths than any other

country on earth, such care is beyond the reach of most of the population.[35] Consequently, neonatal and maternal mortality and morbidity rates remain high.

Screening tests for preterm labour

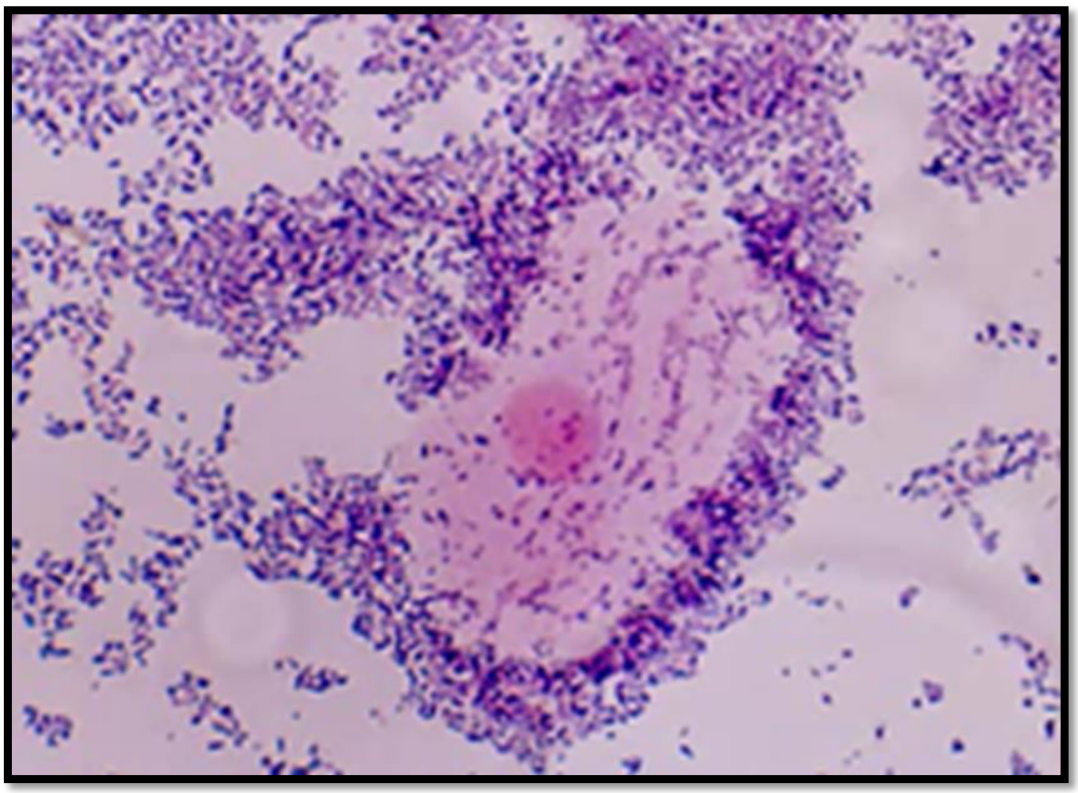
Current screening tests for the prediction of spontaneous preterm labour can be divided into three general categories:

- Risk factor assessment
- Cervical measurement
- Biochemical markers[4]

The most commonly utilized method of screening for BV is the Amsel score, identifying BV by the presence of 3 of 4 criteria, vaginal pH >4.5, abnormal discharge (homogenous non-viscous milky-white discharge adherent to the vaginal walls), clue cells, and positive whiff test.[36] However, the procedure is difficult to reliably implement and unaffordable in rural, developing country PHC's.

Photograph 1. Showing the Nitralazine vaginal pH strip used in the study

Slide1. Clue cell on Histopathology



Slide 2. Showing Nugent's gram stain scoring system

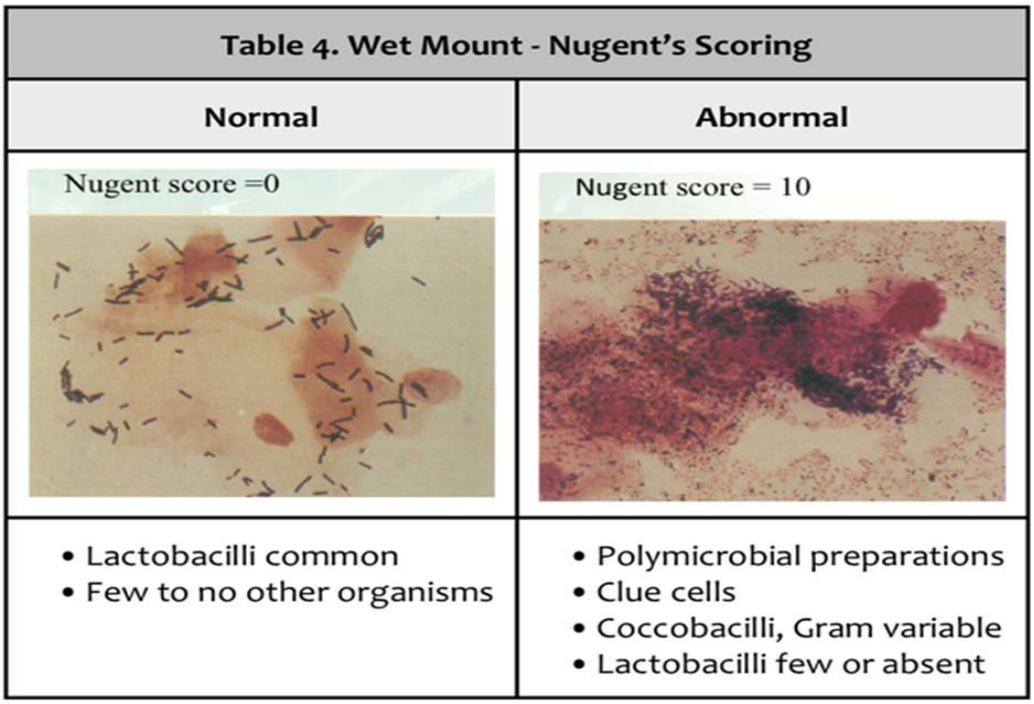


Table 1. Classes of Nugent gram stain scoring system[37]

SCORE*	Lactobacillus morphotypes	*Gardnerella/Bacteroides* spp. Morphotypes	Curved Gram-variable rods
0	**4+	0	0
1	3+	1+	1+ or 2+
2	2+	2+	3+ or 4+
3	1+	3+	
4	0	4+	

* Morphotypes were scored as the average number seen per oil immersion field (minimum of 10-20 fields were examined). Each morphotype was then given a score from the left hand column. The TOTAL SCORE was calculated by adding the individual morphotype scores = Lactobacillus + Gardnerella/Bacteroides + Curved Gram-negative rods.

** QUANTIFICATION SCALE: 0 = no morphotypes seen; 1+ = <1

Table 2. Hay/Ison classification[38]

The Hay/Ison classification.

	Lactobacilli morphotypes	*Gardnerella* morphotypes
Normal (group 1)	Many	Few
Intermediate (group 2)	Equal amount	Equal amount
BV (group 3)	Few	Many

The Nugent scoring system is reliable in diagnosing either BV negative or positive smears, but a problem arises in the intermediate group which is the most difficult to interpret. In a developing country with limited resources such as India, where highly trained skilled manpower comes at a premium, diagnosis of BV by Nugent's score would place a great strain on available resources.[38]

The vaginal pH acidity test is a simple, inexpensive procedure that can be easily administered in rural settings. The pH paper is applied to the high or posterior vaginal fornix to obtain the vaginal pH. The test results are interpreted using a standard color reference chart and the woman's vaginal pH is recorded.

Vaginal pH has a sensitivity and specificity that varies across settings. In Sweden, vaginal pH>4.7 had a sensitivity of 97%, a specificity of 85.8%, a positive predictive value (PPV) of 52.3% and a negative predictive value (NPV) of 99.4% to detect BV.[39]

The Nugent gram stain test, in which a score of ≤3 is negative, 4–6 is intermediate, and ≥7 is positive for BV as described by Nugent et al.[37] is a simpler

and more feasible field procedure than the Amsel test, has high sensitivity and specificity compared with the Amsel score, and may be considered the gold standard.

In a Thai sample where 12% of women were identified with BV by Nugent's score, pH>4.5 had a 100% sensitivity but only 58.9% specificity, with a PPV of 29.7% compared with the Amsel criteria. Compared with Nugent Gram stain score, one U.S. study found pH >4.5 had a sensitivity of 65.6% and specificity of 84.7% and pH ≥4.5 had a sensitivity of 81.3% and specificity of 68.2%.[29]

Using the same Nugent comparison, a multi-center study found PH>4.5 had a sensitivity and specificity of 89.3% and 73.3%, respectively. BV (identified by the Nugent score or using the Amsel criteria) is associated with ~2-times greater risk of spontaneous preterm birth.[40] A pH of ≥5 has similarly been associated with a 45-50% greater rate of preterm birth and with ~2 times higher rates of birth before 32 weeks than women with pH<4.4.

Women with vaginal pH 5.0 or greater than that had a significantly increased incidence of subsequent preterm birth at less than 37, 35 or 32 weeks of gestation. Women whose vaginal pH was 5.0 or less had significantly fewer spontaneous preterm birth and fewer infants weighing less than 2500 grams or less than 1500 grams.

Women with a vaginal pH of 4.5 or greater and a gram stain score of 9 to 10 had a higher risk of preterm delivery than those with a score of 7 or 8. Those with moderate abnormalities of in Gram stain (score7-8) did not have a higher incidence of preterm birth than those with a score of 0-6. Regarding the risk of spontaneous

preterm birth less than 37 completed weeks of gestation, women with a gram stain score of 9 to 10 had a higher risk compared with those having a score of 8 or less.[41]

Risk Factor Assessment

Clinical risk factors for preterm birth include

(i) Demographic characteristics such as low socioeconomic status, poor antenatal care, extremes of maternal age, or malnutrition,

(ii) Behavioral factors including smoking, illicit drug use, alcohol consumption, or heavy physical work,

(iii) Obstetric history including familial (genetic) predisposition, uterine malformation, previous preterm labour or preterm premature rupture of membranes(PROM), previous cone biopsy or cervical surgery, and

(iv) Aspects of the current pregnancy such as multifetal gestation, genital tract bleeding and/or infection, fetal malformation, preterm rupture of membranes, shortened cervix, and other pregnancy complications including preeclampsia and gestational diabetes mellitus.[42,43]

A previous preterm birth before 34 weeks' gestation is amongst the strongest risk factors for subsequent preterm birth with a relative risk of 13.56.[44]

However, in so far as nulliparous women have no past obstetric history to call upon, any such previous history risk factor-based assessment is inapplicable in their situation. Overall risk factor assessment alone is unreliable, as over 50 percent of pregnancies that deliver preterm will fail to be identified.[42,45,46]

Cervical length measurement

Using transvaginal ultrasound, a cervical length below the 10th centile for gestational age increased by 6-fold the risk of delivery prior to 35 weeks' gestation.[45]

A review of 35 studies using sonographically assessed cervical length to predict preterm delivery in *asymptomatic* women and found sensitivities ranging from 68% to 100% and specificities from 44% to 79% with wide variations in their predictive values.[47] A more recent meta-analysis of 28 studies assessing cervical length (<15 mm) in *symptomatic* women with threatened preterm labour found sensitivities ranging from 53% to 67% and specificities ranging from 89% to 92% for delivery within one week . Due to limitations in ultrasound availability and operator expertise, cervical length alone cannot be reliably utilised to predict preterm labour or used as a routine screening tool. Nevertheless, with the exception of modifying lifestyle and/or treating known infection, cervical length determination possibly provides the best avenue for therapeutic intervention, at least at this time in the developed world.[48]

A recent study concluded that women with prior ultrasound-indicated cerclage have similar outcomes whether they receive treatment with ultrasound surveillance and cerclage for cervical length less than 25mm or a planned, history indicated cerclage.[49]

A metanalysis showed at cerclage use has been so frequent in multiple gestations despite the lack of evidence supporting the use of prophylactic cervical cerclage in multiple gestations despite supporting evidence in this settings, despite professional societyrecommendations against routine use with multiple pregnancy and

despite some evidence suggesting that use of cerclage is associated with worse outcomes in these patients.[50]

Biochemical Markers

While the direct study of gestational tissues (e.g., vaginal epithelium, cervix, endometrium, myometrium, placenta, choriodecidua, and fetalmembranes) may provide more accurate localized information on the state of a pregnancy and impending labour, it is the more easily accessible biological fluids including whole blood/serum/plasma, urine, saliva, amniotic fluid, and cervicovaginal fluid (CVF) that are more likely to be amenable to the creation of a rapid bedside biomarker test for predicting preterm labour or preterm PROM. These body fluids provide rich sources of proteins and metabolites that vary in concentration in response to pregnancy and adverse pregnancy states.[51-54]

1. **Amniotic fluid**

Indeed, the procedure *per se* can precipitate preterm labour as well as potentially causing fetal trauma and infection. In the absence of intra-amniotic infection, several protein biomarkers in human amniotic fluid including interleukin-6 (IL-6, symbols in parenthesis assigned by the HUGO Gene Nomenclature Committee)[55,56], interleukin-8 (IL8)[57], interleukin-16 (IL16) , interferon gamma-inducible protein 10 (CXCL10) [56], annexin A2 (ANXA2)[57], and other proinflammatory proteins (CXCL11, ADAM8, SLPI, sICAM1, and vICAM1)[58] have been found to be associated with increased incidence of preterm labour or preterm PROM, yet other studies failed to confirm some of these findings.[59]

Where predictive modelling has been performed, no biomarker in isolation appears to provide adequate predictive efficacy, with generally poor sensitivity and/or specificity.

