RESEARCH ARTICLE

ASSESSMENT OF VAGINAL INFECTION AND IDENTIFICATION OF MICROBES ASSOCIATED WITH UNEXPLAINED PRETERM LABOUR

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Manuscript Info

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Abstract

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Background: Preterm birth (< 37 completed gestation weeks) is a leading contributor to neonatal morbidity and mortality. Despite advances in perinatal medicine in recent decades, preterm delivery continues to pose a challenge to both the obstetricians and the neonatologists. Among many factors that trigger preterm labour, infection is a possible cause. The present study deals with the demographic and baseline characteristics of preterm labour patients, identification of vaginal flora and their relationship with unexplained preterm labour.

Material and methods: For the present prospective observational study, 350 cases of preterm labour were included. All the pregnant women in the present study were enrolled after excluding all exclusion criteria. A thorough systemic and obstetric examination was done along with detailed history of each patient. Vaginal swab samples were taken from the posterior fornix of the vagina. Samples were examined microscopically and also inoculated onto appropriate culture media to identify vaginal flora and degree of infection.

Results: Maximum cases of preterm labour were reported in females of the 20-25 years age group. Females of primigravida showed the highest number of preterm labour as well as infections. Concerning the gestation period (weeks), 34-36 weeks showed a higher frequency of preterm labour as well as infections.Incidence of preterm labour was higher in females who had a previous history of two preterm deliveries and who had a previous history of one abortion. Vaginal infection and urinary tract infection were reported in 34.57% and 24% of total cases studied, respectively. In vaginal swabs *Lactobacillus*, *Gardnerella vaginalis*, fungi(yeast), *Candida*, Gram positive Cocci, *Escherichia coli* and mixed microbes were the prevalent microorganisms identified. Gram staining results of vaginal swabs revealed the presence of *Lactobacillus*,*Gardnerella vaginalis*,*Mobiluncus*, Fungi(yeast),Gram positive bacilli,Gram negative cocci. Among these *Lactobacillus* and *Gardnerella* were the most common.In vaginal swab culture *Enterococusspp*,*Streptococcusaureus*,*Escherichiacoli*,*Proteus mirabilis*,*Pseudomonas*,*Staphylococcus*

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coagulase,*Candida*,*Acetinobacter*,*Klebseilla*and mixed microbes were isolated and identified.Among these *Candida*,*Escherichia coli*and mixed microbes were the most commonly isolated micro-organisms.

Conclusion: Vaginal infection significantly contributes to the cause of preterm labour. It indicates the need to screen, all antenatal women for the precipitating cause of PTL, especially asymptomatic genitourinary infections. There should be measures to manage them as early recognition and timely and prompt treatment can help to reduce preterm labour cases and associated neonatal morbidity and mortality of premature newborns.

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Introduction:-

Preterm birth is one of the most fundamental challenges in Obstetrics and a leading cause of neonatal morbidity and mortality. Despite steadily advanced research in obstetrics, our understanding of the fundamental causes of preterm labour is limited. Vaginal infections may be a basic and significant reason for preterm labour and delivery. Specific microorganisms present in amniotic fluid are thought to come from the vagina. It is a well-known fact that the risk of neonatal mortality increases exponentially with decreasing gestational age and weight. The incidence of preterm birth is 9.9% of all births, worldwide. An estimated 19·8 million live newborns were LBW worldwide in 2020, compared with 22·1 million in 2000 an absolute reduction of 1·9% points between 2000 and 2020.[1] Modern techniques of management for preterm labour and preterm neonates have resulted decrease in neonatal mortality rate. Despite advanced technology and management, PTB in the United States accounts for about 70% of neonatal mortality and about 50% of long-term neurological disorders in children.[2]

The cause of preterm labour is unknown in many cases although several conditions have been associated with an increased risk of preterm birth. 30-40% of all cases related to preterm birth are induced because of maternal or fetal complications and the remaining 60-70% are likely due to subclinical infective or inflammatory causes, especially genitourinary tract, cervical dysfunction, multiple gestation, idiopathic and possible social, environmental and nutritional interactions.[3] The most serious outcome of preterm labour is often associated with adverse maternal and neonatal outcomes related to infection.[4] Association between abnormalities in vaginal flora during pregnancy and preterm labour and delivery has gained a lot of attention. Vaginal infections significantly contribute to the preventable causes of preterm labour.[5,6]

