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Markers for Prediction of Early Onset Neonatal Infection in Pregnancies with Prelabour Rupture of Membranes

Doctoral dissertation

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Abstract

Introduction: Prelabour rupture of membranes (PROM) is a common event in obstetrics and early onset neonatal infection (EONI) is a serious neonatal consequence that can happen related to PROM. Early prediction of EONI is a desirable clinical goal because late diagnosis with delayed treatment increases neonatal morbidity and mortality. Despite ongoing research efforts, identifying efficient predictive markers of EONI in pregnancies with PROM remains a critical challenge.

An additional challenge in developing countries is the availability and reliability of tests and testing.

Aims of the study: The primary aim of this study is to investigate and to determine the predictive value for early onset neonatal infection of maternal serum C- reactive protein and white blood cell count and amniotic fluid glucose concentration, in patients presenting with prelabour rupture of membranes. The secondary aim of the study is to determine associated demographic and socio-economic risk factors for prelabour rupture of membranes in pregnant women of Kosova, a population that has not been studied related to PROM and EONI. This includes the rate of neonatal infection in newborn infants with a maternal history of prelabour rupture of membranes, other associated risk factors for early onset neonatal infection in pregnancies complicated with PROM, most common maternal complications, mode of delivery, and the antenatal care practices in Kosova.

Material and Methods: A cross-sectional design was used to analyse a population of 200 pregnant women presenting to the Obstetrics and Gynecology Tertiary Center in Kosova (during 2013-2015) with prelabour rupture of membranes who gave birth to a single newborns. A questionnaire and evaluation form was used to collect data prospectively at admission and thereafter. Demographic characteristics collected included, antenatal profile, probable risk factors, mode of delivery and maternal and neonatal outcome were recorded and analyzed. Maternal serum, amniotic fluid, and vaginal swab samples were taken at the admission from all the women enrolled in the study with the purpose of determining the CRP, WBC, Glucose and maternal colonization.

Results: In this study, 13 % of the newborns had EONI. The CRP cutoff value of >6 mg/L predicted early onset neonatal infection with a sensitivity of 92.3 %, a specificity of 60.9%, a PPV of 26.1 % and an NPV of 66 %. The area under receiver operating characteristics (ROC) curve for the maternal serum CRP was 0.84 (95 % CI, 0.745-0.934). Analysis of maternal white blood cell count and amniotic fluid concentration showed specificity, sensitivity, PPV and NPV of 57.6 %, 68.3%, 21.4 %, 91.5 % and 57.6 %, 33.9%, 11.5%, 84.3 % respectively. Identified risk factors for PROM included parity, previous PROM, and maternal colonization. Identified risk factors for PROM with EONI included the interval from membrane rupture to delivery, maternal genital tract colonization, newborn's birth weight, and gestational age at birth.

Conclusion: Maternal serum CRP is the most accurate marker for prediction of EONI in pregnancies complicated with PROM, with a predictability of 84 %. This test is available in Kosova and is recommended to be conducted routinely in every pregnant women presenting with PROM. Maternal plasma WBC and amniotic fluid glucose concentration had a poor predictive value in prediction of EONI and may be used only as an additional test in combination with CRP for prediction of EONI in pregnancies complicated with PROM. It is also important to know that PROM- delivery interval, maternal colonization and gestational age and gestational weight at birth are associated risk factors for development of EONI as these can be evaluated critically when planning management of pregnancies complicated with PROM.

Keywords: *markers of infection, prelabour rupture of membranes, early onset neonatal infection, prediction of infection*

АПСТРАКТ

Вовед. Прематурна руптура на мембраните (PROM) е честа појава во акушерството, а раната неонатална инфекција е честа сериозна неонатална последица која е поврзана со PROM. Раното предвидување на EONI е посакувана клиничка цел бидејќи задоцнетата дијагноза заедно со задоцнетиот третман го зголемува неонаталниот морбидитет и морталитет. И покрај континуираните истражувања, идентификувањето на ефикасни предиктори за EONI кај бремености со PROM сè уште претставува голем предизвик. Дополнителен предизвик во земјите во развој е достапноста и сигурноста на тестови и тестирања.

Цели на студијата. Главната цел на оваа студија беше да се испита и да се одреди предиктивната вредност на серумскиот С-реактивен протеин, леукоцитите и концентрацијата на амнионска течност кај мајката во појавата на рана неонатална инфекција кај пациентки со прематурна руптура на мембраните. Исто така, целта на оваа студија беше да се определат асоцираните демографски и социоекономски фактори на ризик за појава на прематурна руптура на мембраните кај бремени жени од Косово, популација која до сега не била испитувана во врска со појавата на PROM и EONI. Беа испитувани: стапката на неонатална инфекција кај новороденчиња од мајки со анамнеза за прематурна руптура на мембраните, другите асоцирани ризик-фактори за рана неонатална инфекција кај бременост искомплицирани со PROM, најчестите компликации кај мајката, начинот на породување, и прегледите во текот на бременоста на пациентките од Косово.

Материјал и методи. Беше спроведена трансферзална, пресечна студија со цел да се анализираат 200 бремени жени со PROM кои се породиле во Терциерниот центар за акушерство и гинекологија во Косово во периодот од 2013 до 2015. Беше користен прашалник и евалуационен формулар за да се соберат податоци проспективно при прием на пациентките и потоа. Беа следени и анализирани следните демографски карактеристики: профилот на пациентките во текот на бременоста, можните ризик-фактори, начинот на породување и исходот кај мајката и новороденчето. При прием во болница од сите вклучени жени во студијата беа земени примероци од серум,

амнионска течност, вагинален брис со цел да се одреди CRP, бројот на леукоцити, гликоза и колонизација.

Резултати. Во оваа студија 13% од новородечињата имаа EONI. CRP cutoff вредноста од >6 mg/L беше доказ за рана неонатална инфекција со сензитивност од 92,3%, специфичност од 60,9%, PPV (позитивна предиктивна вредност) од 26,1% и NPV (негативна предиктивна вредност) од 66%. ROC кривата за серумскиот CRP од мајката беше 0, 84 (95% CI; 0,745-0,934). Анализата на бројот на леукоцитите кај мајката како и концентрацијата на амнионската течност покажа специфичност, сензитивност, PPV и NPV од 57,6%, 68,3%, 21,4%, 91,5% и 57,6%, 33,9%, 11,5%, 84,3%, соодветно. Паритетот, претходна PROM и колонизација беа идентификувани како ризик-фактори за PROM. Ризик-фактори, пак, за PROM со EONI беа: времето поминато од руптура на мембраната до породувањето, инфекции на гениталниот тракт кај мајката, родилната тежина на новороденчињата и гестациската старост при раѓање.

Заклучок. Серумскиот CRP од мајката е најпрецизниот предиктивен идентификатор за EONI кај бремености кај кои се јавува PROM, со предиктивност од 84%. Овој тест е достапен во Косово и се препорачува да се изведува рутински кај секоја бремена жена со PROM. Леукоцитите во серум кај мајката и концентрацијата на амнионската течност беа со слаба предиктивна вредност за предвидување на EONI и може да се користат само како дополнителни тестови во комбинација со CRP за предвидување на EONI кај бремености кај кои се јавува PROM. Исто така, значајно е да се напомене дека интервалот од PROM до породувањето, колонизацијата кај мајката и гестациската старост и тежина на новороденчето при раѓање се придружни ризик-фактори за појава на EONI бидејќи овие фактори може да бидат критички оценувани кога ќе се планира следење на бременост кај кои се јавува PROM.

Клучни зборови: *маркери за инфекција, прематурна руптура на мембрани, рана неонатална инфекција, предвидување на инфекција*

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Abbrevations	
ACOG	American College of Obstetricians and Gynecologists
ANC	Antenatal Care
CI	Confidence Interval
CRP	C Reactive Protein
ECM	Extracellular matrix
EONI	Early Onset Neonatal Infection
EONS	Early Onset Neonatal Sepsis
FDA	Food and Drug Administartion
FM	Fetal Membrane
GBS	Group B Streptococcus
IAI	Intra - amniotic Infection
IL	Interleukine
IP	Interval Period
LP	Latency Period
MMP	Matrix Metalloproteinases
MMPI	Matrix Metalloproteinase Inhibitor
NPV	Negative Predictive Value
PPV	Positive Predictive Value
PROM	Prelabour Rupture of Membranes
PPROM	Preterm Premature Rupture of Membranes
RCOG	Royal College of Obstetricians and Gynecologists
ROM	Rupture of Mmembranes
SIRS	Systemic Inflamatory Response Syndrome
TIMP	Tissue Inhibitor of Metalloproteinases
TNF	Tumor Necrosis Factor
VAFG	Vaginal Amniotic Fluid Glucose
WBC	White Blood Cell
WHO	World Health Organisation

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1. Introduction

In this section, description of terms and definitions used in this dissertation are presented. Additionally, there is a summary of the research literature on this topic. The literature review includes articles from around the world and from multiple language sources.

1.1 Key Terms and Definitions

The key terms for this paper are Rupture of Membrane (ROM), Prelabour Rupture of Membrane (PROM), and Early Onset Neonatal Infection (EONI). These terms are further defined and explained in this section.

1.1.1 Rupture of Membrane (ROM)

The first term to be defined is Rupture of Membranes (ROM) and is a key concept for this dissertation. Spontaneous rupture of membranes (ROM) is part of the normal process of labor and delivery but prelabour rupture of membranes is not. Under normal conditions, the chorioamniotic membrane will rupture during the active phase of labor. For most women, the initial onset of labor is accompanied by contractions that are low frequency, low intensity and irregular. As labor progresses the contractions become more regular, stronger and of increased duration. It is during this more rapid and regular contraction period that the spontaneous rupture of membranes is supposed to happen as the cervical dilatation is progressing. In some women, however, the rupture of membranes happens too early in the prelabor period.

1.1.2 Prelabour Rupture of Membranes (PROM)

Prelabour Rupture of Membranes (PROM) refers to rupture of the chorioamniotic membranes prior to the onset of labor and prior to the onset of clinically apparent labor contractions. It can occur at any gestational age, thus this condition has been classified as "preterm PROM "or "term PROM", depending whether the event occurs before or after 37 weeks of gestation.[1] The time interval between the rupture of membranes and onset of labor pain is called latent period (LP) whereas the time interval between the rupture of membranes and delivery is called interval period (IP). The minimum latency for diagnosis of PROM is one hour. According to Coughey et al, latency after membrane rupture is inversely correlated with gestational age at membrane rupture. At term, 50% of pregnancies complicated with

prelabour rupture of membranes will go into labor spontaneously within 12 hours, 70% within 24 hours, 85% within 48 hours and 95% within 72 hours. Meanwhile among pregnancies complicated with preterm PROM, 50% will go into labor within 24 to 48 hours and 70 -90% within seven days.[2]

PROM is a common obstetric complication but it is difficult to give an accurate incidence because of wide variations reported in existing literature. Recent data reports that PROM occurs in approximately 10 to 12% of all pregnancies. Of these pregnancies, PROM complicates about 8% of the term pregnancies and 2 to 3% of the preterm.[2] A greater incidence of PROM was found in earlier research studies that had narrowed the PROM range incidence of 14 to 17% .[3]

The etiology of prelabour rupture of membranes is almost certainly multi-factorial and in some cases unknown. The biological mechanisms behind the development of PROM include intrinsic membranes weakness, mechanical stress and ascending infection. PROM is linked to a number of adverse maternal and neonatal outcomes. The most frequent maternal consequences associated with PROM are chorioamnionitis, endomyometritis, wound infection, pelvic abscess, bacteremia and postpartum haemorrhage. Meanwhile one of the most serious neonatal consequences associated with PROM is early onset neonatal infection (EONI).

1.1.3 Early Onset Neonatal Infection (EONI)

The majority of current studies define EONI as a neonatal infection within 72 hours after birth.[4] Other studies, as reported by Vergnano et al [4], use a range of onset from 48 hours to 6 days after delivery. This has made it difficult to have consensus in the literature. EONI represents one of the important causes of early neonatal morbidity and mortality. According to Azemi et al.[5], Kosova continues to have a high rate of early neonatal mortality with perinatal infections being one of the main causes of the early neonatal morbidity and mortality.