2. Saliva

Women with a singleton pregnancy and at least one identifiable risk factor for preterm birth. Salivary progesterone was measured at 24 to 28 weeks of gestation and repeated after 3-4 weeks. A single cutoff value for salivary progesterone of 2575 pg/mL produced a sensitivity of 83%, specificity 86%, positive predictive value 60%, and negative predictive value 95%, identifying more than 80% of women who delivered before 34 weeks of gestation. The authors propose that estimating salivary progesterone in high-risk pregnant women may identify those in whom benefit may be derived from supplemental progesterone therapy.[4]

3. Urine

There is a paucity of data examining chemical biomarkers of preterm birth in urine.[4]

4. Blood

A promising study of plasma urocortin concentration in women with symptoms of threatened preterm labour displayed a sensitivity of 80%, a specificity of 100% with a positive predictive value of 100% for preterm delivery within 7 days of sampling.[60]

Using multiplex analyte profiling (xMAP) technology, measured 27 proteins in women presenting with threatened preterm labour. While several proteins were

significantly differentially expressed (interleukin-10 (IL-10), soluble interleukin-6 receptor alpha (sIL6R), tumour necrosis factor-beta (LTA),macrophage inflammatory protein-1 alpha (CCL4), matrix metalloproteinase-9 (MMP9), brain-derived neurotrophic factor (BDNF), granulocyte-monocyte-colony stimulating factor (GM-CSF2), and soluble tumour necrosis factor receptor I (sTNFR1A)), the measurement of cervical length alone provided a greater predictive odds ratio than any of the single biochemical markers studied.[61]

The CVF is a complex mixture of secretions derived from the vagina, endocervix, endometrial decidua, and amniochorion and therefore serves as an important diagnostic site to monitor maternal and fetal health in pregnancy. Unlike the amniotic fluid the CVF is readily accessible and collection is minimally invasive and safe. There are two commonly used clinical biomarker tests for the prediction of preterm labour, namely, fetal fibronectin (fFN) and phosphorylated insulinlike growth factor binding protein-1 (phIGFBP1).[4]

Fetal Fibronectin (fFN)

fFN is a large molecular weight glycoprotein produced by the trophoblast that serves to maintain the chorionicdecidual extracellular matrix. Beyond 16–20 weeks' gestation fFN is not detectible in the CVF. If found beyond 20 weeks' gestation, it may suggest a disruption of the choriodecidual interface and has been identified as a predictor of spontaneous preterm labour.[62]

Measurement of cervicovaginal fetal fibronectin at 22-35 weeks of gestation has been established as the best predictive marker for spontaneous preterm birth in

asymptomatic and symptomatic women predominantly on the basis of its high negative predictive value.[63]

Fetal fibronectin concentrations which were quantified by enzyme linked immunosorbent assay do exhibit a linear co-relation with the risk of spontaneous preterm birth.

A study done to compare the diagnostic accuracy of a bedside quantitative system measurement of cervicovaginal fluid fetal fibronectin in high risk asymptomatic women between 18-21 completed weeks of gelation compared with the standard 22-27 completed weeks of gestational window for prediction of spontaneous preterm birth before 34 weeks of gestation showed that the quantitative measurement taken between 18-21 completed weeks of gestation in high asymptomatic women demonstrated clear discriminative value to using alternative risk threshold of less than 10 ng/ml to define low risk and greater than 200ng/ml to define higher risk. As per the study a low risk woman was conferred a less than 4% risk of preterm birth at less than 34 weeks of gestation.

However, the combined approach of measurement of cervicovaginal fluid fetal fibronectin and cervical length measurement is synergistic and appears to offer the most accurate prediction if resources permit although at 18 weeks of gestation a test result of CVF fFN of less than 10 ng/ml would provide reassurance in the absence of scanning availability hence discriminating between high-risk women who do not need intensive surveillance from those who require further management.[64]

A meta-analysis examining the utility of fFN to predict preterm birth within 7–14 days in *symptomatic* women reported 78–89% sensitivity and 86% specificity.[65]

In the same review the utility of fFN testing in *asymptomatic* women found a lower sensitivity (68–76%) but comparable specificity (88-89%) in predicting spontaneous preterm birth within the 7–14 days.

While the fFN test appears to be more informative in women presenting with threatened preterm labour[66,67], due to its generally poor positive predictive value and limitations due to external factors (e.g.,amniotic fluid contamination, vaginal bleeding, and unprotected sexual intercourse), the fFN test has limited application. fFN is used clinically for its negative predictive value.[68]

Phosphorylated insulin-like growth factor binding protein-1 (phIGFBP1)

phIGFBP1 is secreted by decidual cells and leaks into cervical secretions when fetal membranes detach from decidua. It has been used to clinically assess cervical maturation. Clinical diagnostic trials indicate that, like fFN, phIGFBP1 is a good negative predictor of preterm birth (92% specificity) but lacks suitable sensitivity and positive predictive value in *asymptomatic* women [69,70]. Clearly there is a need for improved biomarker predictive test(s) for preterm labour than currently available tests.

Few studies have analysed the predictive efficacy of these biomarkers and careful consideration is required to distinguish pregnancies experiencing spontaneous preterm labour, spontaneous preterm PROM, and symptomatic (threatened) preterm labour (in the absence of infection).

Using 2D electrophoresis,were the first to publish a "2D map" of the human CVF proteome in pregnancy.[71]

Spontaneous Preterm Labour in At-Risk Women.

Using 2D gel electrophoresis techniques (2D-DIGE and 2D-PAGE), the CVF proteome of women who spontaneously delivered preterm (11 to 22 days prior to labour onset) was compared with gestation-matched women who delivered at term.

Five candidate biomarkers were selected for validation in a large independent cohort of *asymptomatic* women. TXN and IL1RN concentrations in the CVF were found to be significantly reduced up to 90 days prior to spontaneous preterm labour compared to gestation matched women who subsequently delivered at term. TXN was able to predict spontaneous preterm labour up to 28 days from labour with a high positive predictive value and negative predictive value of 75.0% and 96.4%, respectively. IL1RN also showed comparable positive and negative predictive values of 72.7% and 95.7%, respectively.[72]

In a subsequent investigation, Vitamin D binding protein group-specific component (GC) was measured throughout pregnancy. Compared to gestation-matched controls, women destined for a preterm labour had significantly elevated levels of GC up to 100 days before spontaneous labour. GC concentrations were significantly increased by up to 7-fold, 14 days before labour onset. Predictive modelling indicated that GC had a positive predictive value of 82.8% at 3 days and 78.8% at 7 days before labour onset [73]. In a study investigating spontaneous preterm PROM, both ILRN and CSTA in the CVF were found to be significantly reduced 6–23 days before membrane rupture[74].

Threatened Preterm Labour

Once again, using 2D gel electrophoresis techniques (2D-DIGE and 2D-PAGE), the CVF proteome of *symptomatic* women (but with no observable cervical change) who spontaneously delivered preterm within 7 days was compared with gestation-matched women who delivered at term. Four biomarkers, TXN, IL1RN, GC, and ALB were identified and further investigated and all four were significantly altered. From these studies optimal concentration thresholds were determined and predictive modeling was performed. GC displayed 77.8% sensitivity and 98.1% specificity while ALB displayed 83.3% sensitivity and 73.3% specificity.[75]

An important consideration in all of these *term* and *preterm* pregnancy studies was to test the influence of potential confounder variables. Findings indicate that colonization with common vaginal microflora (e.g., *Ureaplasma*spp., Group B *Streptococci* spp., and Candida) have no effect on the expression of these biomarkers nor did multifetal gestation (twin pregnancy).

However, it should be emphasised that women with vaginal bleeding, ruptured fetal membranes, or who had had unprotected sexual intercourse in the preceding 24 hrs or transvaginal ultrasound in the preceding 6 hours were excluded from these studies. [76-79,72,74,75] These investigations indicate that although the "triggers" of labour onset may vary, the terminal mechanisms involved in both *term* and *preterm* labour and parturition are common, namely, matrix remodelling, fetal membranes rupture, and uterine contractions.

These studies have also provided unique insights into the multiple mechanisms that culminate in labour and include inflammation (IL1B, IL1RN); matrix

remodelling (CSTA, SERPINB1, SERPINB3, and SERPINB4), oxidative stress (TXN, SOD1, PRDX2, and GSTP1), and lipid metabolism (FABP5, ANXA3). It is for this reason that multiple biomarkers that target different pathways are likely to prove most beneficial in predicting spontaneous preterm labour, preterm PROM, and threatened preterm labour.

For this reason, the simultaneous quantification of multiple biomarkers, that may include demographic/risk-factor(s), cervical length and biochemical marker(s), and the development of multivariate classification models represent a promising approach to improving diagnostic efficiency.[4]

Antibiotics in the scenario of preterm labour

Trials of antibiotics have yielded mixed results, reflecting the heterogeneity of the studies, medications and patients. Providing antibiotic treatment late in pregnancy, after long-term infection and inflammatory tissue damage have occurred may be ineffective.[80-91]Vaginal creams may result in inadequate tissue penetration of the medication. Some antibiotics, such as clindamycin, may be more effective for BV, while others such as erythromycin, may be more effective for mycoplasma and ureaplasma.[90]

Metronidazole may be most effective for anaerobes like Gardnerellavaginalis and protozoa like Trichomonas vaginalis, but does not cover a broad range of lower genital tract infection microorganisms including: Chlamydia trachomatis, E. coli, Enterococcus faecalis, Group B hemolytic streptococci, Mycoplasma hominis and Neisseria gonorrhoeae and is ineffective in prevention of prematurity.[92]

Clindamycin is recommended by the Centers for Disease Control and American Congress of Obstetricians and Gynecologists to treat women to prevent early onset Group B Streptococcus disease in newborns.[85,86]

For an antibiotic to successfully prevent preterm birth, it may need to be effective against as many of these organisms as possible and provide systemic penetration to eradicate or reduce pathogens in the vagina and the decidua. Oral clindamycin shows promise.

The Cochrane Review [83-89] and other meta-analyses[90,91] suffer from joint analysis of different antibiotics, timing of administration, formulations and/or routes of delivery which demonstrated that antibiotics for treating gestational BV found a 20% (p=0.11) reduction in birth <37 weeks associated with oral or vaginal clindamycin.[83]

Another meta-analysis (5 trials, n=1,523) of clindamycin for second trimester gestational infection found a 32% reduction (p=0.02) in preterm birth.[83]

Study conducted in two hospitals in Great Britain, where the incidence of gestational infection was much lower, around eight percent, where gestation was universally estimated by ultrasound, and where genital tract infection was identified by gram stain using Nugent's criteria. The single published trial of oral clindamycin (n=2,406),[84] included in both reviews found a 52% lower rate of spontaneous birth <37 weeks gestation (p=0.004).[84] That study, conducted in two hospitals in the United Kingdom, provided oral clindamycin at 12 to 22 weeks gestation.

A recently completed hospital based, individually randomized, double-blind, placebo-controlled study to determine whether oral clindamycin is effective in

preventing preterm birth conducted by the Department of Obstetrics and Gynaecology, Jawaharlal Nehru Medical College (JNMC), Belgaum reported a preterm birth incidence of 12% in the clindamycin and 24% in the placebo groups in women with a vaginal pH at or above five between 13 and 17 weeks gestation.

The incidence of <37 weeks delivery was 12% in the clindamycin and 24% in the placebo groups (p=.03; in analyses adjusted for prior preterm or assisted birth p=.04). Excluding abortions, the rate of preterm delivery was 11.1% in the clindamycin and 21.7% in the placebo groups, and the rate of spontaneous (i.e., not forceps or vacuum-assisted) preterm birth was 5.4% in the clindamycin and 19.8% in the placebo groups (both p=.004; in analyses adjusted for prior preterm or assisted birth p=.06). The incidence of <34 weeks delivery was 6% in the clindamycin and 14.7% in the placebo groups (p=.044). The differences were marginally significant (p<.10) when limiting the analyses to spontaneous preterm deliveries and those excluding abortions. There were eight cases of induced prematurity in each study group.[13]

These findings indicate that providing oral clindamycin to women with vaginal pH ≥ 5 early in gestation substantially reduces prematurity and may consequently help reduce global neonatal mortality.[91]

Regardless of its ability to identify BV, a pH ≥ 5 may reflect vaginal flora associated with other gestational infections. Therefore, this study proposes to assess the ability of pH acidity test result ≥ 5 in identification of BV and its consequent accuracy in the prediction of preterm birth. Further, the association between vaginal pH ≥5 at 13-20 weeks of gestation with a number of secondary outcomes such as miscarriage, maternal complications (antepartum and postpartum haemorrhage,

hypertension, and cause of death for any maternal death) through 42 days postpartum, low birth weight, perinatal mortality and neonatal mortality will also be determined. Additionally, a sensitivity analysis will be performed to determine if there is a cut-off for the pH beyond which the preterm birth rate increases or if there is a dose effect (e.g. the higher the pH the greater the rate of preterm birth).