According to the World Health Organization, delivery before 37 weeks of pregnancy is regarded as preterm birth. Preterm birth may be a result of idiopathic or medical and obstetric complications. About 30% of preterm births are unexplained and spontaneous.[7]

Despite the dramatic decrease in the infant mortality rate during the past several years, the percentage of preterm rates remains elevated. Vaginal discharge is the symptom that most often prompts a woman to consult a physician in order to determine the presence of an infection. However, most vaginal infections are nearly asymptomatic. As diagnosis is based on the evaluation of the vaginal ecosystem and demonstration of suspected microorganisms that is why the majority of preterm labour and birth remain unexplained.[8]

Diagnostic criteria for preterm labour:
1. Uterine contraction of 4 in 20 minutes or 8 in 60 minutes plus progressive cervical changes
2. Cervical dilatation of > 2cm
3. Cervical effacement of >80%.

It has been studied that fifty percent of women with vaginal infections which is a significant risk factor for unexplained preterm labour, remain asymptomatic as a result many communities and hospital based clinics do not include screening for these. These women are at increased risk for adverse birth outcomes. Early recognition and prompt treatment of asymptomatic and symptomatic vaginal infections aid in the reduction of the incidence of preterm labour and associated neonatal morbidity and mortality of premature neonates.

Affected women are more likely to develop clinical chorioamnionitis and rupture of membranes compared with women whose cultures are sterile. Moreover, their neonates are also more likely to have perinatal complications such as neonatal sepsis.[9]

According to the National Institute of Health, Bacterial Vaginosis which might cause preterm labour, is one of the infections known to harm the developing baby. Women with Bacterial Vaginosis are at increased risk for preterm birth, low birth weight infants and other adverse birth outcomes. Bacteria can gain access to intrauterine tissues through the placenta, fallopian tube or ascending infection from the vagina and cervix. Ascending infection is considered to be the most common entry route where microorganisms colonise the cervix, decidua, and possibly the membranes, and then may enter the amniotic sac.

The vaginal flora constitutes a balance complex ecosystem that fluctuates during pregnancy causing ecologic disturbance due to overgrowth or imbalance of microbial flora that are normally found in the vagina. Alteration in the balance of vaginal flora may play a significant role in peripartum infection and pregnancy outcomes.[10]

The main reason concerning the selection of the present problem for our study is that vaginal infection is widely prevalent and increasing day by day leading to preterm labour, which if treated effectively and timely, further complications of chorioamnionitis to the fetus and mother can be prevented. Therefore, the present study was designed with the main aim and objectives to determine the significance of vaginal infection in unexplained preterm labour, to find the vaginal flora responsible for PTL and to estimate the relationship of vaginal infection in unexplained PTL.

Material and Methods:-
The present prospective observational study was conducted on 350 pregnant women with 28-37 completed weeks of gestation who experienced preterm labour admitted to the J.K. Lon Mother and Child Hospital, Government Medical College, Kota (Rajasthan) during the year 2022-23.

Inclusion Criteria:
Pregnant women between 28-37 completed weeks of gestation and set into spontaneous labour.

Exclusion Criteria:
1. treated with antibiotics within the past 2 weeks.
2. being treated with vaginal progesterone.
3. diagnosed with placenta previa, placenta abruption or fetal death.
4. pregnant women with APH, PROM, IUD.
5. decline to participate in the study.

All pregnant women in the present study were enrolled after excluding all exclusion criteria. Written informed consent was obtained from enrolled women. Detailed history and examination including age, geography (urban/rural) education, occupation, parity, general physical examination, obstetric examination, and any risk factors in index pregnancy were done and documented in a predesigned proforma. All selected patients in the present study were further monitored regularly.

A thorough systemic and obstetric examination was done. A speculum examination was done to visualize the cervix and vagina and to rule out cervical and vaginal infections.