The early identification of EONI is a desirable clinical goal because late diagnosis with delayed treatment increases neonatal morbidity and mortality. Despite numerous studies focusing on finding efficient markers as predictors of EONI, prediction of EONI remains a critical challenge in pregnancies with PROM. Cost, availability of specimens at the appropriate time, complexity of the assay methods, laboratory turnover time, reliability of the

tests and attitude of attending clinicians are all important factors in determining the suitability of a diagnostic marker for clinical application.[6] Moreover, in countries with limited resources such as Kosova, the lack of laboratory possibilities for advanced diagnostic tests makes diagnostic approach even more difficult. This means that investigation of the diagnostic accuracy of the tests that are available and can be performed and used in prediction of EONI in pregnancies complicated with PROM is of high importance.

Continued research is focused on finding accurate tests or markers to predict ENOI. Recent worldwide studies have focused on determineing the accuracy of maternal plasma C reaktive and leukocyte count and amniotic fluid glucose concentration count as predictors for early onset neonatal infection in pregnancies complicated with PROM. The research has had mixed results with some studies say these tests and predictive markers do predict EONI while other studies say the results are inconclusive or do not predict EONI. Despite not having clear indication if these tests or markers predict EONI, they are the current status of research and the best approach to predicting PROM and are used in this study. Further studies including the one in this dissertation apply these tests and markers to further give evidence if they work to predict PROM. This study presented in this paper is the first known research conducted on PROM with pregnant women of Kosova.

2. Review of the Research Literature

In this section, a review of the research and literature related to this dissertation topic is presented.

2.1 Human Fetal Membrane

2.1.1 Embryonic Development of Fetal Membranes

Fetal membranes are extraembryonic structures which embryonic development is closely related to differentiation of blastocyst into the trophoblast, a blastocyst cavity and embryoblast. The embryoblast, gives rise to the embryo as well as several components of the placenta and fetal membrane, whereas cells of trophoblast only form extraembryonic structures.[7] On the seventh day of development, during implantation stage, the trophoblast undergoes it's differentiation into inner cellular layer and outer layer, meanwhile the embryoblast forms embryonic disc composed of two epithelial layers: the epiblast and the hypoblast.

Amniotic epithelial cells develop from the central region of the epiblast by eight days after fertilization.[8] When first formed, the amnion is in contact with the body of embryo but at about the fourth or the fifth week a small cavity appears in the epiblast layer which enlarges forms amniotic cavity and fluid begins to accumulate in it.

The chorion is formed by the parietal extraembryonic mesoderm and the overlying trophoblast. During the second week, the cytotrophoblast invades the area of syntitiotrophoblast between lacunae and forms finger like projections called the chorionic villi. The chorionic villi initially cover the entire surface termed the chorionic sac, but during the eigth week they disintegrate in some areas forming the smooth chorion and develop in other areas forming the villous chorion. Degeneration occurs in areas composed against the deciduas capsularis as they lose their blood supply and become the smooth chorion and thus the chorionic membrane.[9]

As the amniotic cavity surrounding embryo enlarges, it causes the amnion to expand, thus the mniotic membrane is pushed against the chorion and they form a chorioamniotic membrane, a double membrane sac surrounding the fetus.[10]

2.1.2 Anatomy and Physical Properties of Fetal Membranes

Fetal membrane (FM) is an anatomic structure that consists of amnion and chorion (fetal origin) and portions of deciduas (maternal origin). The chorion is a thick, opaque, friable membrane which varies at term from 0.02 to 0.2 mm thick.[11] Meanwhile, the inner amnion is tough, smooth and translucent and lines the chorion and the surface of placenta, continuing over the outer surface of the umbilical cord. According to Caruso et al. the amniotic membrane's macroscopic feature upon isolation from the placenta at term reveals that it is almost transparent avascular tissue, with a thickness ranging from 0.02-0.5 mm.[12]

The amnion is composed of five histological layers including the epithelium, basement membrane, compact layer, fibroblast layer and the spongy layer. Chorion is composed of a reticular layer, basement membrane and trophoblast layer. Except cellular structure, the other important component of fetal membranes is the extracellular matrix (ECM) which is a dynamic structure that it surrounds and it anchors cell. Collagens are major proteins of ECM and they play a unique contribution to membrane integrity, serving to increase extensibility and tensile strength.[13] The structure of the membranes remains constant from the fourth month until term. The amnion and chorion although slightly adherent, are never intimately connected and usually can be separated easily, even at term. The amnion and chorion grow until up 28 weeks and then increase their size by stretching.[11]

2.2 Mechanism of Prelabour Rupture of Membranes

Normally the fetal membranes maintain their integrity throughout pregnancy and usually the spontaneous rupture occurs at late first phase of labour. Traditionally, this spontaneous rupture associated with labour has been attributed to a general weakening of membranes due to stretching and uterine contractions. Generalized weakness of membranes has been more difficult to establish when membranes ruptured prematurely thus it is considered that membranes that rupture prematurely appear to be focally defective rather than generally weakened. More recent evidence, however, suggests that membrane rupture is

related to biochemical processes including disruption of collagen within ECM and programmed death of cells in the fetal membranes.[14]

2.2.1 Extracellular Matrix Degradation

The ECM components are responsible for providing the main fetal membrane strength. Alterations in its composition lead to membrane weakening and rupture. One of the factors involved in degradation of the ECM components are the potent enzymes termed matrix metalloproteinases (MMP).Endogenous matrix metalloproteinases inhibitors (MMPIs) and tissue inhibitors of matrix metalloproteinases (TIMPs) by inhibiting strictly control these enzymes.[15]. Increased proteolytic activity of MMPs such as MMP-2, MMP-3, MMP-8 and MMP-9 is associated with degradation of collagen and therefore with prelabour rupture of membranes. Ota et al.[16] in their study about the role of MMP-2 in prelabour rupture of membranes at term reported a significantly higher activity of MMP-2 in PROM than in cesarean and normal deliveries. Another study conducted by Maymon et al.[17] evaluated the concentration of MMP-8 among 233 term and preterm gestations with intact and ruptured membranes. Authors report that in both term and preterm gestations, spontaneous rupture of membranes was associated with elevated concentrations of MMP-8.

In a study carried in the United States, Ortega et al.[18] estimated MMP-9 activity in amniotic fluid obtained from normal early gestations, term pregnancies with labour, term pregnancies without labour and pregnancies complicated with prelabour rupture of membranes. The authors conclude that prelabour rupture of membranes is associated with increased levels of MMP-9 and the imbalance between MMP and TIMP may reflect a disorder that promotes prelabour rupture of membranes. Prelabour rupture of membranes and collagen degradation may be triggered by relatively low activity of TIMPs. Ortega et al.[18] in their research has compared the activity of TIMP-1 between normal early gestations, normal term pregnancies without labour and pregnancies complicated with PROM. Their findings suggest a strong association between premature rupture of membranes and reduced levels of TIMP-1.

2.2.2 Apoptosis Mediated Pathway to Prelabour Rupture of Membranes

Accumulating medical research suggests that apoptosis (cell death) is an important pathway of prelabour rupture of membranes. Apoptotic changes may be mediated through inflammatory and non inflammatory pathways. Cigarette smoking induces apoptosis through promotion of proteolysis which is a non inflammatory pathway whereas ascending genital infections, urinary infections and other infections at sites remote from genital tract induce apoptosis through inflammatory cytokines and chemokines .[19]

2.3 Risk Factors for Prelabour Rupture of Membranes

Prelabour rupture of the fetal membranes sometimes occurs in the absence of recognized risk factors. However, histories of previous PROM, maternal age, parity, history of previous abortions, smoking and socio economic factors, have a particular association with PROM.

2.3.1 History of Previous PROM

Previous studies report a history of PROM is a significant factor for recurrence. Pasquier et al.[20] in their Cohort study among 598 women, examined causes and risk factors associated with PROM. They report a positive association between PROM and operative delivery and previous PROM. Based on their research a history of preterm PROM in previous pregnancy confers a high risk of recurrence of about 14.3 % whereas the previous term PROM recurrence rate is reported to be 11.8 %. A study conducted by Ladfors et al.[21] in Swedish population demonstrated the recurrence rate of 20 %.The reported recurrence of PROM in general is approximately 20 %, while the recurrence rate for preterm PROM is reported to be much higher. Asrat et al.[22] analyzed 121 pregnant patients with preterm prelabour rupture of membranes who had a minimum of two consecutive pregnancies under their care. Recurrent preterm PROM occurred in 39 out of 121 patients giving a recurrence rate of 32.2%.

2.3.2 History of Previous Abortions

Data from limited number of studies suggest that a history of previous abortions poses a risk for development of PROM. Makhlouf et al .[23] in their study about adverse pregnancy outcomes among women with prior abortion conclude that women with a history of two or more spontaneous abortions or one or more induced abortions are at increased risk for development of preterm PROM in next pregnancy. In contrast, a study among 111,390 pregnant women in East China indicates that a history of previous abortions is more common among women with term PROM.[24]

2.3.3 Smoking

Cigarette smoking is often cited as an risk factor for several adverse perinatal outcomes. Smoking leads to changes in blood levels of micronutritiens such as ascorbic acid, vitamin B12 and zinc, which may increase the risk for PPROM.[25] Beckman et al.[26] suggest that the risk of PROM is at least doubled in women who smoke during pregnancy. England et al.[27], in a cohort study examined the effect of smoking among pregnant women at different gestational ages. In the study population consisting of 640 pregnant women with PPROM and 40 cases complicated with PROM, they found out that cigarette smoking increases the risk of prelabour rupture of membranes more so at early gestational ages than at term. Similar results are reported by other authors.[28, 29]

2.3.4 Maternal Age

Advanced maternal age is a risk factor for pregnancy complications such as miscarriage, pre eclampsia, gestaional diabetes and cesarean delivery.[30] Few studies show controversy results about advanced maternal age as an additional risk factor for prelabour rupture of membranes. Zieadeh et al .[31] investigated and compared maternal complications in women aged 35 and older with women aged 25-29 years. In conclusion, compared with women aged 20-29 years, women delivering their firs child at or > 35 years were at increased risk of weight gain, obesity, chronic and pregnancy induced hypertension, antepartum haemorrhage and prelabour rupture of membranes. In contrast, a large prospective study conducted in Iran couldn’t find any association between advanced maternal age and prelabour rupture of membranes.[32]

2.3.5 Parity

In obstetrics the term parity applies to the number of births. Parity is often considered an associated factor with specific conditions and complications of pregnancy. In the literature search there are few reports which demonstrate association of parity and PROM.[21, 33]

2.3.6 Socio-economic Status

Several research report association of low economic statuc and PROM. Socio-economic factors may influence the opportunity to antenatal care service, realization of required laboratory tests and consultations but also may be linked to nutrition in pregnancy. Ferguson and colleagues [34] in their case control study show association of low family income with preterm PROM.

2.3.7 Education

There is an inverse relationship between education and pregnancy outcome. Previous studies report a controversy results about association between education and PROM. Ferguson et al (YEAR).[34] have reported a significant association between educational level and PROM. In contrast, a study conducted in Iran couldn't find any association between education and PROM.[35]

2.4 Diagnosis of Rupture of Membranes

Early and accurate diagnosis of PROM is very important. Prompt diagnosis would allow undertaking of specific interventions to optimize perinatal outcome and minimize possible complications. Failure to identify patients with PROM may result in a delay to undertake appropriate care. Conversely, a false diagnosis of PROM may lead to unnecessary interventions, including administration of drugs, hospitalization and induction of labour. According to the American College [36] (ACOG) Practice Bulletin Premature Rupture of Membranes the diagnosis of PROM is based primarily on the patient's history and physical examination. Patients presenting with PROM usually report a watery vaginal discharge or a sudden gush of liquid from genitalia, which is not associated with any labour pain or

contractions. In majority of cases where membrane rupture is recent, diagnosis may be confirmed with sterile speculum examination. The observation of either the pooling of fluid in the posterior fornix of the vagina or the leakage of amniotic fluid from the cervical os is conclusive for membrane rupture. Obtaining information about last menstrual period and/or confirmation of the gestational age it is also necessary to differentiate between term and preterm PROM. In cases when membrane rupture is not recent and no fluid is present in posterior fornix and/or there is no obvious leakage of fluid from the cervical canal, diagnosis can be difficult and create obstetrical dilemma. In these cases additional tests are needed for establishing the diagnosis of rupture of membranes.