Routine screening for gestational infection is affordable but uncommon, in developed country settings. Determination of acidity of the vagina through pH screening at 13-20 weeks gestation by obtaining a sample of fluid from the posterior fornix with pH paper and estimated using a standard color chart may be a simple screening tool for predicting women at risk of preterm birth.

METHODOLOGY

The present study was a prospective cohort study nested within the community based research study of "Clindamycin to Reduce Preterm Birth in a Low Resource Setting: A Randomized Placebo-Controlled Trial" implemented in 12 Primary Health Center areas of Belgaum and Bagalkot districts of Karnataka. The trial will screen permanent residents in the study area and present for antenatal care (ANC) at 13-20 weeks of gestation.

Study design

The study design was a prospective cohort study

Study period

This study was conducted during the period from July 2015 to August 2016.

Source of data

The study was a prospective cohort study nested within the ongoing community based research study of "Clindamycin to Reduce Preterm Birth in a Low Resource Setting: A Randomized Placebo-Controlled Trial" being implemented in 12 Primary Health Center areas of Belgaum and Bagalkot districts of Karnataka. This Clindamycin trial screened permanent residents in the study area who present for antenatal care (ANC) at 13-20 weeks of gestation. Consenting women are screened for vaginal pH and those with pH $\geq$ 5 will be randomized to receive either oral clindamycin or identically appearing placebo.

The study population for our prospective cohort study included 5148 pregnant women after excluding women not conforming to the study's inclusion criteria and inconsistent data, which included women whose vaginal pH ≥ 5 who had not received clindamycin but placebo and women whose vaginal pH < 5 not receiving any medication. Women with vaginal pH ≥ 5 and randomized to the placebo arm (n=809 women) will form the "Exposed group". The cohort of women screened but with vaginal pH < 5 will form the "Unexposed comparison group" (n=4339).

Sample size

The study population for prospective cohort study will be 5148 pregnant women after excluding women not conforming to the study's inclusion criteria and inconsistent data.

Sampling technique

Based on the hypothesis that **30%** of women screened will have a vaginal pH ≥ 5, the study screened approximately 6,479 women to enroll 5148 pregnant women in the 13-20 week gestational window to the present study. Women with vaginal pH ≥ 5 and randomized to the placebo arm (n=809 women) will form the "Exposed group". All women and newborns, even those with vaginal pH < 5 and hence not eligible for randomization, are followed though 42 days postpartum through a **"Maternal Newborn Health Registry"** forming the unexposed comparison group (n=4339).

Gestational age was be determined from estimated date of delivery (EDD) derived by LMP as only less than 10% of the patients have USG from the first trimester.

Selection criteria

Inclusion Criteria:

1. Pregnant women who are permanent residents of the study clusters
2. Gestational age of 13-20 weeks
3. Single gestation

Exclusion Criteria:

1. Consumption of antibiotics within the past 14 days from the date of enrolment
2. Women for whom the primary delivery outcome is unavailable

Ethical clearance

Ethical clearance for the present study was obtained from JNMC Institutional Ethics Committee on Human Subjects Research. The main trial from which the data was procured – "Clindamycin to Reduce Preterm Birth in a Low Resource Setting: A Randomized Placebo-Controlled Trial" and the "Maternal Newborn Health Registry" protocol as part of the "Community Level Intervention for Pre-Eclampsia" trial was also approved by JNMC Institutional Ethics Committee on Human Subjects Research. The main Clindamycin Trial is registered with *Clinical Trial Registry of India – CTRI/2014/01/004352.*

Informed Consent

The main trial – "Clindamycin to Reduce Preterm Birth in a Low Resource Setting: A Randomized Placebo-Controlled Trial" and the "Maternal Newborn Health Registry" protocol as part of the "Community Level Intervention for Pre-Eclampsia" trial was approved by JNMC Institutional Ethics Committee on Human Subjects

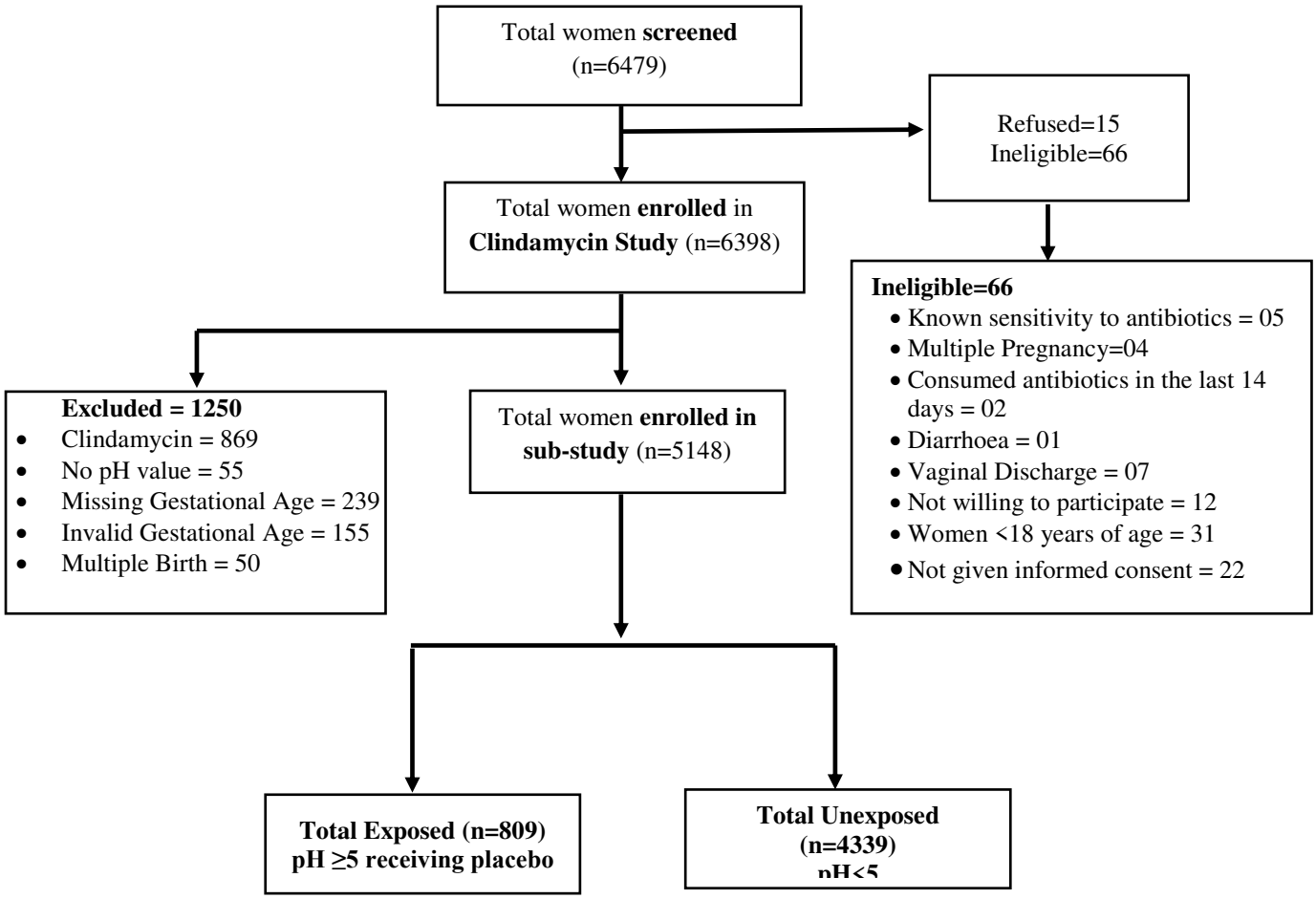
Research where consent from the participants was obtained. Data for this nested prospective cohort study was abstracted from these two studies. As consent for the main studies were obtained from the participants, a waiver of consent was obtained from JNMC Institutional Ethics Committee on Human Subjects Research as it included only collection of data from these two studies.

Method of collection of data

Baseline demographic characteristics, and data relative to the primary and secondary outcomes was accessed from the **Clindamycin and CLIP trial MS Access databases** after resolving any errors or inconsistencies. An analysis dataset to answer the research questions of the proposed prospective cohort study was created.

Kindly note that the hard copy of the Master chart could not be attached in the dissertation copy as it runs in 600 pages and CD of the same is enclosed instead.

CONSORT FLOW DIAGRAM



Statistical analysis

The data obtained was coded and entered into MS Access database 2013. Data were statistically described as mean ± standard deviation or % as appropriate. Association between the vaginal pH ≥ 5 and the incidence of preterm birth will be expressed as risk difference with 95% confidence interval. Descriptive statistics will be used for describing the demographic profile of the study participants such as age, education, gravidity, parity. Pearson Chi-square test and Multivariate logistic regression analysis was done for comparing continuous variables such as age, maternal education expressed as years of schooling, NMR, PNMR etc. Chi-square test

will be used for comparing categorical variables such as rate of preterm birth, gravidity, parity, newborn gender, type of delivery, place of delivery, prior preterm delivery, maternal complications etc. Additionally, logistic regression analysis was done to bolster the cut-off point of vaginal pH ≥ 5 at which point onwards the rate of preterm birth increases.

For comparing categorical data, chi- square test was performed. A probability value ('p' value) of less than or equal to 0.05 was considered as statistically significant. All statistical calculations were done with the use of the computer programs MS Access Database 2013 and SPSS version 21 for Microsoft windows.

RESULTS

Based on the hypothesis that **30%** of women screened will have a vaginal pH ≥5, the study screened approximately 6,479 women to enroll 5148 pregnant women in the 13-20-week gestational window to the present study. Women with vaginal pH ≥ 5 and randomized to the placebo arm (n=809 women) will form the "Exposed group". All women and newborns, even those with vaginal pH < 5 and hence not eligible for randomization, are followed though 42 days postpartum through a **"Maternal Newborn Health Registry"** forming the unexposed comparison group (n=4339 women).