Two vaginal swab samples from the posterior fornix of the vagina were taken using sterile cotton-tipped swabs and sent to the laboratory for examination. One for vaginal culture sensitivity, grams staining and another for KOH mount. Samples were examined microscopically, by gram staining method and also inoculated onto appropriate culture media to identify vaginal flora and degree of infection. Cefixime and Amoxycillin were applied to test antibiotic sensitivity on high vaginal swab cultures. Data were recorded as per Proforma. For data analysis, computer based SPSS-22 was used.

Results:-

The present study consists of 350 cases of preterm labour. All the cases included in the study group satisfied the inclusion criteria. Most of the women in the study were between 20-25 years (40.57%). Most of the pregnant that had preterm labour were pregnant for the first time (primigravida-47.14%) followed by G_2 gravida (25.71%), G_3 gravida (16.57%), and G_4 or more than G_4 gravida (10.57%). Out of 165 preterm labour patients of primigravidae, 90 (54.54%) were found infected which was the highest among all parity, and the lowest infection was observed in G4 or more than G4 gravidae (8.10%). Preterm labour was seen in 34-36 weeks of gestational age at the highest frequency and 28-30 weeks at the lowest frequency and the same trend has been noted for the frequency of infection in preterm labour patients i.e. highest (34.55%) in 34-36 47weeks of the gestation period and lowest (5.25%) in 28-30 weeks of gestation period. The percentages of booked and unbooked patients were 59.14% and 40.85%, respectively. Unbooked patients showed a higher rate of infection (40.55%) than booked (30.43%). History of preterm labour is a strong predictor of preterm labour in the present pregnancy as well. In the present study, 28.85% of females had a previous history of one or more preterm deliveries. Among these, 9.14% had previous one preterm delivery and 13.71% had three or more preterm deliveries. It was found that vaginal infection was present in 34.37% of patients having a history of 1 preterm labour followed by 25% and 5.25% in patients having a history of 2 and >3 preterm labour, respectively. The percentage of preterm labour cases was observed to be 21.14% and 78.85% with respect to rural and urban areas, respectively and the degree of infection was found more in preterm labour patients belonging to rural areas (56.75%) than in urban areas (28.62%). The frequency of preterm labour in females having a previous history of abortion was found to be 10.28%. It was more in the female having previous history of one abortion (6.85%) than two abortions (2.28%) and three or more abortions (1.14%) and the percentage of infection was 29.16 %, 25% and 25%, respectively.

Gram staining results of vaginal swabs in preterm labour showed the presence of *Lactobacillus*, *Gardnerella vaginalis, Mobiluncus,* Fungi (yeast) gram negative bacilli and gram positive cocci. *Gardnerella vaginalis* was observed to be the most common (74.28%) followed by yeast (61.42%), *Lactobacillus* (58%), gram positive cocci (27.71%), *Mobiluncus* (2.85%) and gram negative bacilli (2.57%).

Vaginal infection was observed in 34.57% of females and UTI in 24%. Vaginal microbiology culture showed the presence of *Enterococcusspp.*, *Streptococcusaureus, Escherichia coli, Proteus mirabilis, Pseudomonas, Staphylococcus coagulase, Candida, Acinetobacter, Klebsiella, E. coli + S. aureus*, and mixed microbes. Among these *Candida* (32.57%), *E. coli*(25.71%), and mixed microbes (23.14%) were most common. Other prominent microorganisms seen in the culture were *Streptococcus aureus* (10.57%), *Klebsiella* (11.14%), *Enterococcusspp.* (8.28%) and *Staphylococcus coagulase* (5.42%). Most of the culture positive swab samples were sensitive to commonly used antibiotics Cefixime (87.71%) and Amoxycillin (78.94).

Table 1:- Demographic and Baseline Characteristics of Patients.