Nitrazine test is a method used to identify the presence of amniotic fluid in vagina. It has an overall accuracy of diagnosis of rupture of membranes of approximately 93 %.[37] This colorimetric test is based on the principle of differentiating amniotic fluid from vaginal secretions by pH. Nitrazine test is rapid, easy to perform and because it is inexpensive makes it suitable to be performed even in setting with limited resources. Ultrasound examination has been used widely on initial evaluation of the patients with clinical history of PROM. Evidence of diminished amniotic fluid volume by ultrasound alone cannot confirm the diagnosis, but may help to suggest rupture of membranes. Approximately 50-70 % of women with ruptured membranes have low amniotic fluid volume on initial ultrasonography.[38]

If a nitrazine test is not conclusive and query still exists as to membrane integrity, other tests such as Fern test, Indigo Carmine test, AmniSure test may be performed. While the Fern test is simple, rapid and inexpensive but not satisfactorily accurate test, the Indigo Carmine although it is considered by many authors as a gold standard for definitive diagnosis of membrane rupture, it is too invasive to be used as a routine practice. Lately in 2009, FDA has approved AmniSure test, an rapid, non invasive bed site test for detection of amniotic fluid in vaginal secretions. Although the test is found to be highly diagnostic with a sensitivity of 98.9%,[39] the test is not available in Kosova settings.

2.5 Management considerations of pregnancies complicated with PROM

Management of pregnancies complicated with prelabour rupture of membranes depend if PROM occurs before or after 37 weeks of gestation, or if PROM occurs at term or before term.

2.5.1 Management considerations of pregnancies complicated with Term PROM

Term PROM is the rupture of the fetal membranes prior to onset of labor occurring any time at or beyond 37 weeks of gestation. The reported incidence of PROM varies and is about 6 to 10 %.[40] According to American College of Obstetrician and Gynecologist Practice Bulletin No 80; at term, 50 % of pregnancies complicated by term PROM will go into labour spontaneously within 12 hours, 70% within 24 hours, 85 % within 48 hours and 95 % within 72 hours.[36]

In majority of cases complicated with term PROM, the pregnancy and delivery will end spontaneously without any interventions and consequences; however a number of pregnancies complicated with term PROM may develop maternal - fetal complications. Term PROM may be followed by immediate and delayed complications. The possible immediate complications include: placental abruption, cord prolapsed and cord compression. Although rare, these conditions represent true obstetrical emergencies which may have major impact in maternal and neonatal outcome. These complications are very stressful for obstetrician, impact their decision for management and lead to emergent cesarean delivery. Delayed complications include maternal and neonatal infection. The risk of chorioamnionitis in pregnancies complicated with term PROM has been reported to be less than 10 % and to increase to 40 % after 24 hours of membrane rupture.[41] This highlights the importance of timely and appropriate management of PROM at term.

Initial evaluation of women presenting with term PROM should include confirmation of the diagnosis, confirmation of the gestational age, evaluation of maternal and fetal wellbeing, confirmation of the fetal presentation, assessment of conditions for vaginal birth respectively identification of cases where cesarean delivery is indicated, screening for infection and confirmation of GBS status if available. After initial assessment, the decision for active versus expectant management is taken. Mode of delivery is not compromised by choosing either planned early birth or expectant management, with equal rates of cesarean

and instrumental delivery in both groups. Induction of labour with oxytocin remains the method of choice for most of the pregnancies complicate with PROM, but in sub set of women with an unfavorable cervix, the prostaglandins may be the option. Active management of pregnancies complicated with term PROM is associated with reduced maternal infectious morbidity, reduced neonatal morbidity and increased maternal satisfaction without increasing rate of cesarean delivery.[42]

Sometimes the management of these patients depends on their desires and preferences. If after counseling regarding the risks and benefits of active versus expectant management women elect for expectant management, waiting for spontaneous labour may be considered for the first 12-24 hours. Risk of infection is much greater after 24 hours so the use of expectant management after the first 24 hours is questionable.[43] When the decision for delivery is made, group B streptococcal prophylaxis should be given.

2.5.2 Management Considerations of Pregnancies Complicated with Preterm PROM

Preterm premature rupture of fetal membranes (PPROM) is rupture of membranes with premature gestations (<37 weeks of gestation). It occurs in about 3% of pregnancies, meanwhile is responsible for about 30 % of all preterm births.[44] Preterm PROM is a complication of pregnancy that carries risk for development of several maternal and neonatal adverse outcomes, during antenatal, intrapartal and postpartal period. The initial evaluation of a patient with preterm PROM includes: 1) accurate assessment of gestational age; 2) estimation of fetal weight and presentation; 3) evaluation of the possible of infection; 4) determination of lung maturity; 5) assessment of fetal wellbeing and 6) exclusion of occult cord prolapsed.

In pregnancies complicated with preterm PROM, latency after membrane rupture is inversely correlated with the gestational age at membrane rupture. The most extensive study of spontaneous course of preterm PROM was reported by Nelson et al.[45], who evaluated the outcome following expectant management of 511 pregnancies with preterm PROM between 20 and <36 weeks of gestation. 52% of patients delivered within 48 hours. Over 47.8% of the patients continued their pregnancy beyond 48 hours, whereas 12.9% of cases continued their pregnancy by > or = seven days.

Preterm PROM may be associated with maternal complications such as infection, placental abruption, retained placenta and increased likelihood of operative delivery. Clinically evident intra amniotic infection, develops in 13 to 60 % of women complicated with preterm PROM and postpartum endometritis, occurs in 2 % to 13 % of women of women with preterm PROM.[46] Maternal infection is more commonly associated with prolonged rupture of membranes, membrane rupture at early gestationale age and multiple vaginal examinations. Placental abruption is another severe complication associated with preterm PROM which occurs in 4-7 % of cases.[47]

This significant association of preterm PROM and placental abruption is seen especially in patients with early midtrimester PROM and is one of the causes that leads to fetal distress and fetal death. Retained placenta is another less common but serious maternal complication which is commonly followed by hemorrhage and that requires instrumental intervention.[46] Preterm PROM is also linked with increased likelihood of instrumental and operative delivery. Noor et al. in their research about a prevalence of preterm PROM and its outcome report a instrumental delivery rate of 20 % and a cesarean delivery rate of 14 %.[48] Maternal sepsis and death has been reported to occur rarely, at 0.8 % and 0.14 % respectively.[49]

Neonatal complications are related primarily to prematurity and infection thus the management of pregnancies complicated with preterm PROM is closely related to the gestational age at the time of membrane rupture.

At 34 to 36 weeks of gestation, the occurrence of severe neonatal complications due to immaturity is low. Corticosteroids are generally not recommended to accelerate lung maturity after 34 weeks.[50] Group B streptococcal prophylaxis, should be given based on prior culture results or intrapartum risk factors. Expectant management in this group of women leads to a significant increase in the risk of infection without any benefit to the fetus.[49] So the patients with PROM at 34- 36 weeks of gestation should be transferred to a tertiary center with good neonatal care facility and delivery is recommended. If expectant management is continued beyond 34 weeks of gestation, the balance between benefit and risk should be considered and discussed with the patient, and expectant management should not extend beyond 37 weeks of gestation.[51]

In contrast to gestations at 34-36 weeks of gestations, pregnancies complicated with preterm PROM between 32 and 34 weeks of gestation need assessment of lung maturity. Amniotic fluid for fetal lung maturity testing and for diagnosis of infection may be collected by amniocentesis or from a vaginal pooled amniotic fluid. Recent studies suggest that there is a little benefit to be gained by brief pregnancy prolongation when fetal pulmonary maturity is evident with PPROM after 32 weeks, and the risk of infection increases with conservative management.[52] Therefore, if testing indicates lung maturity, expeditious delivery is recommended.

At this gestational age antenatal antibiotics and corticosteroid therapies have clear benefits and should be offered to all women if not contraindicated. Close fetal and maternal monitoring as an inpatient is recommended and delivery should be considered after corticosteroid benefit has been obtained. If testing indicates amniotic fluid infection, delivery should be considered.[53] According to ACOG there is no role of expectant management in any woman with preterm PROM beyond 34 weeks of gestation, therefore delivery is recommended.[36] Spontaneous rupture of membranes at 28 to 32 weeks of gestations is one of the important challenges for the obstetricians, as it is associated with controversies and clear guidelines are yet to be framed.

Once PROM is confirmed at 28 to 32 weeks, the patient ideally needs to be managed at specialized centers with well equipped neonatal intensive care. Expectant management at this gestational age carries the risk for development of infection, while immediate delivery carries the risk of prematurity. In general, when preterm PROM occurs at 28 to 32 weeks of gestation, expectant management is favored over active intervention in the absence of chorioamnionitis, placental abruption or fetal distress.[54]

After initial assessment, if both mother and fetus are stable, expectant management is recommended as an inpatient with close observation which involves: 4 hourly measurement of maternal temperature, heart rate, respiratory rate and blood pressure, daily cardiotocographs, twice or thrice weekly maternal cell count, C-reactive assays and culture of vaginal swabs, all in an effort to detect intrauterine infection. Generally a single course of corticosteroids is recommended.[55]

Delivery should be considered once 34 weeks of gestation is reached, as the risk of expectant management outweigh the advantages after 34 weeks.[56] Delivery before 32 weeks of gestation is associated with a significant risk of neonatal complications, including severe acute morbidity and death. Because of this, stable gravida with preterm PROM at 24 to 28 weeks of gestation is generally best served by conservative management in an attempt to prolong pregnancy. Conservative management consists of initial continuous fetal and maternal monitoring combined with subsequent modified bed rest, if no maternal and fetal contraindications exist, until 33 completed weeks of gestation. The adjunctive therapy during conservative management of preterm PROM at 24 to 28 weeks of gestation consists of prophylactic antibiotic to prolong latency and a single course of corticosteroids to reduce the risk of infection and gestational age depended morbidity in the newborn.[57]

Expectant management of patients presenting with preterm PROM at 24 to 28 weeks of gestation is today the standard of care and expeditious delivery before 34 weeks and regardless of gestational age is recommended only in case of development of infection, placental abruption and fetal distress.[58]

6. Early Onset Neonatal Infection and Prelabour Rupture of Membranes

Early-onset neonatal infection is a significant cause of mortality and morbidity among newborn babies. [59] According to the World Health Organization, more than four million neonates die annually, with a global neonatal mortality rate of 23/1,000 live births. [59]

About a million of these deaths are attributable to neonatal infections [60] Within the neonatal period an estimated 50% of all deaths are within the first 24 hours while 75% are within the first week of life[61]

Most European countries have experienced declines in their neonatal mortality rate but there are still wide variations in early neonatal mortality rates between developed and in developing countries. The European Perinatal Health Report represented the early neonatal mortality rate during 2010 for 33 countries. The reported early neonatal mortality rate for Finland, Sweden and for Portugal was 1.1 per 1.000 whereas for Denmark, Norway and for Luxemburg it was 1.5.

Basis on this report the highest early neonatal mortality rate was 4 per 1.000in Malta.62

In Kosova; the early neonatal mortality rate got reduced by half during the period 2000 – 2011. It was 14.80 ‰ in 2000, 9.02 ‰ in 2005 whereas in 2011 it was 8.90 ‰. According to report on perinatal situation in Kosova 2014, the trend of the early neonatal mortality rate continued to decline, but in 2014 the decline was substantial, from 6.2 ‰ in 2013 to 3.7 ‰ [63] It is still high, however, compared to other European countries and there is still lot to be done. The leading causes of early neonatal death globally are complications of preterm birth, intra partum related causes and infections.[64] Similarly according to yearly reports on perinatal situation of Kosova, infection is one of the three main causes of early neonatal death.

Neonatal infections, defined as bacteremia/sepsis, pneumonia, and meningitis, cause approximately 23.4% of neonatal deaths worldwide each year. Approximately half of the deaths caused by infection occur during the first week of life. Out of these, neonatal sepsis is a frequent cause of morbidity and mortality with an annual incidence of 2-6/1000 live births in the developed countries of the world [65].In the developing countries, the estimated incidence is 3-4 times higher and neonatal sepsis remains one of the most common reasons for admission in neonatal units [66.]