CONSORT FLOW DIAGRAM

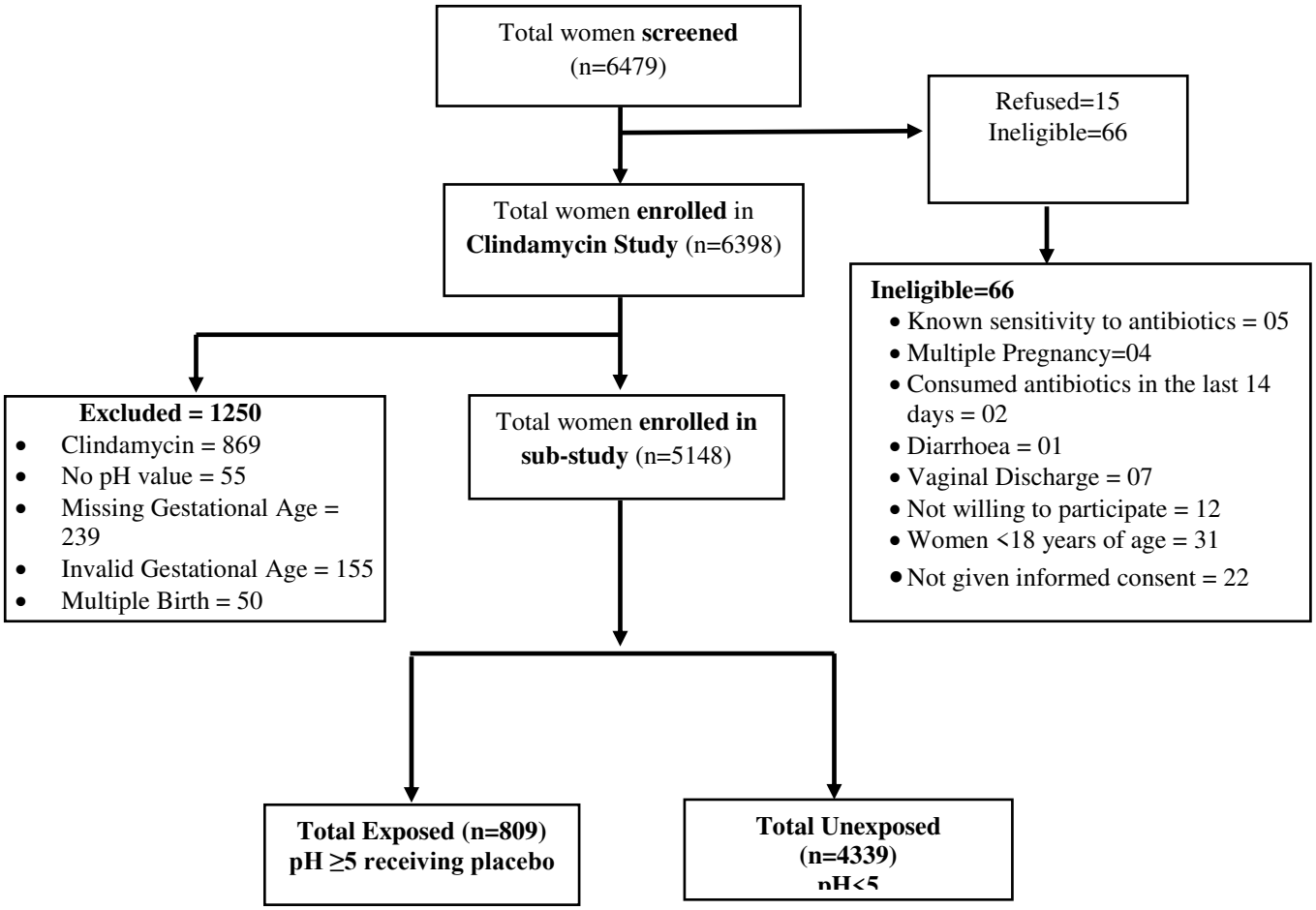


Table 3. Baseline Characteristics of Participants

Baseline characteristics	Exposed (n=809)	Unexposed (n=4339)	P value
Age [years, Mean (SD)]	23.18(3.217)	22.71(3.166)	0.026
Level of maternal schooling • **No formal schooling illiterate** • **No formal schooling literate** • **Schooling**	 18% 3.6% 78.4%	 13.7% 2.9% 83.4%	0.003
Years of maternal schooling [Mean (SD)]	8.70(2.810)	9.20(2.849)	0.101
BPL card holder • **Yes** • **No**	79.5% 20.5%	79.8% 20.2%	0.961
pH value [Mean (SD)]	5.15(0.229)	4.366(0.227)	0.00
Parity [Mean (SD)] • **Nulliparous** • **1-3** • **>3**	294(36.6%) 494(61.6%) 15(1.8%)	1609(37.3%) 2595(60.2%) 103(2.5%)	0.591
Maternal height [in cms, Mean (SD)]	151.63(6.049)	152.44(6.089)	0.13
Maternal weight [in kgs, Mean (SD)] (at ANC visit >12 wks gestation earliest possible)	45.37(7.252)	45.59(16.208)	9.20
BMI [kg/m^2, Mean (SD)]	19.73(2.965)	19.68(7.37)	0.53
Hemoglobin [gm%, [Mean (SD)]	9.76(0.966)	9.79(0.910)	0.001
Systolic Blood Pressure [mmHg, Mean (SD)]	109.42(10.403)	111.07(7.581)	0.90
Diastolic Blood Pressure [mmHg, Mean (SD)]	70.92(7.433)	71.74(6.186)	0.004

Statistical analysis was performed for the all the patients who have undergone screening and met the eligibility criteria. Even though there were some differences between the exposed and the unexposed groups within the study cohort, the inclusion of all the study participants in the analysis increases the universal generalizability of the conclusions.

The characteristics of the study subjects with respect to their socio-demographic profile and baseline examination showed statically significant differences in categories of Age, Level of maternal schooling, Hemoglobin and Diastolic blood pressure. However, these differences were clinically not significant.

Statistical significance with respect to the baseline parameters could be attributed to small differences between the two cohorts getting inflated on account of the difference in the size of the two groups within the cohort, the ratio between exposed to un-exposed comparison group being 1:5 respectively.

The mean pH value was (5.15) in the exposed and (4.388) in the un-exposed group.

Identifying that the relationship between BMI and preterm birth may not be linear, we chose to examine the rate of preterm birth by the WHO BMI categories. This data is displayed in the below table:

Table 4. WHO Criteria for categorization of BMI

BMI Category	RR	95%CI
<18.50	1	REF
18.50 to <25.0	0.98	0.84 to 1.17
25.0 to <30.0	1.1	0.74 to 1.64
30.0+	2.06	0.93 to 4.56

Table 5. Multivariate logistic regression analysis for BMI and Preterm Birth

Preterm birth < 34 weeks		
TERM	**RR**	**95% CI**
Vag pH≥5.0	1.53	1.11 to 2.10
25.0 to <30.0	1.84	1.12 to 3.04
Preterm Birth < 37 weeks		
Vag pH≥5.0	1.32	1.06 to 1.62
30+	2.03	0.92 to 4.47

Recognizing that we had identified a number of covariates that were associated with preterm birth, we chose to further clarify these associations between preterm birth (both < 37 weeks and <34 weeks) and each other by performing stepwise logistic regression modelling. Terms that did not at least demonstrate a trend were excluded.

BMI more than 25 had clinically significant association for higher rates of spontaneous preterm birth i.e. RR-1.84, 95%CI 1.12 to 3.04 for the gestational age cut-off of 34 weeks and RR-2.03, 95% CI 0.92 to 4.47 at 37 weeks for BMI more than 30.

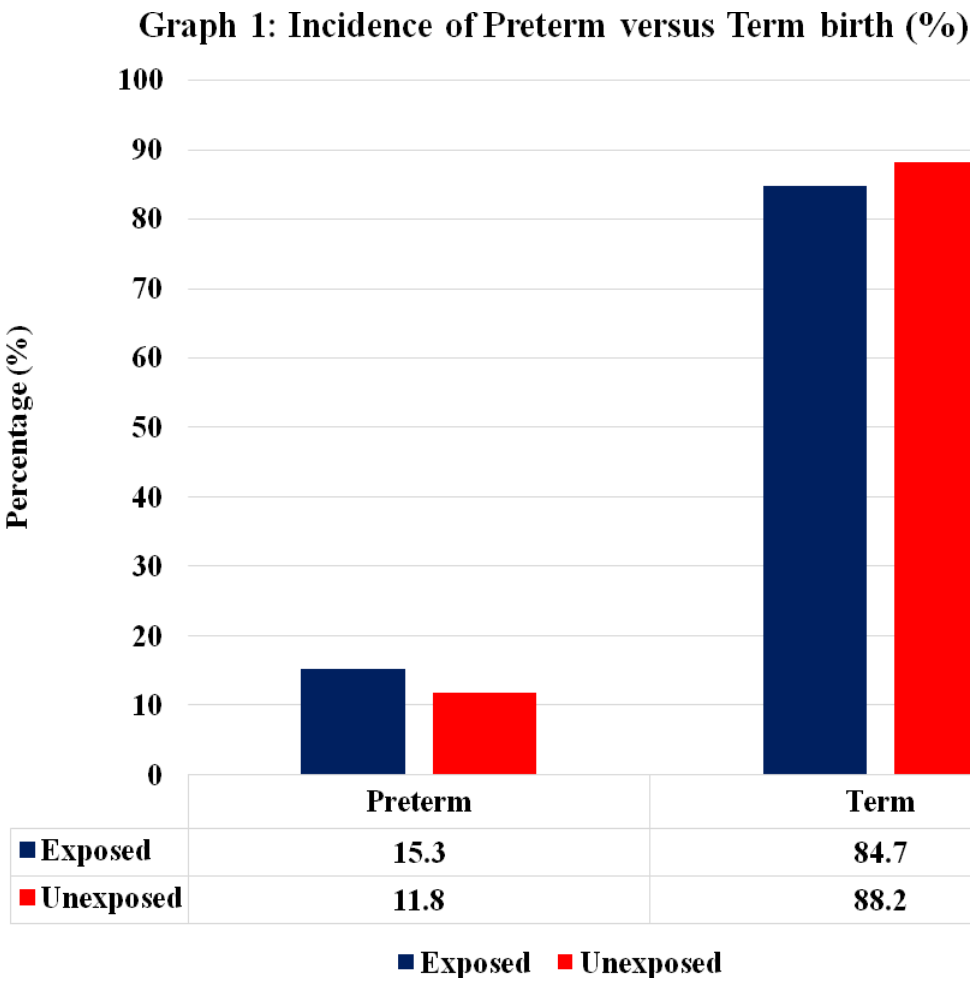


Table 6. Incidence of preterm birth versus term birth

p value-0.005

The incidence of preterm birth in the exposed group is 15.3% compared to 11.8% in the un-exposed group with an absolute difference in the rate of preterm birth being 3.5%. The **RELATIVE RISK** of preterm birth**,** expressed as **ratio** of absolute difference (**3.5%**) of the incidence of preterm birth rate in the untreated group (**15.3%**), is **23%** greater in women with vaginal pH $\geq$ 5 than in women with vaginal pH less than 5. This difference is both statistically and clinically significant.

Graph 2: Gestational age at birth (%)

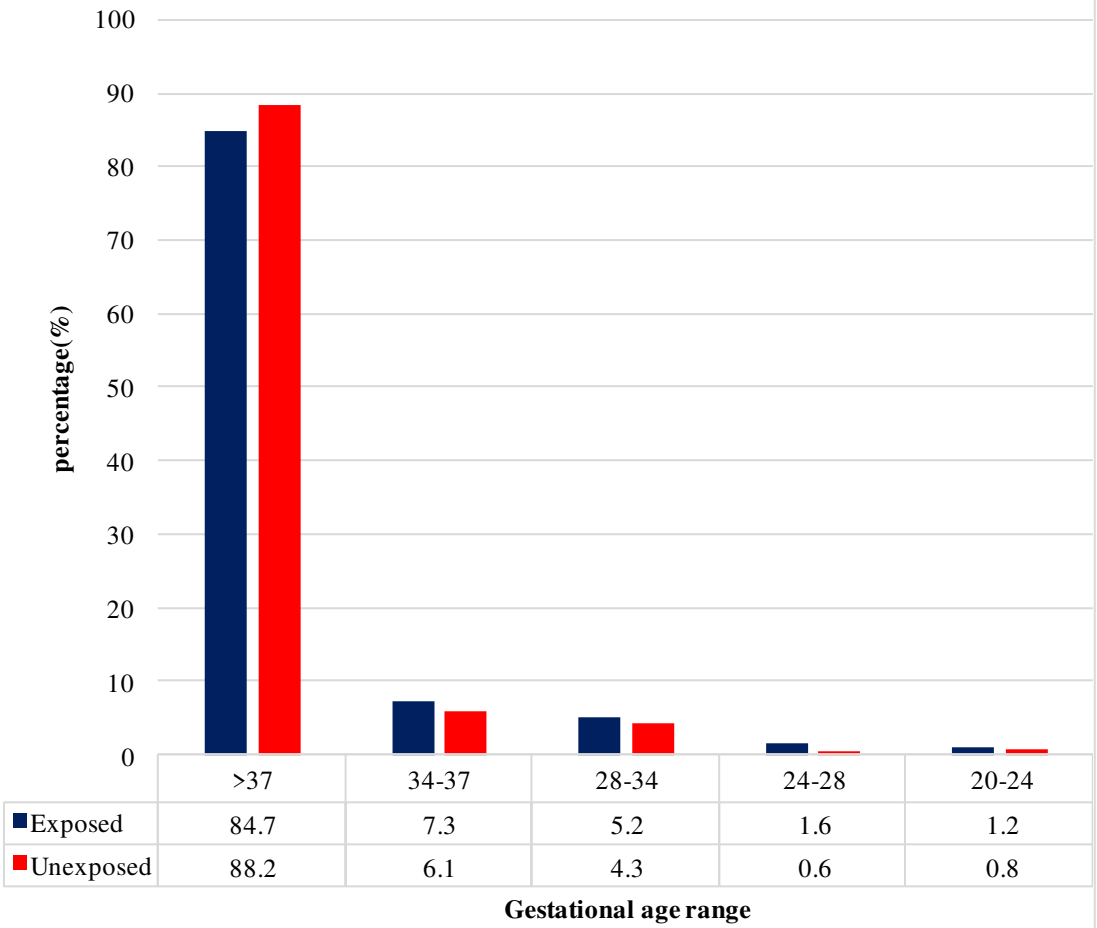


Table 7. Incidence of preterm birth versus term birth at different gestational age ranges

p value-0.009

The rates of preterm birth are higher across all gestational age ranges in women with vaginal pH ≥ 5 when compared to women with vaginal pH < 5 indicating that there is a significant risk of having a preterm birth (p value-0.009) when the vaginal pH ≥ 5.