Characteristics	No. of Patients (N=350)	No. of PTL patients with infection	Percentage
Age			
<20	25	10	40.00
20-25	142	55	38.73
26-30	129	43	33.33
31-35	38	11	23.94
>35	16	2	12.50
Parity			
Primigravidae	165	90	54.54
G2	90	21	23.33
G3	58	7	12.06
≥G4	37	3	8.10
Gestation Period			
28-30	21	4	5.25
30-32	37	11	29.72
32-34	47	14	29.78
34-36	245	92	37.55

Booked/Unbooked			
Booked	207	63	30.43
Unbooked	143	58	40.55
Previous History of Preterm Deliveries			
1PTL	32	11	34.37
2PTL	48	12	25.00
3PTL	21	5	5.25
Residence			
Rural	74	42	56.75
Urban	276	79	28.62
Previous History of Abortion			
One Abortion	24	7	29.16
Two Abortion	8	2	25.00
Three Abortion	4	1	25.00
Urinary Tract Infection			
Yes	84	36	42.85
No	266	76	28.57

Table 2:-Gram Staining Results of Vaginal Swab in Preterm Labour.

Agent	No. of Patients (N=350)	Percentage
Lactobacillus	203	58.00
Gardnerellavaginalis	260	74.28
Mobiluncus	10	02.85
Fungi (Yeast)	215	61.42
Gram-negative Bacilli	9	02.57
Gram-positive Cocci	97	27.71

Table 3:- Results of Vaginal Microbiology Culture.

Microbes	No. of Patients (N=350)	Percentage
Enterococcus Spp.	29	08.28
Streptcoccus aureus	37	10.57
Escherichia coli	90	25.71
Proteus mirabilus	10	02.85
Pseudomonas	11	03.14
Staphylococcus coagulase	19	05.42
Candida	114	32.57
Acenetobacter	8	02.28
Klebsiella	39	11.14
E. coli + S. aureus	11	03.14
Mixed Microbes	81	23.14

Discussion:-

Vaginal infections greatly contribute to cause preterm labour.[11] Despite this fact, the pathogenic process that causes preterm labour is still not fully understood. It is a challenging research problem to define abnormal vaginal microflora in pregnant women because of the hormonal changes that occur during pregnancy and it is also believed that the morbific role of particular microbes in the vagina as a risk factor for preterm labour varies. It is also possible that the typical vaginal microbiota varies racially or ethnically and also geographically. Usai et al. stated that lack of vaginal lactobacilli was a greater predictor of preterm birth at 33 weeks of gestation than the presence of *Mycoplasma hominis* but this predictor's sensitivity and positive predictive value were only 28% and 25%, respectively.[12]

There is a possibility that the colonization rate of bacteria in the vagina is pretentious not only by methodological elements such as the approach of detection and the sample collection site but also by inner and outside factors that are specific to everyone.[13] In the present study, the maximum number of females in the PTL were reported to be in

the 20-25-year age group.   Rathore *et al.,* also reported the maximum number of females in the PTL were in the 21-25-year of age group.[14] Yarlagadda *et al.* showed that the maximum number of females having PTL were in < 20 years of age (32.75%) and 22.4% of females were between 22-25 years.[15] Shannon et al. reported PTL in only 15.8% and 18.8% of cases in extremes of age groups.[16]

Maximum PTL cases and degree of infection were reported to be in primigravidae, which is a similar finding to earlier studies conducted by Jayakrishnan *et al.*and Patel *et al.*[17,18] With increasing parity incidence of vaginal infection decreases as with each pregnancy awareness about perineal hygiene increases. Contrary to this Yarlagadda et al. observed 21.55% cases of PTL in primigravidae and 78.44% in multigravidae.[15]

Around 40.85% of pregnancies were unbooked and the frequency of infection was found to be higher in them than in booked. Most of these females belonged to lower socio-economic strata. It had been found that these patients remain untreated for regular antenatal checkups leading to preterm labour.

In the present study, the frequency of preterm labour and infections both were observed to be increased from 28 weeks to 36 weeks and the highest frequency was seen in 34-36 weeks of the gestational period. Similar results were reported by Jayakrishnan.[17]

The number of patients visiting the J K Lon Mother and Child Hospital, Kota were mostly from the urban area. Still, the percentage of vaginal infection was found to be greater in the rural area possibly due to a lack of awareness and poor perineal hygiene.