Early onset infectious morbidities in newborn implies infections that occurs from birth to seven completed days after birth and are considered to be one of the major threats in patients with PROM. PROM increases the risk of neonatal infection from 0.1 % to 1.4 %.

The risk increases dramatically to 8 % with the presence of chorioamnionitis,[67] whereas, the risk for developing neonatal sepsis increases progressively with the time elapsed between rupture of membranes and eventual delivery. A five-fold rise in sepsis is seen when comparing incidences at 24 hours versus 72 hours of premature rupture of membranes.[68]

Several studies have evaluated this association between PROM and neonatal infection. Wu J et al .[69] in their research have evaluated influence of premature rupture of membranes on newborns health among 711 pregnancies complicated with PROM. Authors report that among all patients with PROM, 25% (178/711) of the newborns had different infectious diseases: 92 (12.9%) had pneumonia and 63 (8.9%) had septicemia, which totally accounted for 87.1% of the patients with infectious diseases, 23 (3.2%) newborns had other infectious diseases including peritonitis, purulent meningitis, urinary system infection, skin infection, perianal infection and conjunctivitis, which accounted for 12.9% of the patients with infectious diseases.

Another recent study that evaluated the development of early neonatal infections in prelabour rupture of membrane was conducted over 2 years period in Abha Maternity Hospital, Kingdom of Saudi Arabia .[70] The authors reported that early onset neonatal infection was present in 14% of the infants but in only 6% was septicemia documented. Plucinska et al.[71] in a study conducted in Poland evaluated the impact of premature rupture of membranes (PROM) on neonatal outcome, particularly on the incidence of intrauterine infections. The study included 428 newborns, born after PROM. Authors reported an incidence of 29 % (124 /428 newborns) of neonatal infection among neonates born from pregnancies complicated with PROM. These data support a association between PROM and early neonatal infectious morbidity.

Physicians and especially obstetricians have long debated whether intrauterine infection is a cause or consequence of PROM and it seems likely both pathways are possible. The case for intrauterine infection being a consequence of PROM appears to be proved. Prior to rupture of membranes, the amniotic cavity nearly always is sterile. The physical properties of the intact placental membranes usually represent an effective barrier in preventing entry of

bacteria. With rupture of membranes bacteria from the lower genital tract typically enter the amniotic cavity [72]. This ascending route is the most common pathway for development of intrauterine infection. Based on this pathway, with rupture of membranes, the clock of infection starts to tick; from this point on isolation and protection of the fetus from the external microorganisms ceases.[73] Indeed its now being suggested that the neonatal morbidity and mortality, largely due to infection increases with the time from rupture of membranes to the onset of labor.

The other possible pathway that prelabour rupture of membranes is a consequence of intra amniotic infection is not absolutely established; however it is certainly possible that subclinical infection of the uterine cavity and amniotic fluid may lead to substantial adverse pregnancy effects such as prelabour rupture of membranes and early neonatal infection.[74]

The microorganisms most commonly associated with early-onset infection include group B Streptococcus (GBS), Escherichia coli, coagulasenegative Staphylococcus, Haemophilus influenzae , and Listeria monocytogenes .[75] Fungal pathogens may be associated with early-onset neonatal sepsis, and *Candida* spp. are most likely, occurring among term or preterm infants .[76]The relative frequencies of such microorganisms may show variations between different population and different places and also this pattern changes in the same geographical area over a period of time.

2.7 Postnatal Diagnosis of Early Onset Neonatal Infection

Accurate and timely diagnosis of early onset neonatal infection is very important and in the same difficult. Just after the birth, the challenges for clinicians are threefold: (1) identifying neonates with a high likelihood of sepsis promptly and initiating antimicrobial therapy; (2) distinguishing “high-risk” healthy-appearing infants or infants with clinical signs who do not require treatment; and (3) discontinuing antimicrobial therapy once sepsis is deemed unlikely.77 A number of neonates born with neonatal infection and sepsis may have signs and symptoms such as poor activity, temperature instability, hypotension, poor perfusion with pallor, mottled skin and respiratory distress. These signs are nonspecific and are observed with other noninfectious conditions also.

In a number of neonates, the physical examination reveals normal and is evidence that sepsis is not present, bacteremia can occur even in the absence of clinical signs.[77]

Other diagnostic methods used in neonatal units for detection and diagnosis of EONI include a complete blood cell count with a white blood cell differential, blood culture, chest x-ray, and a lumbar puncture.[78] Isolation of bacteria from blood is a standard and most specific method used to diagnose neonatal sepsis. Positive culture ranges from 8% to 73 % in the diagnosis of potential neonatal sepsis. An additional drawback of culture diagnosis is that culture reports will be available only after 48-78 hours. [79] Among above mentioned methods for a full work up, a wide variety of biomarkers have been investigated in newborns with suspected early onset neonatal infection. Cytokines such as interleukin 6 (IL-6), interleukin 8 (IL-8), and tumour necrosis factor (TNF) have been studied as markers for diagnosis of early onset neonatal infection. These markers have been shown to be highly accurate for the diagnosis of early onset neonatal infection, thus these markers may be performed in developed countries with advanced laboratory facilities, but in a country with low resources such as Kosova none of them is available for routine clinical use.

Other markers, such as C reactive protein and procalcitonin, are the most common acute phase reactant investigated in neonates with suspected EONI. According to the American Academy of Pediatrics the sensitivity of a CRP determination is low at birth because of insufficient inflammatory response of the newborn whereas procalcitonin undergoes to a physiologic increase within the first 24 hours of birth. These diagnostic markers are useful just if obtained after several hours after the birth.[80]

Nonspecific clinical manifestation, low sensitivity and specificity of routine laboratory investigations, rapidity of deterioration in neonates with true sepsis implies limitations of the postnatal diagnosis of EONI, which causes a unnecessary use of antibiotics in non-infected newborns meanwhile in a number of newborns with true EONI ,starting of treatment is delayed. Thus antenatal prediction of EONI ,based on perinatal risk factors such as PROM and based on antenatal investigation of markers of infection would lead to decrease in the infectious morbidity rate of the newborns, first by helping the obstetrician to recognize the need for antibiotic treatment, cessation of tocolysis, or decide early delivery and, also would enable the neonatologists to more accurately select the population of newborn infants that represent risk group for the development of early onset neonatal infection.

2.8 Antenatal prediction of EONI in pregnancies complicated with PROM

Intraamniotic infection remains as the major risk factor as well as consequence of PROM which may have impact in maternal, fetal and neonatal wellbeing and influences obstetrical management. In every case of pregnancy complicated with PROM, with careful evaluation the presence of intraamniotic infection should be proved or excluded with the aim of decision making for further pregnancy management respectively in order to make a decision for expectant management or termination of pregnancy.

Intraamniotic infection (IAI) refers to inflammation or infection of the placenta and fetal membranes (amnion and chorion).[81] The most common route of the intraamniotic infection is the ascending bacteria from the lower genital tract mostly in the presence of ruptured membranes. Research studies indicate that IAI complicates up to 10 % of all pregnancies and approximately 0.5 to 2.0% of term pregnancies. The etiology of IAI associated with PROM is usually polymicrobial and in majority of cases is caused by a combination of aerobic and anaerobic pathogens.[82,83] The pathogens that are most frequently isolated in the amniotic fluid of patients with IAI are those that are found in the vaginal flora including: Gardnerella vaginalis, Ureaplasma urealyticum, A,B and D streptococci, Peptostreptococcus and Escherichia colli.

IAI is associated with adverse pregnancy outcomes. While maternal mortality caused by IAI is rare, neonatal mortality directly related to IAI is between 1% to 4 % for term infants and more than 10 % for preterm.[82] once intra-amniotic infection develops, the fetus may become infected also. The infected fetus is prone to develop pneumonia, meningitis, enteritis and systemic infection respectively sepsis[81]. Depending on the course, the intraamniotic infection may be clinical or subclinical.

In the last 5 years, new information regarding incidence of intraamniotic infection (IAI) has developed. It previously had been stated that clinically evident intrauterine infection occurred in approximately 1 % of pregnancies[81]. Studies published in the last few years report rates of 4 to 10 % among all pregnancies whereas in pregnancies complicated with PROM the reported rate of IAI is 10% [84]. Clinical intraamniotic infection is diagnosed in the presence of a rise in temperature to at least 37.8 C° along with two or more of the following criteria –maternal tachycardia, fetal tachycardia, uterine tenderness and foul odour of the

amniotic fluid.[84] Clinical criteria are neither specific nor sensitive thus diagnosis of clinical IAI requires high index of suspicion which must be supported by laboratory tests[81]. Subclinical or silent IAI is the term reserved for the cases in which bacterial invasion of the amniotic fluid is not associated with clinical signs of infection. Subclinical intra –amniotic infection is far more frequent than the clinically evident IAI.[85] Table 1 shows that the probability for development of IAI in pregnancies with PROM is inversely correlated to the gestational age at the moment of membrane rupture.

Table A: Probability for development of intraamniotic infection in pregnancies with PROM

Gestational age at the rupture of membranes	Probability for the development of IAI
At less than 28 weeks	40 %
At 28 to 34 weeks	20 %
At more than 37 weeks	5 %

Because of the strong association between PROM and IAI which may be clinical with no specific clinical markers or silent without any clinical signs, the management of pregnancies with PROM in certain cases poses dilemma for obstetricians. The early identification of IAI is a desirable goal in patients with term, or particularly with preterm prelabour rupture of membranes.

Antenatal prediction of early onset neonatal infection would help the obstetrician to recognize the need for antibiotic treatment, cessation of tocolysis or early delivery and also it would enable the physicians to more accurately differentiate infected from non infected infants respectively select the population of newborns that require antibiotic treatment.[86] Despite the fact of numerous studies focusing on finding efficient markers as predictors of EONI prediction of EONI remains a critical challenge in pregnancies with PROM [87].

Existing research reports on the different invasive and non-invasive methods and markers to predict EONI in pregnancies complicated with PROM. Cost, availability of specimens at the appropriate time, complexity of the assay methods, laboratory turnover time, reliability of the tests, and attitude of attending clinicians are all important factors in determining the suitability of a diagnostic marker for clinical application [6].

Table B: Characteristics of an ideal infectious marker

Clinical characteristics

1. A well defined optimal cut off that is comparable
2. Favourable diagnostic utilities:
   - Sensitivity (approaching 100 %)
   - Specificity (.85 %)
   - Positive predictive values (.85 %)
   - Negative predictive value (.85 %)
3. Detects infection at an early stage
4. Monitors progress of treatment
5. Prognostication

Laboratory characteristics

1. Stable compound
2. Sustained increase or decrease in level for at least 48 hours after onset of clinical manifestation
3. Quantitative measurement
4. Easy method of measurement
5. Quick laboratory turnover time
6. Results comparable between laboratories
7. Low cost

*Adapted from article in P C Ng: Diagnostic markers of infection in neonates, Arch Dis Child Fetal Neonatal Ed. 2004

2.8.1 Markers and Invasive Tests for Prediction of EONI

Numerous studies have demonstrated prediction of early onset neonatal infection can be achieved by tests carried on amniotic fluid obtained via amniocentesis.