Graph 3: Rate of preterm birth (%)

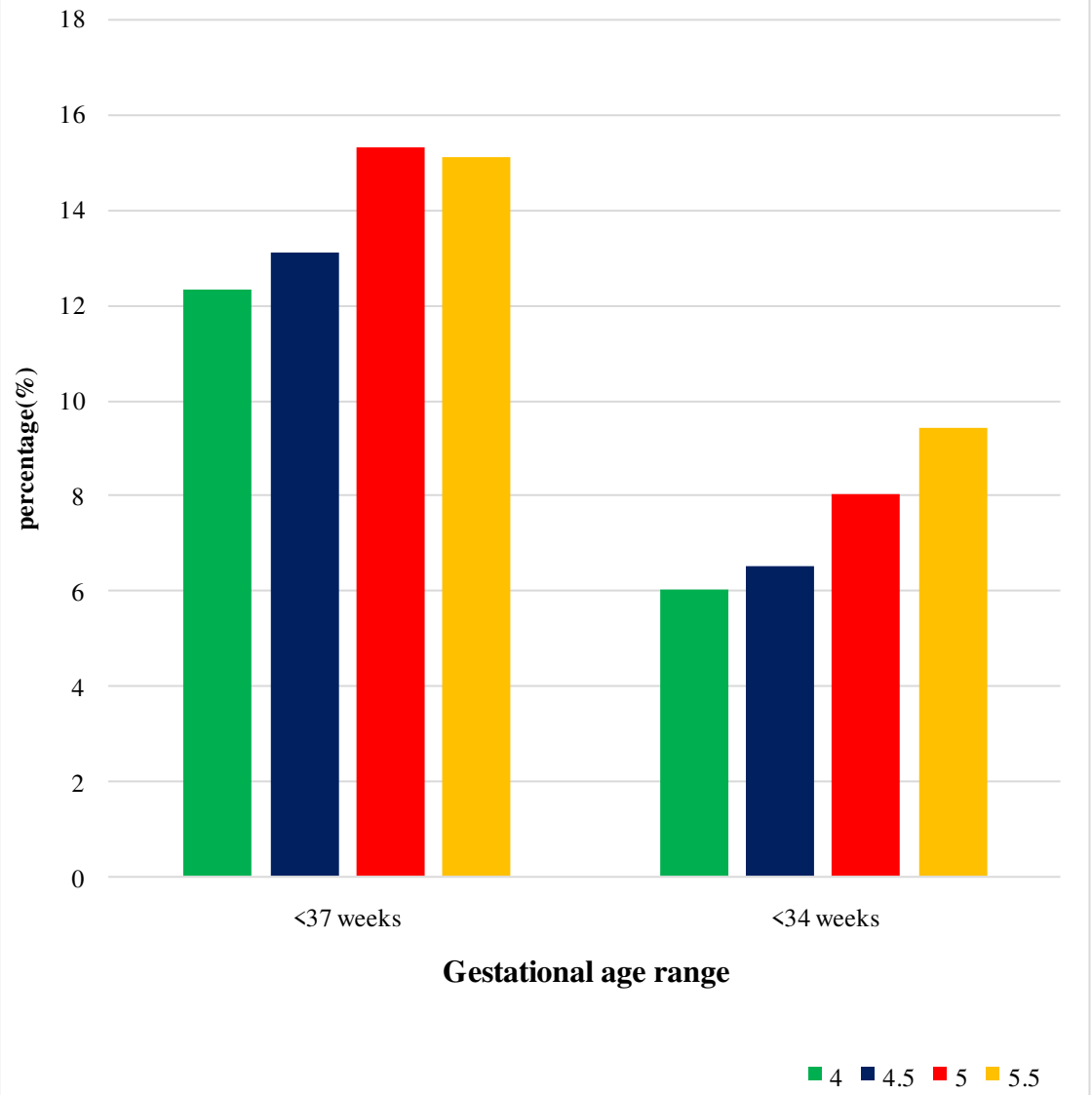


Table 8. Rate of preterm birth at different vaginal pH cut-off points

Gestational Age in weeks	Rate of preterm birth			
	Vaginal pH			
	4	4.5	5	5.5
<37	12.3%	13.14%	15.3%	15.16%
p value	0.693	0.001	0.005	0.172
<34	6%	6.5%	8%	9.4%
p value	0.8	0.009	0.011	0.025

There is a linear increase in the rate of preterm birth with increasing values of vaginal pH. The rise starts from the upper limit of normal vaginal pH 4.5 (p value=0.001). The highest rate of preterm birth is found with a vaginal pH 5 (p value-0.005) signifying that vaginal pH 5 can be used as a clinically useful cut-off point for predicting the occurrence of preterm birth.

A similar significant linear co-relation between vaginal pH and rates of spontaneous preterm birth is also observed for the gestational age groups of less than 34 weeks with vaginal pH of 4.5 ($p < 0.009$), 5 ($p < 0.011$) and 5.5 ($p < 0.025$).

Graph 4 : Logistic Regression: Vaginal pH and Preterm Birth at < 37 weeks of gestation

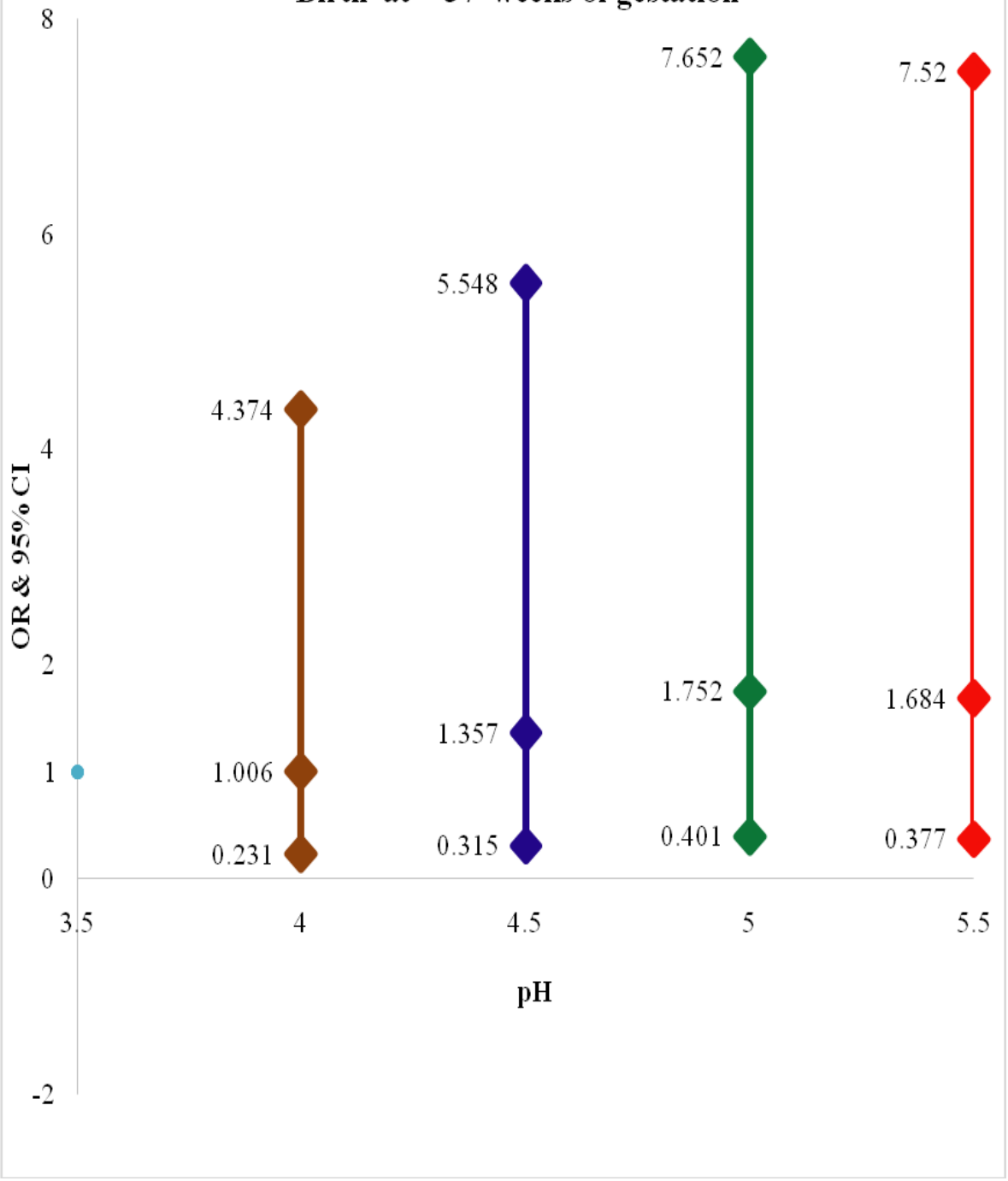


Table 9. Logistic regression analysis of vaginal pH versus rate of preterm birth at < 37 weeks of gestation

pH	OR	95% CI		p-value
3.5	1	**REF**		-
4	1.006	0.231	4.374	0.994
4.5	1.357	0.315	5.548	0.682
5	1.752	0.401	7.652	0.456
5.5	1.684	0.377	7.52	0.495

Table 9. indicates that vaginal pH ≥ 5.0 is significantly associated with increased risk of preterm birth.

Graph 5. Logistic Regression: Vaginal pH and Preterm Births < 34 weeks of gestation

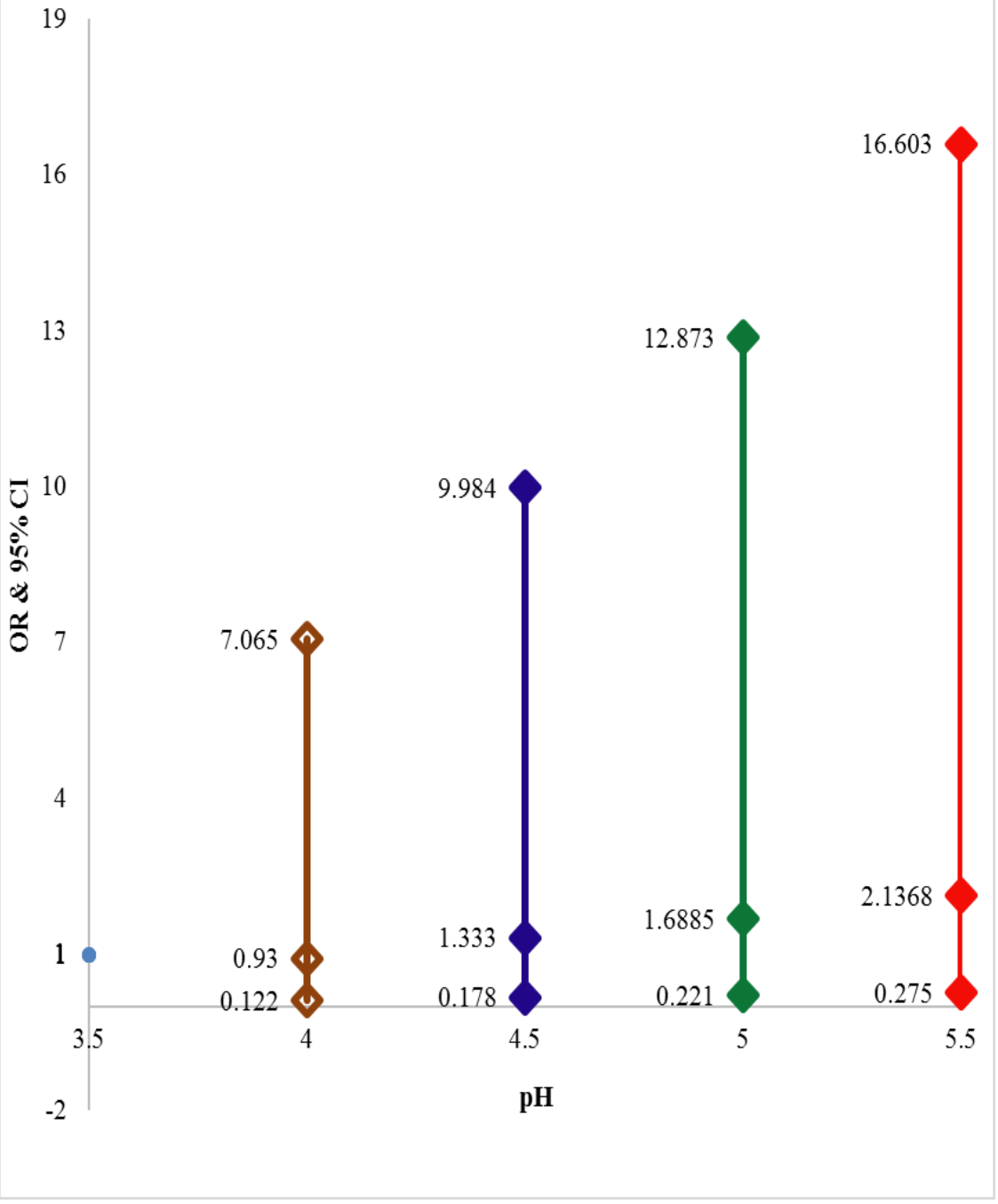


Table 10. Logistic regression analysis of vaginal pH versus preterm birth at < 34 weeks.

pH	OR	95% CI		p-value
3.5	1	**REF**		-
4	0.93	0.122	7.065	0.994
4.5	1.333	0.178	9.984	0.779
5	1.6885	0.221	12.873	0.613
5.5	2.1368	0.275	16.603	0.468

Table 10. demonstrates the linear relationship between vaginal pH and the risk of Preterm Birth and maybe considered as a good predictor of both early (< 34 weeks) and overall (< 37 weeks) preterm birth.