Out of 350 cases studied, 101 (28.85%) females had a previous history of one or more delivery and 36 (10.28%) females had a previous history of one or more abortions. Yarlagadda *et al.* reported that 38.79% and 32.75% of females have a previous history of abortion and preterm delivery.[15] In the study by Shannon *et al.,* 35% of females having preterm labour had a previous history of abortion and 12-11 had a previous history of preterm birth.[19]

Urinary tract infection was found to be 24% of the total cases studied. As the number of patients with a history of UTI was found to be a higher vaginal infection rate and landed up into preterm labour thus it should be detected earlier and treated efficiently to reduce the incidence of preterm labour thereby improving foetal-maternal outcomes. Bernado et al. showed that the risk of pregnant mothers with urinary tract infections developing preterm labour is twice that of those without urinary tract infection.[20]

Gowri and Begum reported *Candida* as the most common microorganism in vaginal swab culture.[21] In the present study also, *Candida* was found to be in 32.57% of all samples analysed in culture. It was found to be most common followed by *Escherichia coli* and mixed microbes. Similar results were also reported by Patil *et al.* in vaginal swab cultures of females having PTL.[22] Rathore *et al.* observed mixed microbes (27.27%) as commonest isolated organisms from vaginal discharge culture.[14]

In the present study, the percentage of vaginal infection was observed to be 34.5% in the females having preterm labour. Similar results were obtained in various earlier studies (Khan *et al.,* Yarlagadda *et al.,* Vrishali *et al.* etc.).[11,15,23] Incidence of vaginal infection was reported to be high in the present study when compared to the study conducted by Shannon and Savita who reported an incidence of 8.29% and Samim *et al.* found 7.24% of women with vaginal infection in preterm labour.[17,24]

Among 350 cases with preterm labour *Lactobacillus* was positive in 203 (58%) whereas *Gardnerella vaginalis* was in 260 (74.28%). Nguyen *et al.* also found a similar result in a case-control study from Vietnam.[25] DiGiulio *et al.* suggested that the risk of PTB is associated with the presence of *Gardnerella* or *Ureoplasma*.[26] Diversity in *Lactobacillusspp.* present in vaginal discharge leads to better pregnancy outcomes.[27]

Fungal (yeast) infection was reported in 61.42% of females having PTL. Nguyen *et al.* also reported similar results.[25] Yeast infection in the early stages of pregnancy may increase the rate of PTB.[14]

The sensitivity pattern of the organisms isolated suggests that most of the organisms isolated were sensitive to the commonly used antibiotics – cefixime and amoxycillin.

Conclusion:-

Vaginal infection significantly contributes to the cause of preterm labour. It indicates the need to screen, all antenatal women for the precipitating cause of PTL, especially asymptomatic genitourinary infections. There should be necessary measures to manage them as early recognition, and timely and prompt treatment help to reduce preterm labour cases and associated neonatal morbidity and mortality of premature newborns.