Culture of amniotic fluid, use of gram stain, analysis of biochemical markers such as leukocyte esterase and glucose and analysis of intraamniotic cytokines including matrix metalloproteinase -9 ,interleukin -6,TNF a,IL 8, IL1 have all been tested for diagnosis of infection on amniotic fluid obtained by transabdominal amniocentesis.[88-92]

Table C: Invasive diagnostic tests of infection

Test	Sensitivity	Specificity	Reference
Culture	100 %	76 %	Broekhuizen et al.[88]
Gram stain	44.8 %	97.6 %	Romero et al.[89]
Leukocyte esteraze	80 %	84%	Gauthier et al.[90]
Glucose	41 %	94%	Greig et al.[91]
MMP-9	77 %	73 %	Harirah et al.[92]
IL-6	100 %	79 %	Harirah et al.[92]

Although these diagnostic methods are highly accurate, they require the performance of amniocentesis, which regrettably is an invasive technique, and may be difficult to perform when the amniotic fluid volume is significantly reduced.[93] Therefore, because of the invasive nature of the procedure and insufficient evidence to recommend the use of amniocentesis in the diagnosis of intrauterine infection, in current practice amniocentesis is not performed in the majority of cases. Therefore, alternative indirect non invasive methods are being proposed to assess the microbial status of the intrauterine environment and to predict early onset neonatal infection .[93]

2.8.2 Markers and Non-Invasive Tests for Prediction of EONI

Development of antenatal non invasive test that would provide early prediction of early onset neonatal infection in pregnancies complicated with prelabour rupture of membranes is very important to both obstetricians and neonataologists. Non-invasive accurate prediction of EONI in pregnancies complicated with PROM would enable obstetricians appropriate clinical management of pregnancy in the meaning of to do conservative management or immediate delivery meanwhile neonatologist would benefit in the meaning of differentiation of neonates at high risk for infection and start the appropriate management of the newborn without delay, in an early period just after birth, even before the postnatal test results are available.

Prediction of EONI would lead to appropriate and on time actions by obstetricians and neonatologist, and indirectly this would influence the reduction of infectious neonatal morbidity and mortality.

Maternal serum is an easily reachable material in every pregnant women while in pregnancies complicated with PROM low vainal pool amniotic fluid is another easy approach compartment which offers the possibility of obtaining biological material in almost noninvasive way.[93] The most common inflammatory markers used in clinical practice are C – reactive protein, leukocytes and glucose.

C-reactive protein (CRP) first described by Tillet and Francis in 1930, is an acute-phase protein produced by liver cells in response to inflammation. In healthy young adults, the median concentration of CRP is 0.8 mg/L, the 90th centile is 3.0 mg/L, and the 99th centile is 10 mg/L. According to Pepys et al.[94] de novo hepatic synthesis of CRP, starts rapidly after a infectious stimulus, serum concentrations rising above 5 mg/L by about 6 hours and peaking around 48 hours. The plasma half life of CRP is 19 hours and when the inflammatory process ceases, the circulating CRP concentration falls rapidly.

CRP concentrations also have been evaluated in pregnant women. Previous studies indicate that CRP values in normal pregnancies appear to be higher than the standardized values for non pregnant individual with the median value of 2 mg/L reported. [95] No consistent change in CRP levels with gestational age was found among serially sampled women not in labor. Thus this marker is used as an indicator of infection among pregnant

women, especially in pregnancies complicated with prelabour rupture of membranes. To date, several studies have evaluated the accuracy of CRP in prediction of either intra amniotic infection or EONI. The conflicting results reported may be partially explained by the different thresholds used and different gestational ages of the study population included in the studies.

WBC are another marker of inflammation/infection. Leukocytosis is defined as a white blood cell count greater than $11x10^9$/L and typically reflects the normal response of bone marrow to an infectious or inflammatory process. Pregnancy is associated with mild leukocytosis, however according to different studies normal leukocyte count in pregnancy usually ranges from 5.00 to 12.00 $x10^9$ [96] According to a number of previous studies, number of maternal leukocytes count is one of the indicators that can be used in detection of intraamniotic infection and prediction of EONI.[97]

Despite maternal serum, the amniotic fluid is another compartment in which analysis of markers of infection may be performed. In pregnancies complicated with prelabour rupture of membranes, amniotic fluid compartment becomes achievable and specimens of amniotic fluid may be collected in a almost non invasive way, from a vaginal pool. Amniotic fluid samples obtained vaginally may be used to determine the concentration of different markers which are important for prediction of either fetal lung maturity or for prediction of intrauterine infection in infant. Amniotic fluid assessment has been studied in patients with PROM to investigate possible infection. Amniotic fluid indicators of infection include: elevated levels of TNF, elevated leukocyte count, elevated levels of matrix metaloproteinases, and the presence of bacteria by culture and low glucose concentration.

Glucose concentration in amniotic fluid is one of the markers that have been suggested to be useful in prediction of intraamniotic infection in pregnancies complicated with PROM. Approximately 30 years ago, Weiss and al.[98] found that in normal pregnancy, the mean amniotic fluid glucose concentration rises between 14^{th} and 17^{th} week of gestation and then declines steadily toward term, from 45.9 mg/ dl between weeks 16 and 17 to 15.8 mg/dl during weeks 40 to 42. Several studies have shown inverse association between amniotic fluid glucose concentration and intra-amniotic infection respectively. It has been suggested that in the presence of infection the amniotic fluid glucose concentration is reduced due to the metabolism of glucose by microorganisms and due to consumption of glucose by

activated neutrophils. Having into considerations the amniotic fluid glucose concentrations in normal pregnancy and variations according to gestational age, a cut off value of ≤ 14 mg/dl for glucose positive result has been suggested in investigation of accuracy of glucose concentration in prediction of EONI [99.]

As summarized in the table below, previous studies have investigated the diagnostic accuracy of different markers such as WBC, CRP, procalcitonin, IL 6, lipopolysaccharide binding protein, and glucose obtained from the maternal serum or amniotic fluid in prediction of early onset neonatal infection.

Table D: Markers for prediction of EONI

Marker	Sensitivity	Specificity	Reference
CRP	67.7 %	63.3 %	Lee et al. [100]
Procalcitonin	53 %	45 %	Tita et al.[101]
IL6	81 %	76 %	Pfeiffer et al. [102]
WBC	48%	85 %	Tita et al. [101]
VAF Glucose	82 %	56%	Buhimschi et al. [103]

3. Summary of Research and Literature Review

Because of varying results reported about tests and markers to predict EONI in the literature, further investigation is warranted. The results report differences in thresholds used and differences in gestational age of the study population included. This makes it difficult to definitively say what tests are recommended and their validity. Further investigation is necessary to establish the role of maternal serum CRP and WBC and amniotic fluid glucose concentration. These simple non-invasive and inexpensive markers in prediction of EONI in pregnancies complicated with PROM

4. Motivation

Neonatal morbidity and mortality are major global health challenges with 3 million deaths occurring in the first seven days of life during early neonatal period. Knowing that up to two thirds of newborn deaths can be prevented, effective health measures and research in this field are very important. This is especially relevant for low- and middle-income countries that have limited resources and have the highest reported rates of neonatal morbidity and mortality. The three major causes of early neonatal morbidity and mortality are infection, preterm birth, and birth asphyxia.

Despite improvements in obstetrics and neonatal care, Kosova, remains one of the countries with the highest neonatal mortality rates in Europe. Prelabour rupture of membranes (PROM), both term and preterm, are common events in obstetrics and a risk factor for development of infection. When this infection occurs it is one of the most serious and threatening complications for both mother and fetus.

Prediction of early onset neonatal infection (EONI) in pregnancies complicated with PROM would help obstetricians choose appropriate management of these cases and also would help neonatologists to early differentiate neonates at risk of infection. Proper management of pregnancies with PROM and early differentiation of neonates at risk for infection may result in decreasing the early neonatal morbidity and mortality related to infection.

5. Aims of the study

5.1 Main objective

Main objective of this study is to investigate the predictive value for early onset neonatal infection in patients presenting with prelabour rupture of membranes of these markers:

- Maternal serum C- reactive protein
- Maternal serum white blood cell count, and
- Amniotic fluid glucose concentration

5.2 Secondary objectives

Secondary objectives of the study aim to determine:

- Demographic and socio-economic risk factors for prelabour rupture of membranes in pregnant women of Kosova
- Maternal colonization respectively the frequency of different pathogens involved in PROM
- The rate of neonatal infection in newborn infants with a maternal history of prelabour rupture of membranes
- Associated risk factors for EONI in pregnancies complicated with PROM
- Mode of delivery and maternal-neonatal outcome in pregnancies complicated with PROM

6 Hypotheses

X1. C reactive protein alone is not an effective predictor of early onset neonatal infection in patients with PROM.

X2. Prenatal markers used in combination are not useful markers for prediction of EONI or for planning the management immediate delivery versus conservative management in patients presenting with PROM.

X3. There is no relationship between abnormal genital tract microflora and infection of the genital tract with early onset neonatal infection.

X4. Demographic and socio-economic factors do not play a major role in development of prelabour rupture of membranes in Kosovo pregnant women.

7 Material and Methods

7.1 Study design

This cross sectional study and has been implemented between September 2013 and July 2015, at the Tertiary Obstetrics and Gynecology Clinic-University Clinical Center of Kosova. The study participants include pregnant women admitting for prelabour rupture of membranes. Selection of the study participants is based on the defined inclusive and exclusive criteria.

Inclusive criteria:

- Pregnancy at or > 24 weeks of gestation
- Prelabour Rupture of Membranes
- Giving birth within 72 hours after PROM
- Not on corticosteroid treatment
- Not on antibiotic treatment

Exclusive criteria

- Pregnant women presenting with labour contractions
- Giving birth beyond 72 hours after PROM
- Hypertensive disorders on pregnancy
- Diabetes on pregnancy
- Fetal malformations

7.2 Population

A total of 200 pregnant women presenting with PROM who fulfilled study's inclusion/exlusion criteria and their newborns (N=200) are included in the study.

7.3 Interventions and Data Collection

Informed consent form was obtained from all potential participants. A specific questionnaire and evaluation form was prepared and used to collect data prospectively at admission and thereafter. Data covering demographic characteristics, antenatal profile, and probable risk factors were recorded and analyzed.

Confirmation of the gestational age was based on the last menstrual period and in the cases with irregular cycle or unknown last menstrual period, the gestational age was

determined based on the medical records of the first trimester ultrasound examination. Confirmation of the diagnosis of rupture of membranes was documented by sterile speculum examination confirming the pooling of amniotic fluid in the posterior vaginal fornix or/and direct visualization of fluid leakage from the cervical canal. When needed, a nitrazine test was performed also. An ultrasound examination was performed to confirm fetal wellbeing.

Maternal serum, amniotic fluid, and vaginal swab samples were taken at the admission from all the women. The blood samples are used for estimation of CRP (expressed as mg/L) and leukocyte count (expressed as cells /liter). WBC equal and greater than 14×10^9 and a CRP greater than 6 mg/L are deemed positive respectively pathological**.** The amniotic fluid was collected vaginally and samples are used to determine the glucose concentration (expressed as mmol/L). Pathological amniotic fluid glucose concentration was considered value $\leq$ 0.777 mmol/L. A high vaginal swab sample is taken and cultured at the Microbiology Department of the University Clinical Center of Kosova.

Data covering time elapsed from PROM to delivery, mode of delivery, maternal complications, and time from admission to discharge are extracted from the delivery ward registers and from the patient's history. Immediately after the delivery, the physical condition of the newborn was evaluated using Apgar score by a Neonatologist who was present at birth. The newborns were observed during the first seven days of life respectively during early neonatal period. Early onset neonatal infection was the main outcome registered and studied. The occurrence of early neonatal infection was diagnosed by a Neonatologist.

With the aim to determine the differences in risk factors, time elapsed from membrane rupture to delivery, mode of delivery, indications for cesarean delivery, maternal complications and days of hospitalizations according to gestational age, all the participants were divided in two groups: Group 1.Women with Term PROM $\geq$ 37 weeks of gestation and 2.Women with Preterm PROM < 37 weeks of gestation.

After obtaining all the relevant data, with the purpose to determine the predictive value for early onset neonatal infection of CRP, WBC and Glucose all the newborns were divided into two groups based on the presence of the neonatal infection:

Group 1. Newborn's without neonatal infection and Group 2. Newborn's with neonatal infection.

7.4 Statistical analysis

Statistical programs STATISTICA 7.1; SPSS 17.0 were used for the statistical analysis; collected data was processed using the following statistical methods:

- Database was formed by using specific computer software and was processed by using standard descriptive and analytical methods.
- Attribute statistical data were analyzed by determination of rates and odds ratio and statistical significance between detected difference – Difference test
- Qualitative data were analyzed by measuring the central tendency and probability distribution (mean and standard deviation)
- T-test was used to determine the significance of the difference found between quantitative data with normal distribution
- Pearson chi-squared test was used to determine the associative relations
- Sensitivity and specificity were used for binomial classification

    i. Sensitivity (true positive rate) measures the proportion of positives that are correctly identified as such
    ii. Specificity (true negative rate) measures the proportion of negatives that are correctly identified as such

- Reciever operating characteristic (ROC), or ROC-curve was used for analysis
- Shapiro-Wilk's test tested the normality of distribution of variables
- For CI (confidence interval ± 95% CI) was defined statistical significance at level of standard error less than 0.05 (p)
- The results are shown in tables and graphs

8. Results

The study examined early neonatal infection in pregnancy with prelabour rupture of membranes and it comprised a total of 200 pregnant women and their newborns.