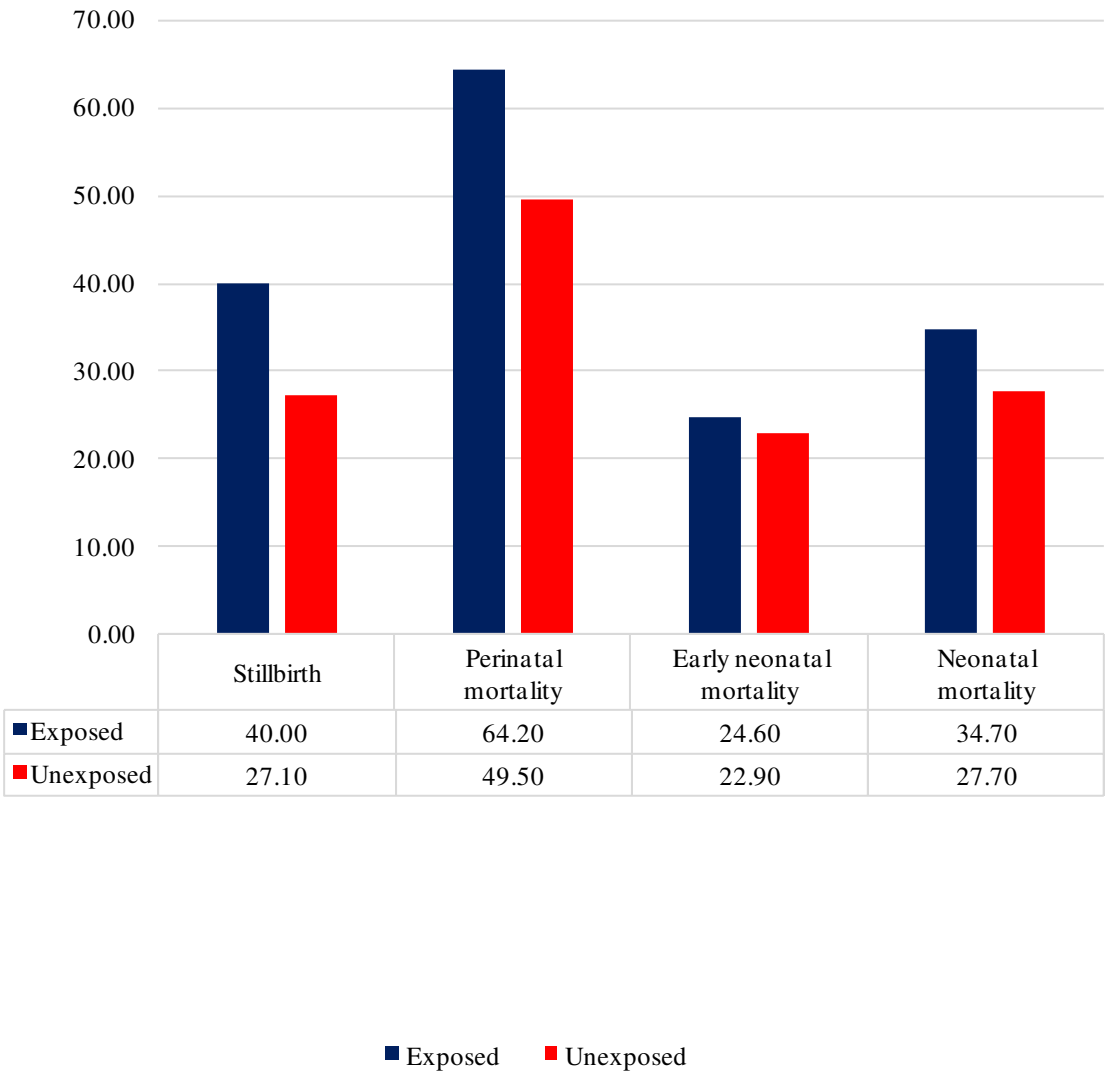
Table 11: Logistic regression analysis performed cumulatively at gestational age overall < 37 weeks and at < 34 weeks.

pH	OR	95% CI		p-value
Less than 37 Weeks				
<5.0	**1**	**REF**		
≥5.0	**1.372**	**1.114**	**1.69**	**0.003**
Less than 34 weeks				
<5.0	**1**	**REF**		
>5.0	**1.489**	**1.131**	**1.961**	**0.005**

A logistic regression analysis was performed to determine whether the risk of developing preterm birth was associated with increasing vaginal pH.

There was a statistically significant correlation with reference to cut-off of vaginal pH > 5 with increased risk of spontaneous preterm birth, in both early (OR - 1.489, 95% CI 1.131-1.961, p value-0.005) and overall (OR-1.372, 95% CI 1.11-1.69 p value-0.003) preterm birth.

Graph 6: Foetal / Neonatal Outcome



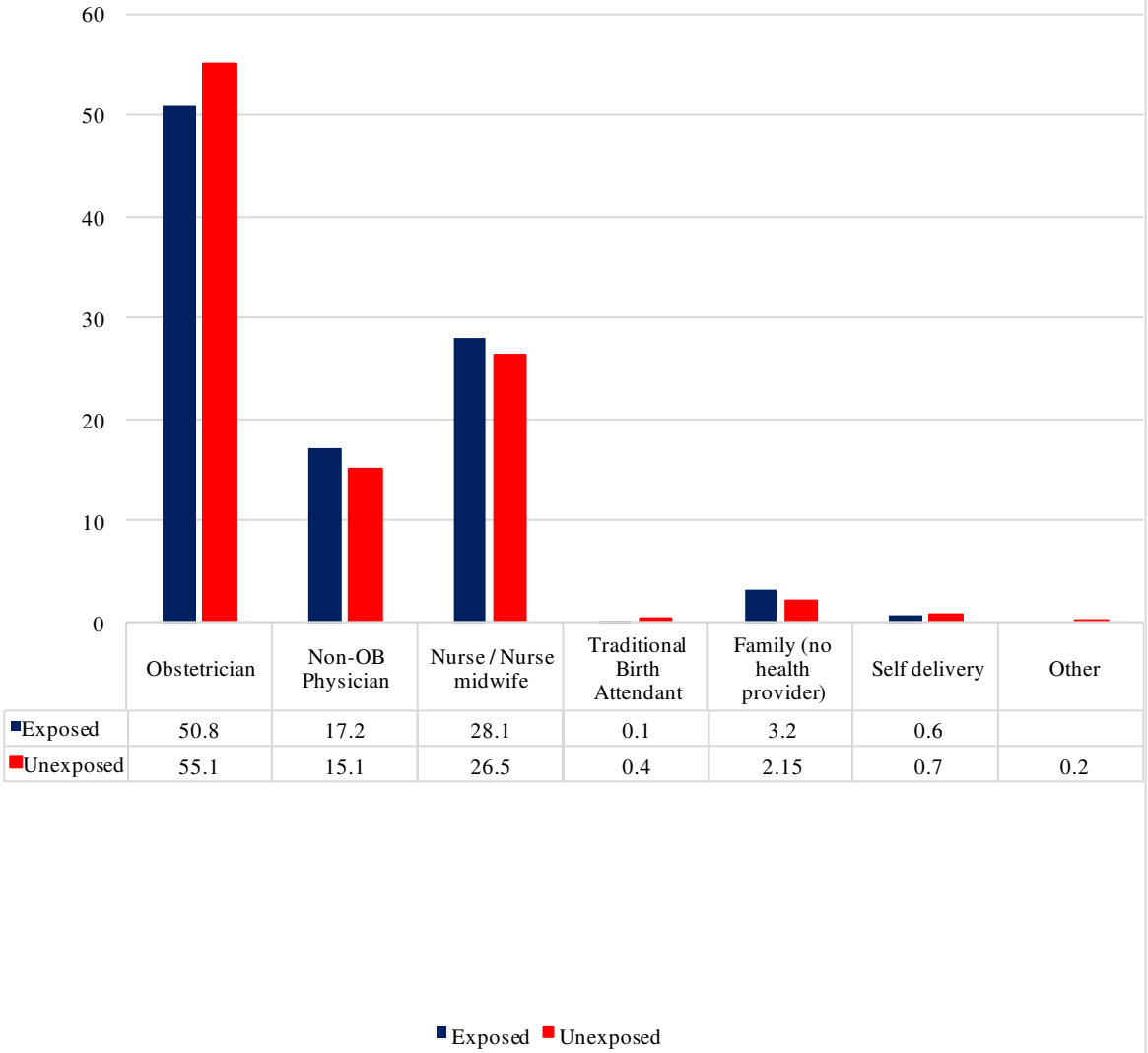
	Stillbirth	Perinatal mortality	Early neonatal mortality	Neonatal mortality
Exposed	40.00	64.20	24.60	34.70
Unexposed	27.10	49.50	22.90	27.70

Table 12. Foetal and neonatal outcome

p value-0.002

The adverse fetal outcomes such as stillbirth rate and perinatal (per 1000 births), early neonatal and neonatal mortality rates (per 1000 live births) were significantly higher in the exposed groups ($p < 0.002$).

Graph 7: Delivery Attendant (%)



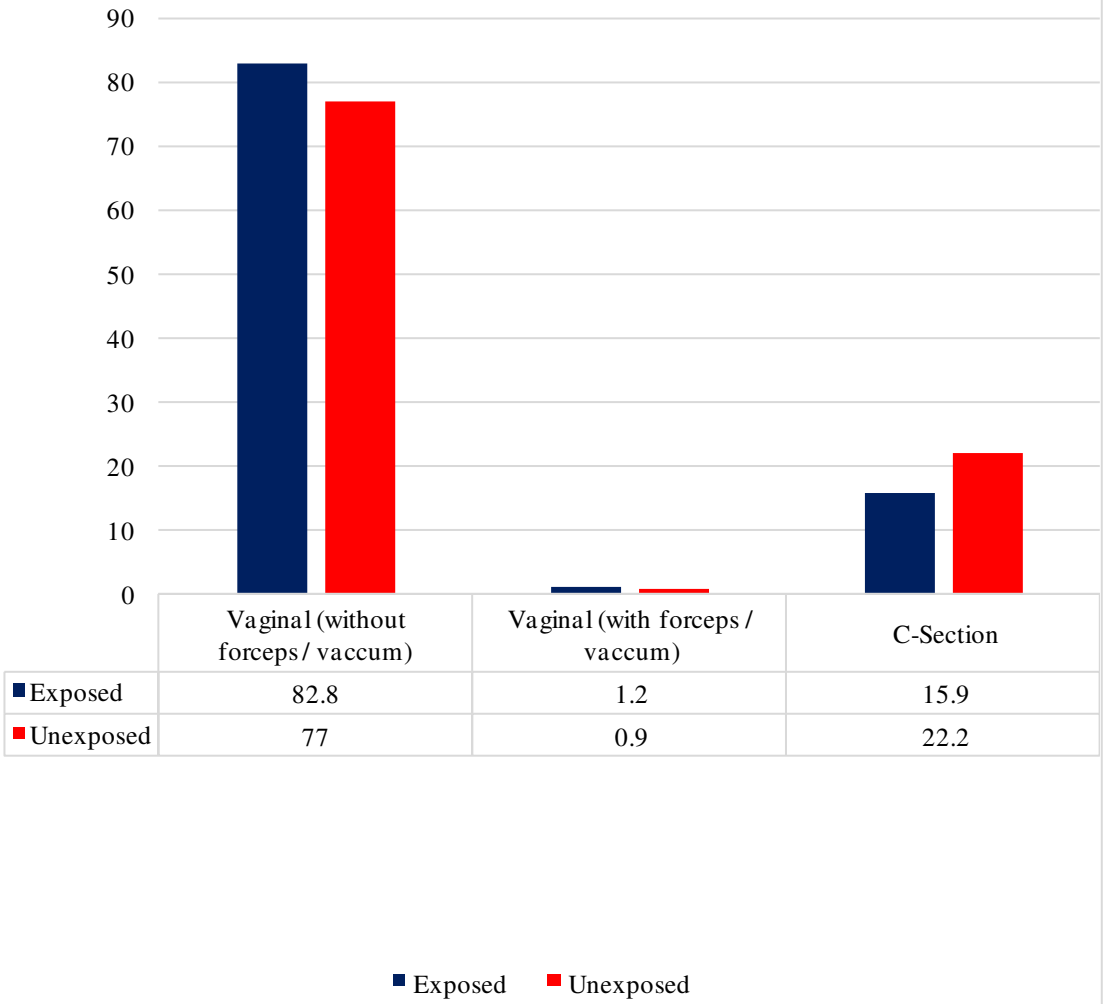
	Obstetrician	Non-OB Physician	Nurse / Nurse midwife	Traditional Birth Attendant	Family (no health provider)	Self delivery	Other
Exposed	50.8	17.2	28.1	0.1	3.2	0.6	
Unexposed	55.1	15.1	26.5	0.4	2.15	0.7	0.2

Table 13. Type of delivery attendant

p value-0.071

The type of delivery attendant was similar between the two groups (p value-0.071).

Graph 8: Mode of delivery (%)



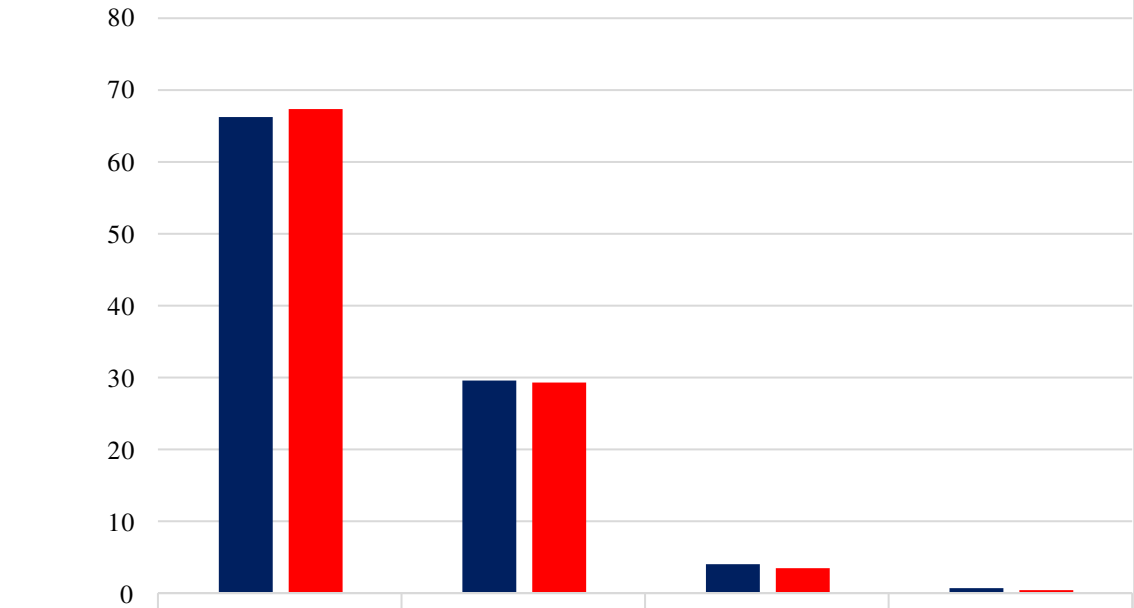
	Vaginal (without forceps / vaccum)	Vaginal (with forceps / vaccum)	C-Section
Exposed	82.8	1.2	15.9
Unexposed	77	0.9	22.2

Table 14. Mode of delivery

p value-0.00

There was a 6.3% higher rate of C-section in the unexposed group which is statistically significant ($p < 0.05$).