References:-

1. Okwaraji YB *et al*.: National, regional, and global estimates of low birth weight in 2020, with trends from 2000: a systematic analysis. Lancet. 2024; 16, 403(10431):1071-1080.
2. Casanova R; Chuang A; Goepfert AR;Hueppehen NA; Weiss PM, Beckman CRB; Ling FW; Herbert WNP; Laube DW; Smith RP : Preterm Labour. In: Beckmann and Ling's Obstetrics and Gynecology, Eighth Edition. London : Wolters Kluwer Health.2018 : 147-51.
3. Lumley J.: Defining the problem: the epidemiology of preterm birth. BGOG. 2003; 110:3-7.
4. Ziaei S; Sadrkhanlu M; Moeini A; Faghihzadeh S.: Effect of bacterial vaginosis on premature rupture of membranes and related complications in pregnant women with a gestational age of 37-42 weeks. GynecolObstet Invest. 2006; 61(3):135-138.
5. Hay PE; Lamount RF; Taylor-Robinson D; Morgan DJ; Ison C: Pearson J. Abnormal bacterial colonization of the genital tract and subsequent preterm delivery and late miscarriage. BMJ 1994;308:295-8.
6. Lee SE; Romero R; Kim EC; Yoon BH :A high nugent score but not a positive culture for genital Mycoplasma is a risk factor for spontaneous preterm birth. J Maten Fetal Neonatal Med. 2009;22:3212-17.
7. Hass DM. : Preterm birth, in clinical evidence. 2006; 16th edition, BMG Publishing Group, London.
8. Chao TT; Bloom SL; Mitchell JS; Mclntire DD; Leveno KJ: The diagnosis and natural history of false preterm labour. Obs Gyn. 2011;118(6):1301-08.
9. Villamor-Martinez E; Lubach GA; Rahim OM: Association of histological and clinical chorioamnionitis with neonatal sepsis among preterm infants: a systematic review, meta analysis. Front Immunol. 2007;11:972.
10. Hillier S; Nugent R; Eschenbach D; Krohn M; Gibbs R; Martin D; Cotch M; Edelman R; Pastorek J; Room; Rao A; McNellis D; Regan J; Carey J;KlebenoffM. : Association between bacterial vaginosis and preterm delivery of a low birth weight infant. N Engl J Med 1995;337:1737-42.
11. Khan RI *et al.*: Prevalence of vaginal infection in preterm labour, PJMHS, 2022; 16(4): 990-992.
12. Usui R. *et al.*: Vaginal lactobacilli and preterm birth. J. Perinat Med. 2002; 30: 458-466.
13. Olsen P.: The impact of oral probiotics on vaginal group B streptococcal colonization rates in pregnant women. A pilot randomised control study women and birth. 2018; 31(1): 31.37.
14. Rathore P *et al.*:A case control study evaluating association of preterm Labor with vaginal infection. International Journal of Clinical Obstetrics and Gynaecology. 2020; 4(5) : 152-156.
15. Yarlagadda S; Sajana G; Prasuna J; Narra L. : Association of vaginal infections in preterm labour. Int J Repd Cout, Obs Gyn. 2018; 7(6) : 2176-79.
16. Shannon FF. and Savita C. : A study of risk factors for preterm labour. Int. J. Repd. Contra. Obs. Gyn. 2015; 4 : 1306-1312.
17. Jayakrishnan C. *et al.*: Role of Bacterial vaginosis in threatened preterm and preterm labour, and its screening. Int. J. Obs. Gyn. Res. 2016; 3(2) : 115-120.
18. Patel VM *et al.*: A case control study on correlation between genitourinary infection and preterm labour. NJMR. 2016; 5(1) : 83-86.
19. Shannon FF and Savita C.: A study of rick factors for preterm labour. Int. J. Repd. Contracept. Obs. Gym. 2015; 4: 1306-1312.
20. Bernardo F *et al.*: Association of genitourinary infection and cervical length with preterm childbirth. Braz. J. Med. Biol. Res. 2020; 54(1).
21. Gowri M. and Begum S.: Perinatal outcome following genitourinary tract infection in preterm labour: A hospital based cross-sectional study. J. Cli. Diag. Res. 2021; 15(8): 10-12.
22. Patel VM *et al.*: A Clinical study of preterm labour. Int. J. Repd. Contra. Obs. Gyn. 2016; 8 : 4503-4510.
23. Vrishali G. *et al.*: Urogenital infection – A cause of pre-term labour. Int. J Contp. Med. Res. 2017; 4(4): 888-891.
24. Samim *et al.*: Risk factors for preterm birth in Iraq: A case-control study BMC Pregnancy childbirth. 2006; 6: 13.
25. Nguyen QHV; Le HN; NU VAT; Nguyen ND; Le MT: Lower genital tract infections in preterm premature rupture of membranes and preterm labour. a case-control study from Vietnam. 2021; 15(6): 805-11.

26. DiGiulio DB *et al.*: Temporal and spatial variations of the human microbiota during pregnancy. Proc. Nat Acad. Sci. USA. 2015; 112: 11060-11065.
27. Petricevic L *et al.*: Characterization of the vaginal lactobacillus microbiota associated with preterm delivery. Sci. Rep. 2014; 9: 18963.