8.1. Demographic characteristics of the patients presenting with PROM

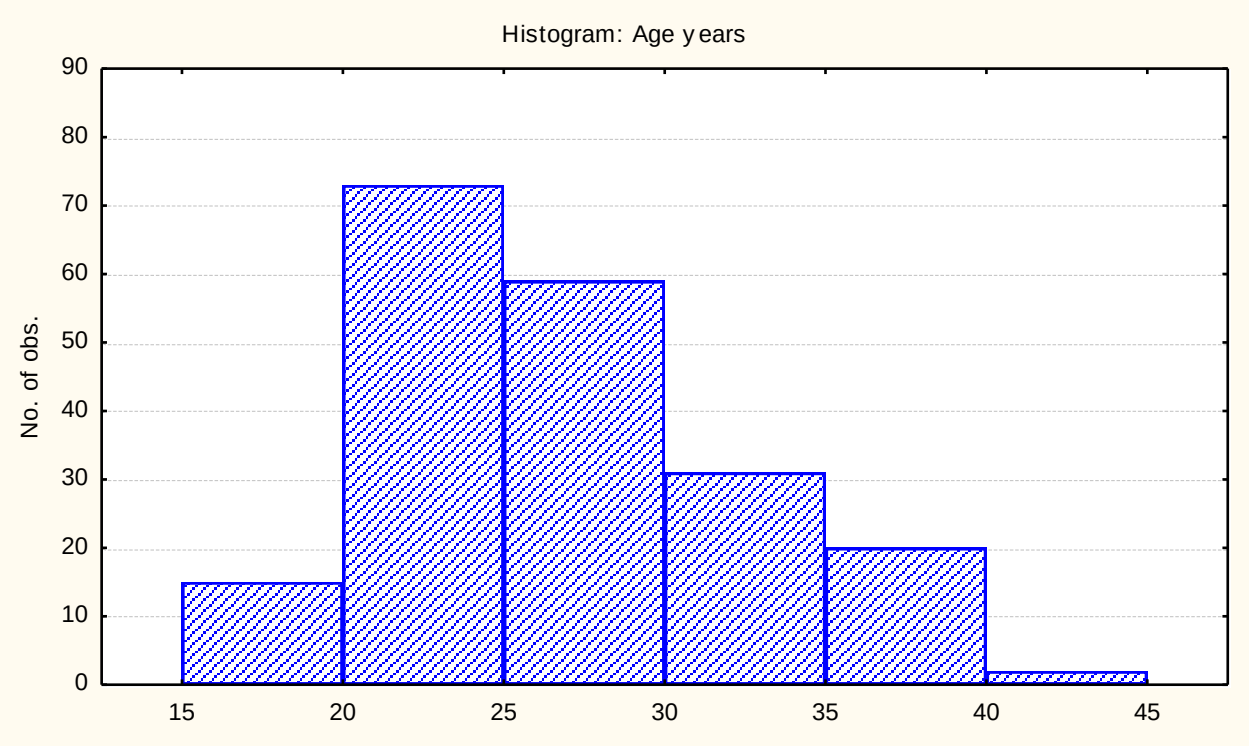
The mean age of patients included in the study was 27.5±5.5 yrs., minimum 18 and maximum 43 years (Table 1 and Figure 1). 132 (66.0%) patients were at the age between 20 and 30 yrs, 53 (26.5%) were older than 30 yrs and 15 (7.5%) were younger than 20 years (Figure 1b).

Table 1: Mean maternal age

	Valid N	Mean	Minimum	Maximum	Std.Dev.
Age years	200	27,5	18,0	43,0	5,486246

Figure 1a: Mean maternal age

Figure 1b: Distribution of patients according to age groups



The mean age of patients in gestational week <37 was 28.1±5.5 yrs, and of those >=37 weeks of gestation was 27.2±5.5 yrs (Table 2 and Figure 2). There was no statistically significant difference (p>0.05) between the two groups with regard to maternal age (p=0.286202) (Table 2).

A statistically significant association was found between maternal age and gestational age before and after 37 weeks of gestation (p<0.05) (Pearson Chi-square: 9.33216, p=0.025190).

Table 2: Mean age of patients according to gestational week

gestational week/ Age years	**Mean** <37g w	**Mean** >=37g w	**t-value**	**p**	**Valid N**	**Valid N**	**Std.Dev.**	**Std.Dev.**
	28.1	27.2	1.069377	0.286202	56	144	5.540143	5.462626

Figure 2: Mean age of patients according to gestational week

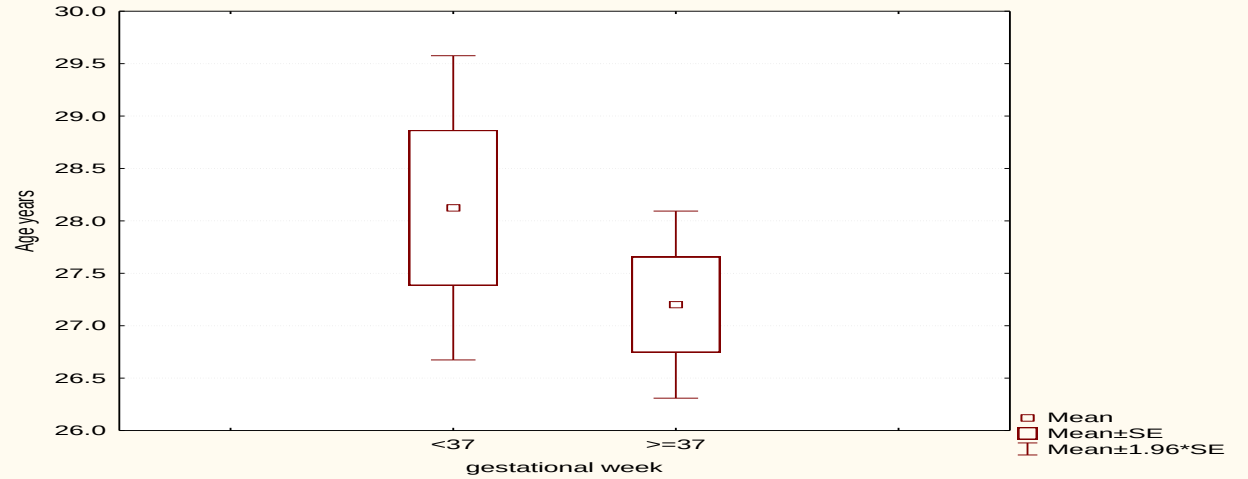
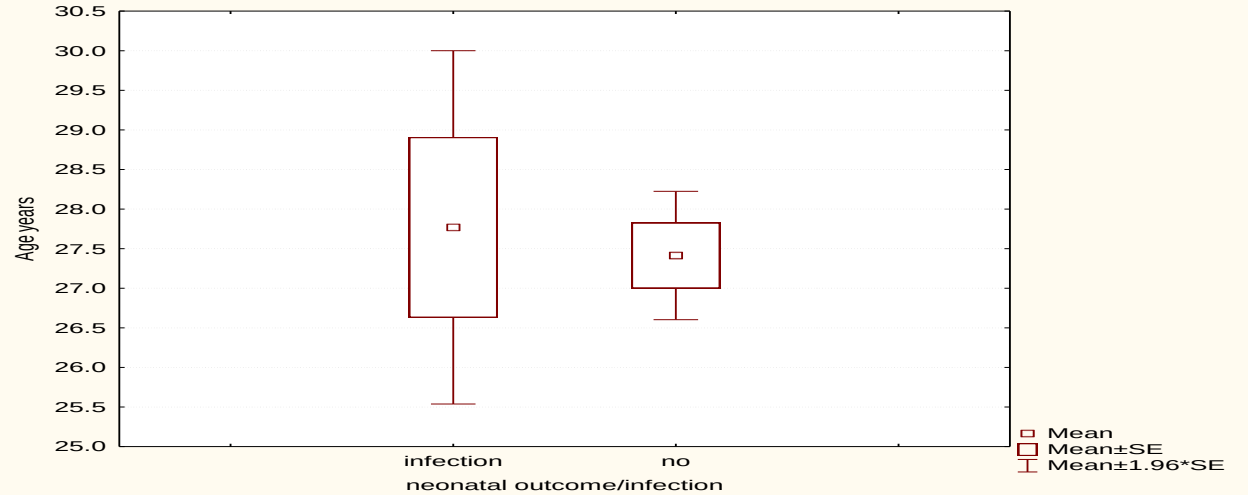


Table 3: Mean age of patients according to neonatal infection

neonatal outcome-infection/ Age years	Mean Infection	Mean no	t-value	p	Valid N	Valid N	Std.Dev	Std.Dev
	27.8	27.4	0.307429	0.758840	26	174	5.80556	5.45300

Figure 3: Mean age of patients according to neonatal infection



The mean age of our patients with registered neonatal infection was 27.8±5.8 yrs, and of patients without neonatal infection 27.4±5.4 yrs (Table 3 and Figure 3). There were no statistically significant differences between the two groups (term and preterm-PROM) regarding mean maternal age ($p>0.05$) ($p=0.75884$) (Table 3).

A statistically significant association was found between maternal age and registered neonatal infection ($p<0.05$) (Pearson Chi-square: 17.6460, $p=0.000521$).

The highest percentage of patients with PROM had completed high school (44.5%), 30.5% of patients had university degree and 25.0% had only primary education (Table 4 and Figure 4). The percentage difference between high school education versus university and primary education was statistically significant ($p<0.05$) ($p=0.0021$, $p=0.0000$).

Table 4: Distribution of patients according to level of education

Education	Count	Percent
elementary	50	25,0
secondary	89	44,5
university	61	30,5
total	**200**	**100.0**