Graph 9: Delivery Location(%)



	Hospital	Clinical/Health Center	Home in village	Other
Exposed	66	29.4	4	0.6
Unexposed	67.1	29.3	3.25	0.4

Table 15. Delivery location

p value-0.553

There was no difference in the delivery location between the two groups. (p - 0.553)

Graph 10: Sex of the baby(%)

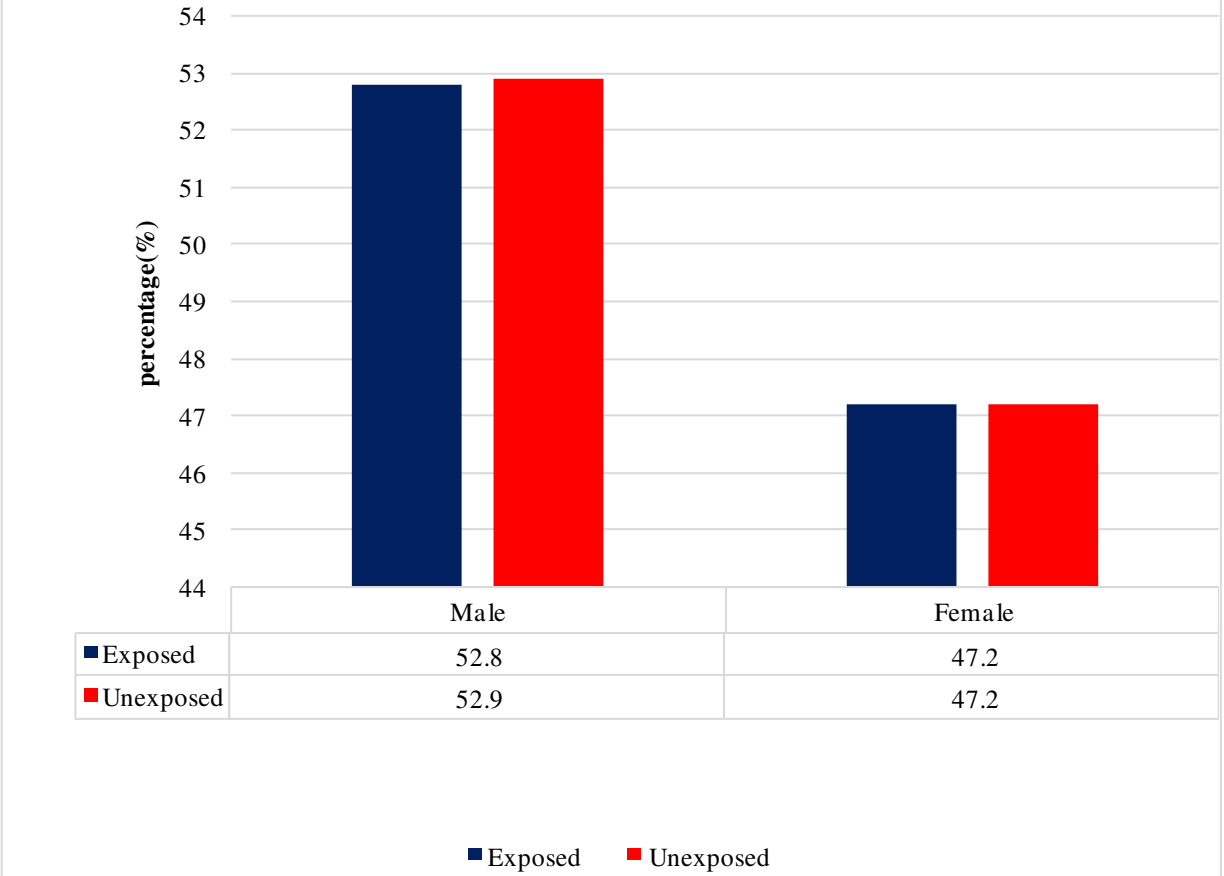


Table 16. Sex of the baby

p value -0.71

There is no statistically significant difference in the gender of the babies born in the two groups (p <0.71).

Graph 11: Birth weight (%)

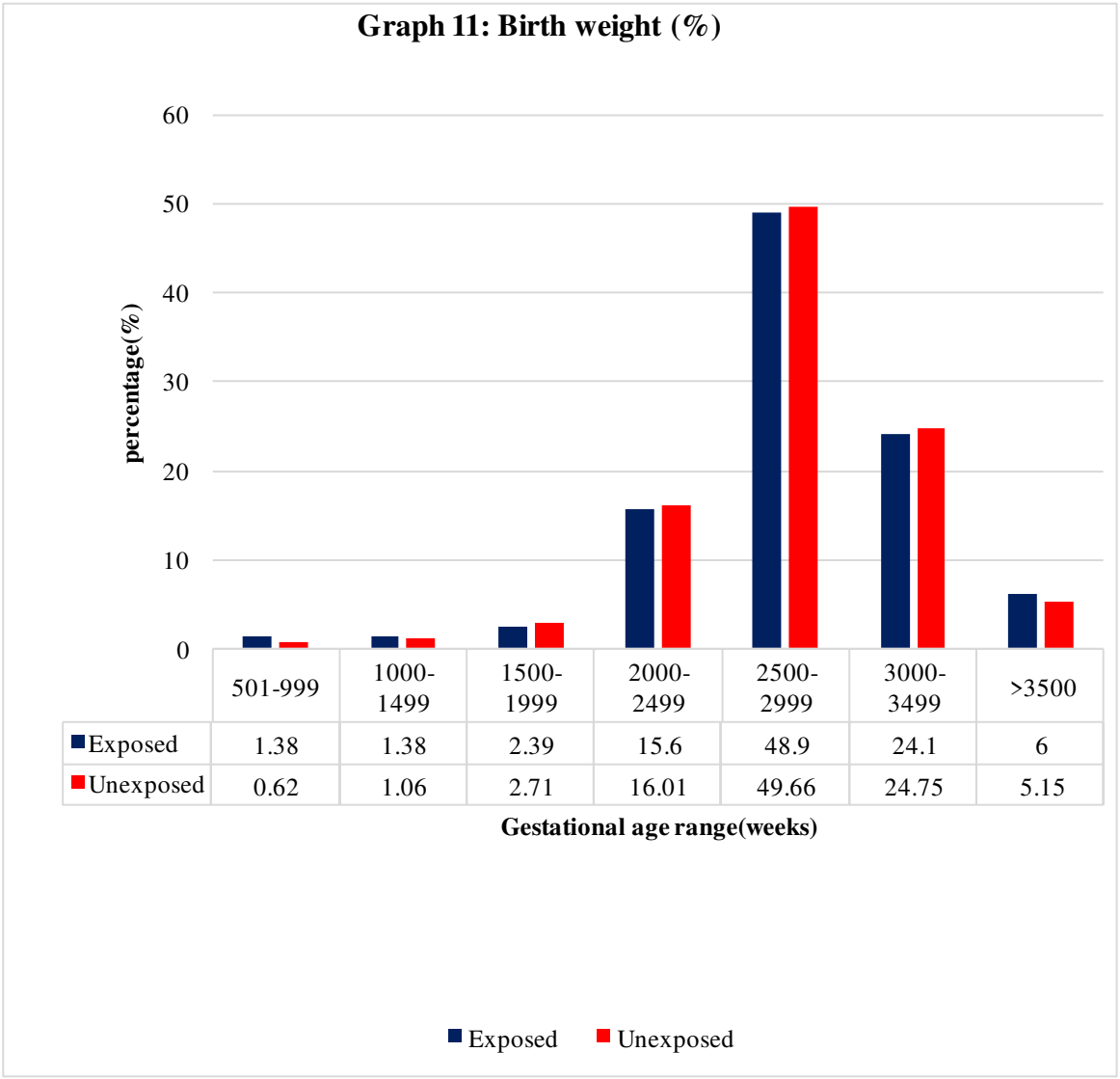


Table 17. Birth weight

p value-0.235

The birth weights among different gestational age ranges appeared to be higher in the unexposed group. However, the differences were statistically not significant. (p <0.235). The mean birth weight in two comparison groups was 2716 in the exposed and 2738 in the unexposed group with no statistically significant difference.

DISCUSSION

Baseline characteristics

The sociodemographic profile and baseline parameters were well matched between the two groups with no statistical or clinical association positive for increased risk of spontaneous preterm birth.

Multivariate logistic regression analysis

Recognizing that we had identified a number of covariates that were associated with preterm birth, we chose to further clarify these associations between preterm birth (both at <37 weeks and <34 weeks) and each other by performing stepwise logistic regression modelling. All candidate variables identified as being associated (p-value <0.1) with preterm birth was performed using forward stepwise logistic regression (p=0.1). Terms that did not at least demonstrate a trend were excluded. Routine model checking and assessment for interactions was performed.

BMI more than 25 was the parameter which demonstrated clinically significant association for higher rates of spontaneous preterm birth i.e. RR-1.84, 95%CI 1.12 to 3.04 for the gestational age cut-off of 34 weeks and RR-2.03, 95% CI 0.92 to 4.47 at 37 weeks for BMI more than 30.

Incidence of preterm birth

- The incidence of preterm birth in women with vaginal pH $\geq$ 5 was 15.3% versus 11.8% in study subjects with vaginal pH < 5.

- The absolute measured difference between the incidences of preterm birth between the two groups is 3.5%.

- The **RELATIVE RISK** for preterm birth **[**expressed as **ratio** of absolute difference (**3.5%**) of incidence of preterm birth rate in the untreated group (**15.3%**)], is **23%** greater in women with vaginal pH $\geq$ 5 than in women with vaginal pH less than 5. The observed difference, the effect size, of 23% is both statistically and clinically significant.

- The results of the present study therefore suggest that having a vaginal pH $\geq$5 translates to a 23% greater risk of occurrence of spontaneous preterm birth in these women.

- Bacterial vaginosis represents abnormal vaginal microbial colonization that leads to adherence, invasion and host inflammatory response. This altered vaginal microenvironment makes it conducive to infection and consequently results in higher rates of preterm delivery.

- In a multicenter Bacterial Vaginosis trial, enrolling women between 8-22 weeks of gestation to identify early pregnancy vaginal markers predictive of subsequent preterm birth, the incidence of spontaneous preterm birth at vaginal pH $\geq$ 5 was found to be 12.4% and 7.1% at gestational age of 37 weeks and 32 weeks respectively. These rates are similar, albeit lower than, to the rates observed in the present study.[41]

- The present study was performed on a predominantly rural population with lower socioeconomic status, and potentially poorer state of personal hygiene thereby accounting for the higher rate of incidence of preterm birth in the

present study (15.3%) versus the lower incidence in the reference study (12.4%).

- The progressively higher rates of preterm birth across all gestational age cut-offs in women with vaginal pH $\geq$ 5 has been observed which is clinically and statistically significant (p value-0.009).

- Similar higher spontaneous preterm birth rates have been noted in women with increased vaginal pH values in the previous study.[41]

Vaginal pH threshold effect

- The highest rate of preterm birth is found with a vaginal pH 5 (p value-0.005) suggesting that vaginal pH 5 can be used as a clinically useful cut-off point for predicting the occurrence of preterm birth.

- The 0.2% difference in the preterm birth rate between vaginal pH 5 and 5.5 is an incidental finding which is statistically and clinically not significant. It could also be explained on the basis that due to the almost identical appearance with a subtle difference in the colors representing the vaginal pH 5 and 5.5 on the nitralazine pH strip, there's a possibility that there could be misclassification of vaginal pH for some women accounting for the 0.2% difference.

A similar significant linear co-relation between vaginal pH and rates of spontaneous preterm birth is also observed for the gestational age groups of less than 34 weeks with p values for vaginal pH of 4.5(0.009),5(0.011) and 5.5(0.025).[41]

Logistic regression analysis

When a logistic regression analysis was done to test the hypothesis as to whether the risk of preterm birth, both early (< 34 weeks) and overall (<37 weeks), increases linearly as vaginal pH increases.

Although at each individual pH cut off points there is no demonstrable statistically significant co-relation of preterm birth with raised vaginal pH values, there appears to be a positive association as the 95% confidence intervals do not cross zero.