Figure 4: Distribution of patients according to level of education

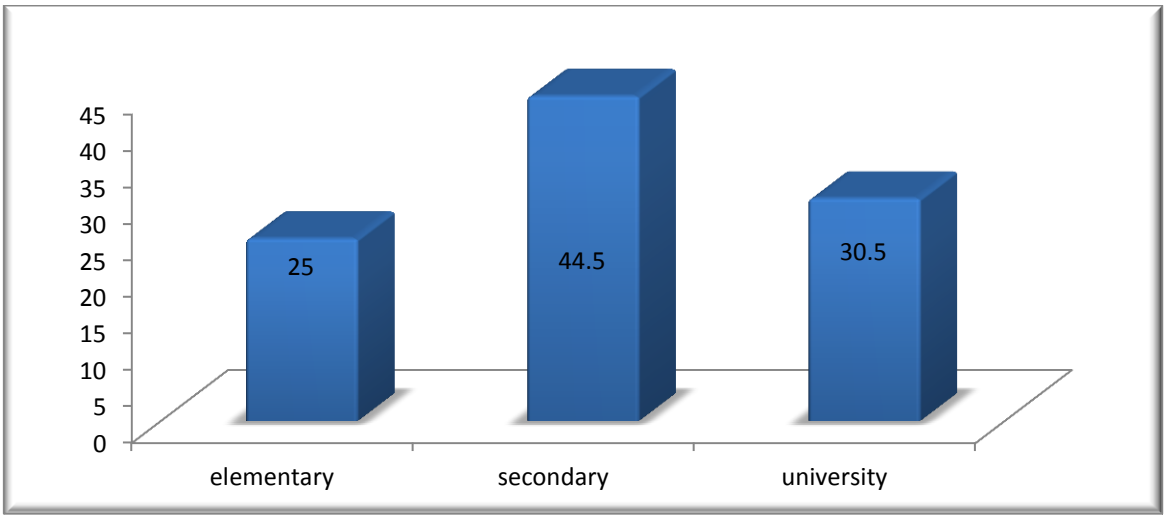
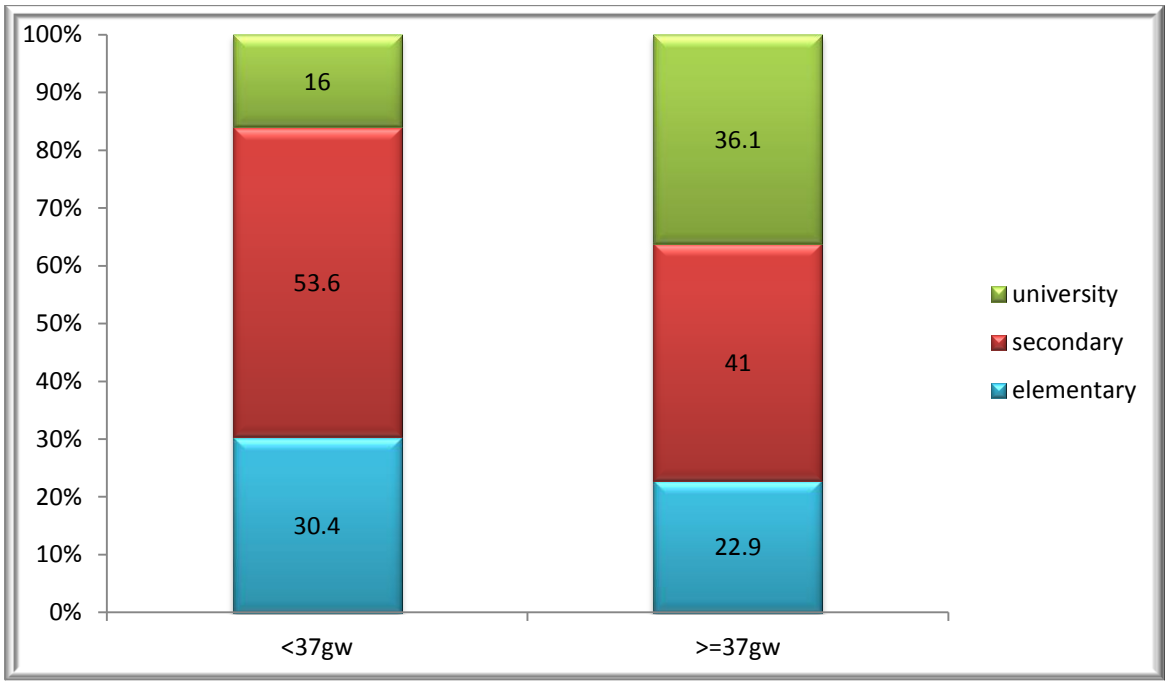


Table 5: Distribution of patients according to level of education and gestational week

Education	gestational week<37		gestational week >=37	
	Count	Percent	Count	Percent
elementary	17	30.4	33	22.9
secondary	30	53.6	59	41.0
university	9	16.0	52	36.1
total	**56**	**100.0**	**144**	**100.0**

Figure 5: Distribution of patients according to level of education and gestational week



The highest percentage of patients with preterm-PROM (<37gw) had high education - 53.6%, 30.4% of patients had primary education and 16.0% had university degree (Table 5 and Figure 5).

The highest percentage of patients with term-PROM (>=37gw) were with high education - 41.0%, 36.1% of patients had completed higher education and 22.9% had primary education (Table 5 and Figure 5).

According to the difference test, the percentage difference between high school education versus university and primary education in patients with preterm-PROM (<37gw) was statistically significant ($p<0.05$) ($p=0.0129$, $p=0.0001$).

There was a statistically significant difference between high school education versus university and primary education in patients with term-PROM (>=37gw) ($p<0.05$) ($p=0.0011$, $p=0.0155$).

A statistically significant association between level of education of patients and preterm-PROM and term-PROM was found ($p<0.05$) (Pearson Chi-square:7.64002, df=2, $p=0.021931$)

Neonatal infection was registered in the highest percentage of patients with PROM who had completed high education (57.7%), followed by patients with primary education (26.9%) and those with university degree (15.4%) (Table 6 and Figure 6).

Difference test revealed that the percentage difference registered between high school education versus university and primary education in patients whose infants were found to have neonatal infection was statistically significant ($p<0.05$) ($p=0.0290$, $p=0.0025$).

According to difference test, the percentage difference registered between high school education versus university and primary education in patients whose infants were not found to have neonatal infection was statistically significant ($p<0.05$) ($p=0.0004$).

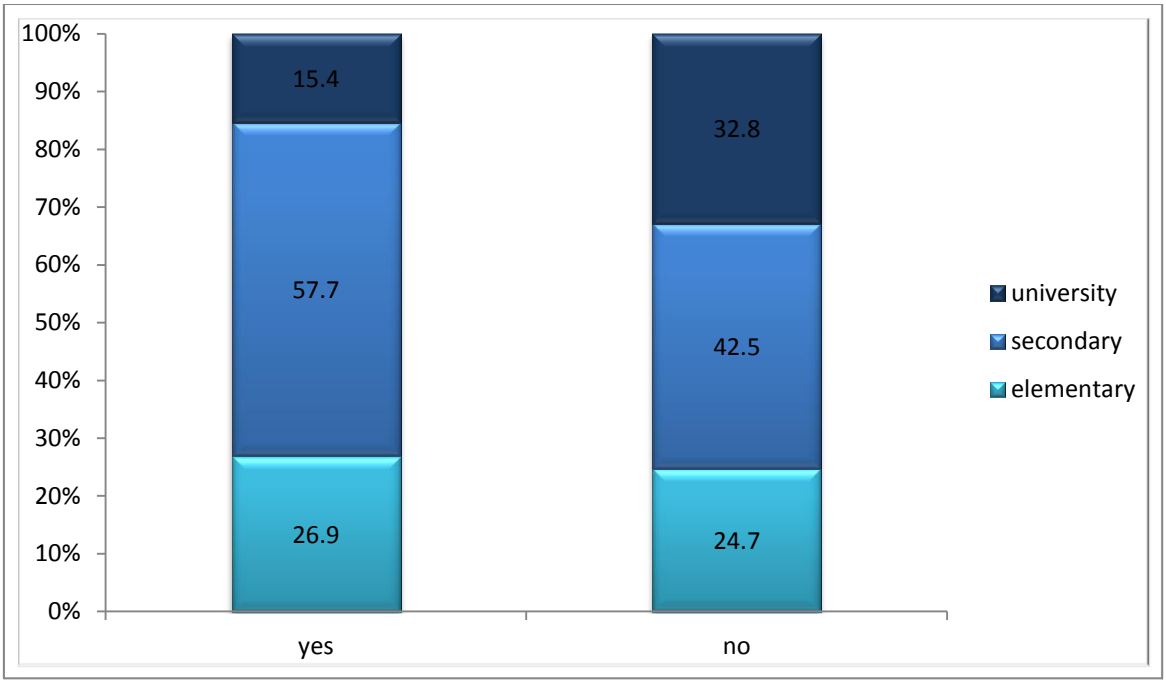
The percentage difference registered between patients with high school education versus patients with university education and whose infants were without neonatal infection was statistically not significant ($p<0.05$) ($p=0.0627$).

No statistically significant association was found between level of education of patients with PROM and neonatal infection ($p>0.05$) (Pearson Chi-square: 3.45168, df=2, $p=0.178029$).

Table 6: Distribution of patients with PROM according to level of education

Education	**neonatal outcome/infection**		**neonatal outcome/** no **infection**	
	Count	Percent	Count	Percent
elementary	7	26.9	43	24.7
secondary	15	57.7	74	42.5
university	4	15.4	57	32.8
total	**26**	**100.0**	**174**	**100.0**

Figure 6: Distribution of patients with PROM according to level of education



There was an equal percentage of patients with PROM (50%) who lived in urban and rural areas. No statistically significant association was registered between the patients' place of living and preterm-PROM and term-PROM ($p>0.05$) (Pearson Chi-square: 0.892857, df=1,p=0.344707. No significant association was found between the place of living of patients with PROM and neonatal infection ($p>0.05$) (Pearson Chi-square: 0.176835, df=1, p=0.674109).

8.2. Socio-economic characteristics of the patients presenting with PROM

The highest percentage of patients with PROM was unemployed - 82.5% and only 17.5% were employed (Table 7 and Figure 7). Difference test showed that the percentage difference registered between employment and unemployment status was statistically significant ($p<0.05$) ($p=0.0000$).

89.3% of patients with preterm-PROM (<37gw) were unemployed, whereas 79.9% were patients with term-PROM (>=37gw) (Table 8 and Figure 8).

No statistically significant association was registered between patients' employment status and preterm- and term-PROM ($p>0.05$) (Pearson Chi-square: 2.48059, df=1, p=0.115262).

Table 7: Distribution of patients according to employment status

employed	Count	Percent
no	165	82,5
yes	35	17,5
total	**200**	**100.0**

Figure 7: Distribution of patients according to employment status

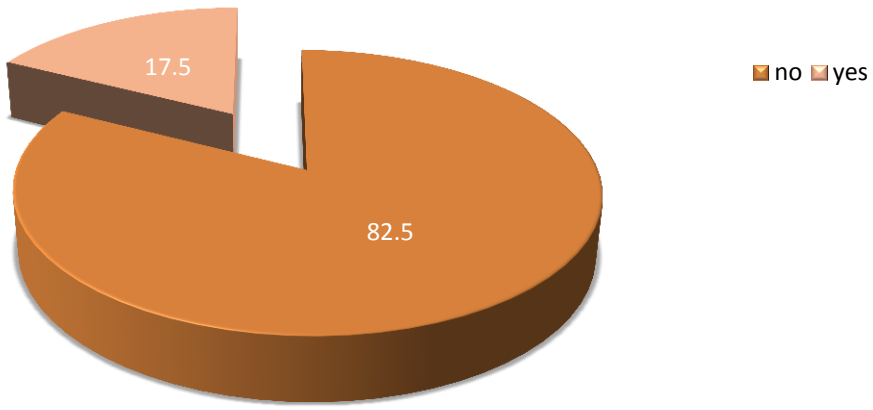
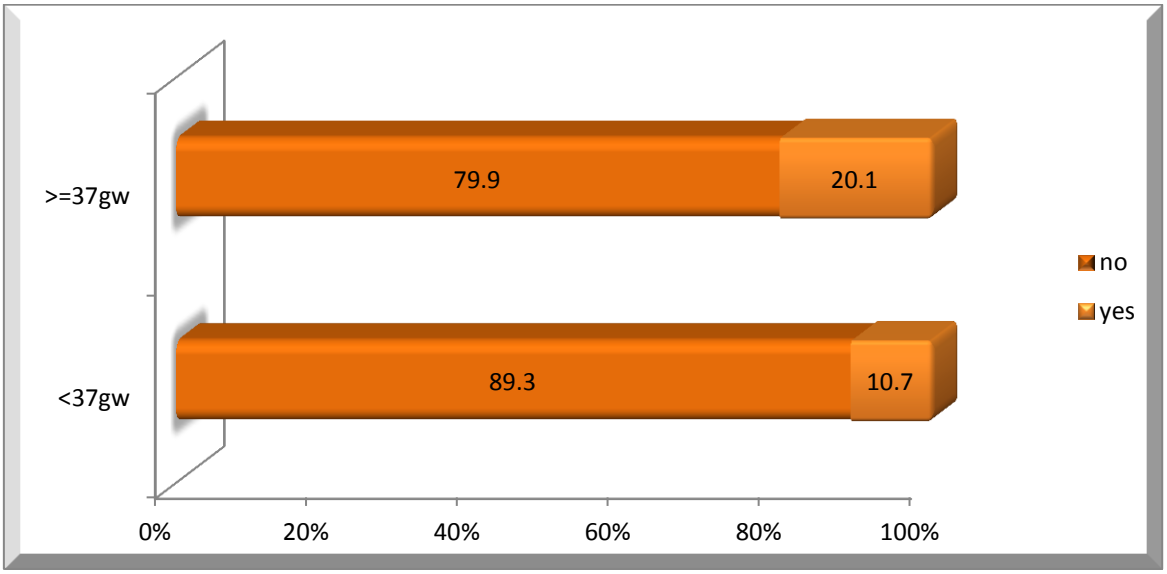


Table 8: Distribution of patients according to employment status and gestational week

employed	gestational week <37gw		gestational week >=37gw	
	Count	Percent	Count	Percent
no	50	89.3	115	79.9
yes	6	10.7	29	20.1
total	**56**	**100.0**	**144**	**100.0**

Figure 8: Distribution of patients according to employment status and gestational week



Neonatal infection was registered in 96.2% of patients with PROM who were unemployed, and no infection was registered in 80.5% of unemployed patients (Table 9 and Figure 9). A statistically significant association was found between patients' employment status and neonatal infection ($p<0.05$) (Pearson Chi-square: 3.85898, df =1, p=0.049483).