The occurrence of bacterial vaginosis as indicated by a pH ≥ 5 at 20 weeks of gestation was associated with an increased risk of spontaneous preterm birth between < 37 weeks gestation (Odds ratio 1.37; 95% CI 1.114 to 1.69, p value-0.003) and preterm birth at < 34 weeks of gestation (Odds ratio 1.49; 95% CI 1.131 to 1.96, p value-0.005).

Similar significant co-relation between gestational age cut-off at <37, <35 and <32 weeks with vaginal pH = 5 in comparison to vaginal pH< 5 has been reported with p values of 0.03, 0.01 and 0.005 respectively.[41]

For comparison of pH < 5.0 versus pH ≥ 5 applied to the same gestational age groups as mentioned before, the p values were all less than 0.001 showing positive clinical association of increased incidence of preterm birth and increased pH value.

The highest rate of preterm birth is found with a vaginal pH 5 (p value-0.005) signifying that vaginal pH 5 can be used as a clinically useful cut-off point for predicting the occurrence of preterm birth.

Fetal outcome

The numerous adverse sequelae of having born preterm synergistically act together to culminate eventually in loss of valuable life. This is reflected by greater incidence of preterm birth in women with vaginal pH $\geq$5 with analogous increase of stillbirth (40 per 1000 births), perinatal (64.2 per 1000 live births), early neonatal (24.6 per 1000 live births) and neonatal mortality (34.7 per 1000 live births) rates in this cohort of women (p value-0.002). The adverse fetal and neonatal outcomes are significantly higher in the exposed group with higher rates of preterm birth than the unexposed group.

Prematurity is the leading cause of early neonatal deaths and a significant long-term loss of human potential amongst survivors all over the world. In addition to its substantial contribution to mortality, the effect of preterm birth amongst some survivors continues for life. Being born preterm increases the baby's risk of death due to other causes especially early neonatal sepsis with preterm birth estimated to be a risk factor in at least 50% of all neonatal deaths.

Delivery location

More than 95% of the population covered had an institutional delivery in the hands of a trained health professional reflecting on the upcoming outlook of rural Indian women of this requirement for safe motherhood with good antenatal care and fetal outcome.

Delivery attendant

More than 50% population were delivered by an Obstetrician in a tertiary care hospital attached to a medical college or in a private hospital across both groups

emphasizing the growing awareness of women of the need for institutional delivery by a skilled birth attendant or a trained health professional.

Mode of delivery

The increased rates of C-section in the unexposed group (6.3% higher rate) could be due to higher rates of term births.

When correlation of birth weights was done there was no association as birth weights are equally distributed across all gestational age ranges between the exposed and the unexposed groups.

Sex

Preterm birth is both more common in boys, with around 55% of all preterm births occurring in males and is associated with a higher risk of dying when compared to girls born at a similar gestation.

The present study did not show any positive association for gender with the incidence of preterm birth (p value-0.71).

Birth weight

The present study showed no clinical or statistical association between the incidence of low birth weight in women with vaginal pH > 5. However, in a majority of the higher birth weight categories, the proportion of babies born to women with vaginal pH < 5 was higher. This may partially explain the higher rate of Caesarean deliveries observed in the unexposed group.

In the BV trial, women whose vaginal pH was 5.0 or less also had significantly fewer subsequent spontaneous preterm births (p value-<0.001) and fewer newborn infants weighing less than 2500 g or less than 1500 g (p value<0.0005).[41]

CONCLUSION

Lactobacillus present in abundance normally in the vaginal micro flora produces hydrogen peroxide by a series of oxidation and reduction reactions that converts the glycogen present in normal vaginal epithelium to lactic acid maintaining the acidic medium of the vagina at 4.5 and most importantly preventing the overgrowth of other pathogenic anaerobes in vaginal ecosystem.

The sustenance of this system of vaginal homeostasis is vital to the prevention of altered vaginal microbiology deterring the development of a vaginal pH >4.5 and precluding the woman from having an unnecessary detrimental spontaneous birth prematurely.

Vaginal pH is a simple screening test that

- Can be administered easily in a community setting to predict the risk for preterm birth where the maximum burden of the disease lies
- It's less expensive
- Does not require expertise

This simple test administered in the early pregnancy between the gestational age ranges of 13-20 weeks will give us an idea of the future risk of preterm birth.

The rates being higher in the exposed groups supports the data that the higher the vaginal pH, greater the incidence of spontaneous preterm birth and higher the mortality rates due to the associated morbidities of having delivered prematurely.

Hence appropriate treatment administered for women with vaginal pH more than 5 will help reduce the incidence of spontaneous preterm birth, burden of prematurity by significantly impacting the mortality rates.

The present study has explored the utility of vaginal pH as an effective mass screening tool to identify BV in resource limited settings for timely administration of appropriate treatment for the prevention of preterm birth.

SUMMARY

- The present study was a prospective cohort study nested within the community based randomized controlled trial to test the efficacy of oralClindamycin forreducing the incidence ofpreterm birth implemented in 12 Primary Health Center areas of Belgaum and Bagalkot districts of Karnataka.

- The study population for prospective cohort study was 5148 pregnant women after excluding women not conforming to the study's inclusion criteria. While 809 women randomized to the placebo arm of the trial formed the exposed group, 4,339 women with vaginal pH <5 formed the unexposed group.

- The objectives of the present study were to determine the association between vaginal pH ≥ 5 at 13-20 weeks of gestation and the incidence of preterm births and to perform a sensitivity analysis to determine if there is a cut-off for the pH beyond which the preterm birth rate increases or if there is a dose effect (e.g. the higher the pH the greater the rate of preterm birth).

- The characteristics of the study subjects with respect to their socio-demographic profile and baseline examination showed statically significant differences in categories of Age (p-0.026), Level of maternal schooling (p-0.03), Hemoglobin (p-0.001) and Diastolic blood pressure (p-0.004). However, these differences were clinically not significant.

- The multivariate logistic regression analysis was done on the sociodemographic parameters and the examination characteristics which were statistically significant. BMI more than 25 was the parameter which

demonstrated clinically significant association for higher rates of spontaneous preterm birth i.e. RR-1.84, 95%CI 1.12 to 3.04 for the gestational age cut-off of 34 weeks and RR-2.03, 95% CI 0.92 to 4.47 at overall 37 weeks for BMI more than 30.

- The incidence of preterm birth in the exposed group is 15.3% compared to 11.8% in the un-exposed group with an absolute difference in the rate of preterm birth being 3.5%. i.e. 23% greater risk of preterm birth in women with vaginal pH ≥ 5.

- The rate of preterm birth across all gestational age ranges was higher in the exposed group than in the unexposed comparison group (p-0.009).

- The highest rate of preterm birth was 15.3 is found with a vaginal pH 5 (p value-0.005).

- On performing logistic regression analysis,there was a statistically significant correlation with reference to cut-off of vaginal pH > 5 with increased risk of spontaneous preterm birth, in both early (OR -1.489, 95% CI 1.131-1.961, p value-0.005) and overall (OR-1.372, 95% CI 1.11-1.69 p value-0.003) preterm birth.

- Vaginal pH ≥ 5.0 is significantly associated with increased risk of preterm birth and maybe considered as a good predictor of both early (< 34 weeks) and overall (< 37 weeks) preterm birth.

- The adverse foetal outcomes such as stillbirth rate (40 /1000 births), and perinatal (64.2/1000 births), early neonatal (24.6/1000 live births) and

neonatal mortality rates (34.7/1000 live births) were significantly higher in the exposed groups (p <0.002).

- The personnel conducting delivery was similar between the two groups (p value-0.071) where more than half of the population in both the groups i.e. 50.8% in the exposed versus 55.1% in the unexposed group were delivered by an Obstetrician.

- The present study has reported that 88.2% women in the exposed group with a 6.3% higher rate of C-section rate in the unexposed group with no significant association.

- 66% of women in exposed had an institutional delivery and 67.1% in the unexposed group reflecting than more than two-thirds of the population had an institutional delivery.

- There is no statistically significant difference in the gender of the babies born in the two groups (p <0.71).

- The mean birth weight in two comparison groups was 2716 in the exposed and 2738 in the unexposed group with no statistically significant difference (p-0.235) with the incidence of higher birth weights reported in unexposed group.

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ANNEXURE-I
DATA COLLECTION INSTRUMENT

"Association between vaginal pH ≥ 5 at 13-20 weeks gestation and the incidence of preterm birth: A prospective cohort study"

1. Study ID No: ☐☐☐☐☐☐

2. Clindamycin Screening ID No: ☐☐☐☐☐☐

3. MNH Participant ID No: ☐☐☐☐☐☐☐☐

4. Age: _____

Screening

5. Is the pregnant woman in 13 to 20 weeks of gestation?

a. Yes b. No ☐

6. Is the woman a permanent resident in the study cluster?

a. Yes b. No ☐

7. Is it a multiple pregnancy?

a. Yes b. No ☐

8. Has the woman taken antibiotics within the past 14 days?

a. Yes b. No ☐

9. Do you have excessive vaginal discharge that is causing trouble to you?

a. Yes b. No ☐

10. Is the woman **enrolled** in the study? ☐

a. Yes b. Refused c. Ineligible

11. Results of pH assessment (99 = Refusal) ☐☐.☐☐

12. Is the pH level > 5? ☐

13.Level of maternal schooling**:**

b. No formal schooling, illiterate ☐
c. No formal schooling,literate
d. Schooling

14. Years of maternal schooling**:** ☐

15.BPL card holder ☐

a. Yes b.No

16. Maternal height (in cms)**:** ☐☐☐

17. Maternal weight (at ANC visit > 12 wks gestation earliest possible): ☐☐☐

18. Hemoglobin (gm%) : ☐☐☐

a. unavailable

19.Systolic blood pressure(mmHg) ☐☐☐

20.Diastolic blood pressure(mmHg) ☐☐☐

MATERNAL OBSTETRIC INFORMATION:

21. Gravida: ☐☐

22. Para: ☐

a. Nulliparous

b.1-3

c. >3

23. Outcome of previous pregnancies

a. Total number of miscarriages ☐☐

b. Total number of Medical Termination of Pregnancy MTPs ☐☐

c. Total number of stillbirths ☐☐

d. Total number of live births ☐☐

e. Total number of early [0-7 days] neonatal deaths ☐☐

f. Total number of late [8-28 days] neonatal deaths ☐☐

g. Total number of living children ☐☐

24. Record estimated date of delivery by LMP ☐☐ ☐☐ ☐☐☐☐
dd mm yyyy

25. Gestational age as per LMP Weeks ☐☐ Days ☐

26. Has the participant had an Ultrasound done at any time during this pregnancy? ☐
a. Yes
b. No

27. Estimated Delivery Date by Ultrasound:
dd mm yyyy ☐☐☐☐☐☐☐☐

28. Date Ultrasound performed: ☐☐☐☐☐☐☐☐

dd mm yyyy

29. Gestational age at that ultrasound: Weeks ☐☐ Days ☐

a. Yes b. No ☐

30. Date of delivery:
dd mm yyyy ☐☐☐☐☐☐☐☐

31. Who conducted the delivery: ☐
a. Obstetrician
b. Non-OB Physician
c. Nurse / Nurse midwife
d. Traditional Birth Attendant
e. Family (no health provider)
f. Self Delivery
g.Don't know
h. Other _____

32. Where did delivery occur? ☐
a. Hospital
b. Clinic / Health Center
c. Home in Village

33. Type of delivery: ☐
a. Spontaneous
b. Induced

34. Mode of delivery: ☐
a. Vaginal (without forceps / vacuum)
b. Vaginal (with forceps / vacuum)
c. C-section
d. Miscarriage
e. Medical termination of pregnancy (MTP)

35. Maternal status at the time of delivery: ☐

a. Alive b. Dead

36. If died, date of death: ☐☐☐☐☐☐☐☐

dd mm yyyy

37. Clinical assigned cause of death (if available). Please check primary cause: ☐

a. Abortive-related outcome
b. Hemorrhage
c. Infection
d. Pre-eclampsia/Eclampsia
e. Obstructed/prolonged labor
f. Other – specify: _____

Neonatal conditions and outcome

38. Delivery outcome: ☐
a. Live birth
b. Stillbirth
c. Miscarriage (<20wks)
d. Medically terminated pregnancy (MTP)

39. Sex of the baby: ☐
a. Male
b. Female

40. Birth weight (in grams if unknown enter '9999') ☐☐☐☐

41. If birth weight unknown, baby appeared to be: ☐
a. Very small (<1000g)
b. Small (1000–1499g)
c. Small to normal (1500–2499g)
d. Normal >2500g
e. Don't Know

42. Gestational Age at birth (wks): ☐☐

43. Date of Death: ☐☐☐☐☐☐☐☐
dd mm yyyy

44. Was the death a stillbirth (no heartbeat, no breath and no movement at birth)?

a. Yes
b. No
c. Don't Know
d. Maternal Death before labor

Data to help determine cause of death

45. Were there signs of maceration? ☐

a. Yes
b. No
c. Don't Know

46. Major malformation at birth ☐ ☐

a. Neural tube defect
b. Abdominal wall defect
c. Other: _____

47. Cause of death assigned by medical provider (if available). Please check primary cause of death if available ☐ ☐

a. Prematurity
b. Asphyxia
c. Sepsis
d. Congenital anomalies
e. Other. Specify: _____
f. No official cause assigned