Table 9: Distribution of patients according to employment status and registered neonatal infection

employed	neonatal outcome/infection yes		neonatal outcome/infection no	
	Count	Percent	Count	Percent
no	25	96.2	140	80.5
yes	1	3.8	34	19.5
total	**26**	**100.0**	**174**	**100.0**

Figure 9: Distribution of patients according to employment status and registered neonatal infection

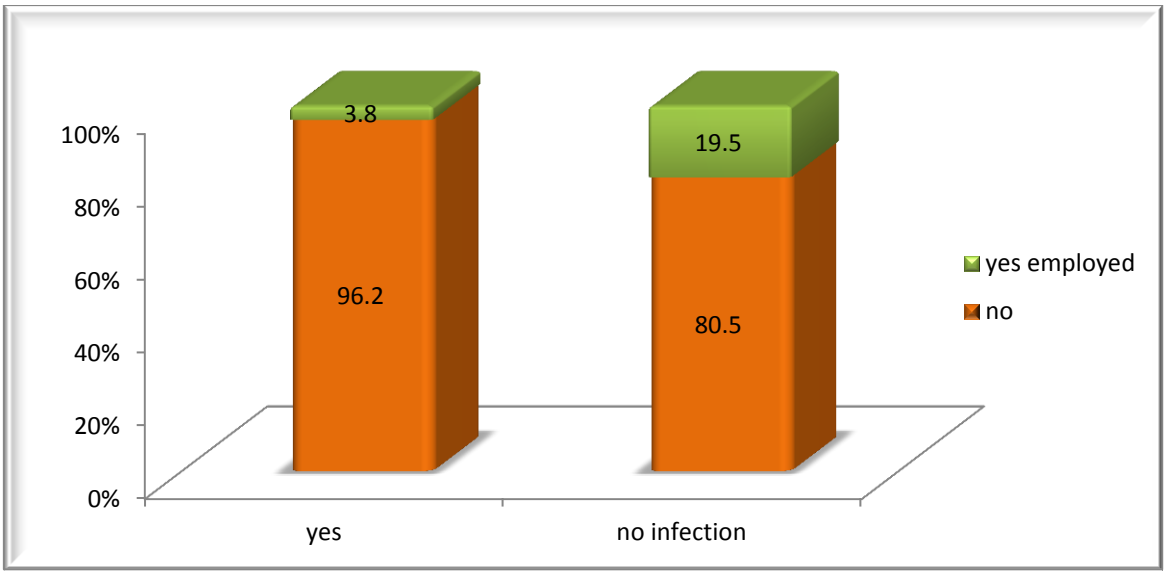
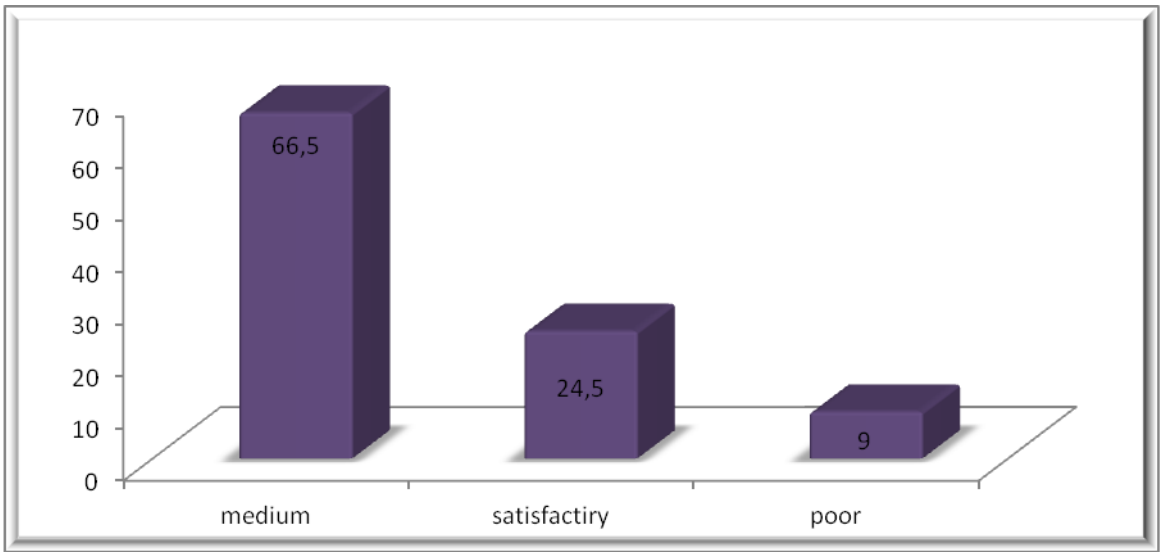


Table 10: Distribution of patients with PROM according to satisfaction with standard of living

Standard of living	Count	Percent
medium	133	66,5
satisfactory	49	24,5
poor	18	9,0
total	**200**	**100.0**

Figure 10: Distribution of patients with PROM according to satisfaction with standard of living



The highest percentage of patients with PROM were moderately satisfied with the standard of living - 66.5%, followed by 24.5% of patients who were satisfied, and only 9.0% who were not satisfied, that is, were poor (Table 10 and Figure 10).

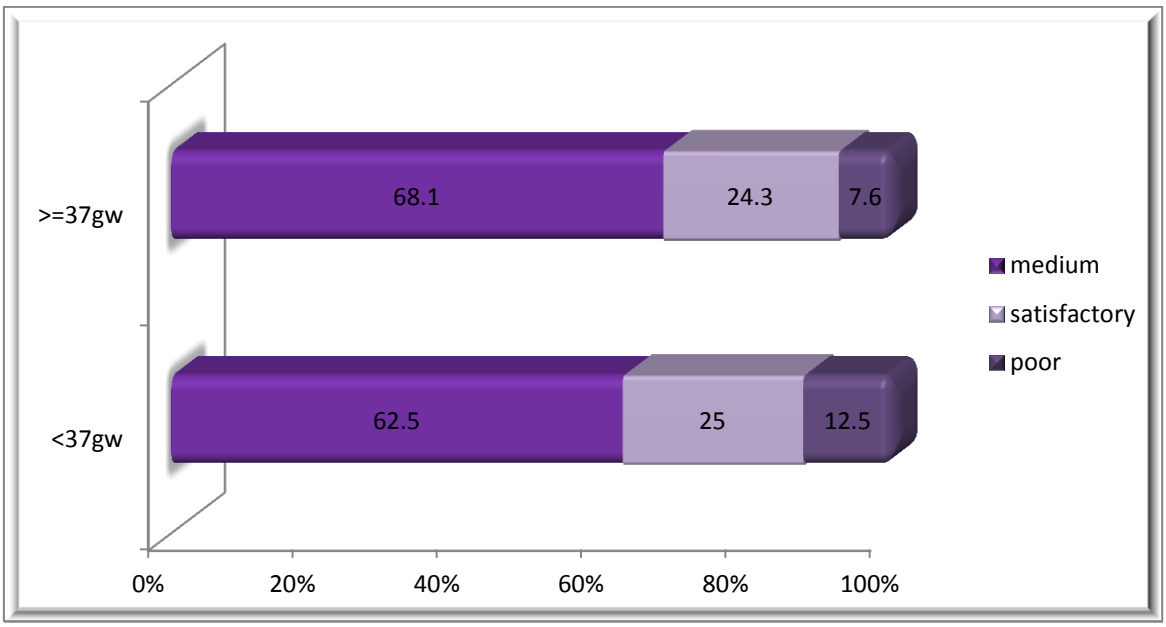
Difference test showed that percentage difference registered between the standard of living was statistically significant for $p<0.05$ ($p=0.0000$) between those who were moderately satisfied and those who were not satisfied.

62.5% of patients with preterm-PROM (<37gw) were moderately satisfied with the standard of living and 68.1% were patients with term-PROM (>=37gw) (Table 11 and Figure 11).No significant association was registered between the living conditions of patients and preterm- and term-PROM ($p>0.05$) (Pearson Chi-square: 1.25371, df=2, $p=0.534271$).

Table 11: Distribution of patients according to standard of living and gestational age

Living conditions	gestational age <37gw		gestational age >=37gw	
	Count	Percent	Count	Percent
medium	35	62.5	98	68.1
satisfactory	14	25.0	35	24.3
poor	7	12.5	11	7.6
total	**56**	**100.0**	**144**	**100.0**

Figure 11: Distribution of patients according to standard of living and gestational age



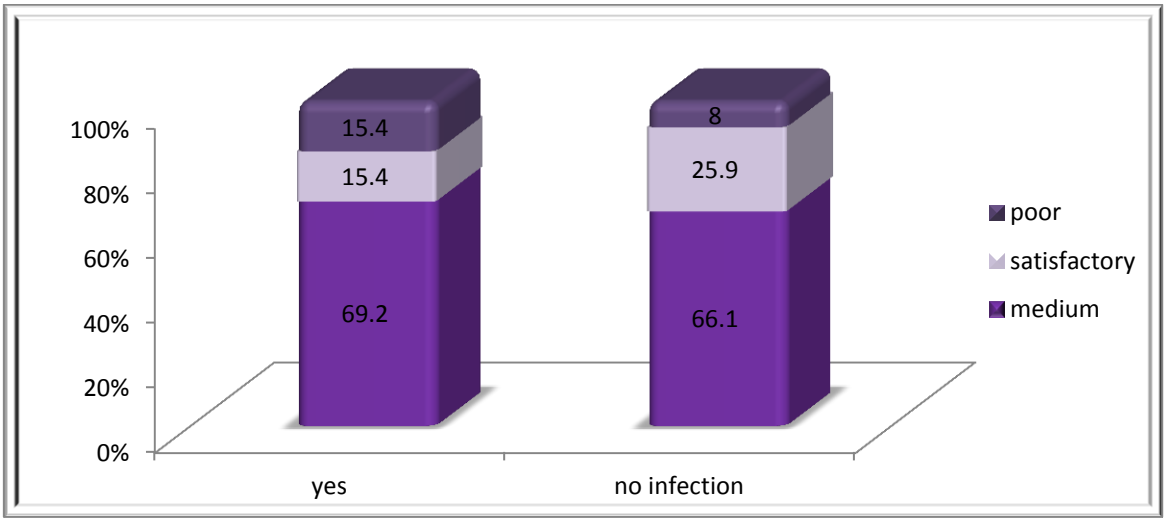
In 69.2% of patients with PROM who were moderately satisfied with the standard of living neonatal infection was found and in 66.1% of patients who were moderately satisfied with the living conditions neonatal infection was not found (Table 12 and Figure 12).

No statistically significant association was detected between the satisfaction of patients with the standard of living and neonatal infection ($p>0.05$) (Pearson Chi-square: 2.40062, df=2, p=0.301106).

Table 12: Distribution of patients according to standard of living and registered neonatal infection

Standard of living	neonatal outcome/infection yes		neonatal outcome/infection no	
	Count	Percent	Count	Percent
medium	18	69.2	115	66.1
satisfactory	4	15.4	45	25.9
poor	4	15.4	14	8.0
total	26	100.0	174	100.0

Figure 12: Distribution of patients according to standard of living and registered neonatal infection



The average number of family members was 6.3±3.7, minimum 2 and maximum 22 (Table 13 and Figure 13a).

More than half of the patients with PROM (51.5%) had a family with 2 to 4 members; in 35.5% of patients the family consisted of 5 to 10 members; in 10.5% of patients there were 10 to 15 family members, etc. (Figure 13b).

Difference test showed that percentage difference registered between the number of family members, from 2 to 4 members, versus other groups was statistically significant for $p<0.05$.

Table 13: Average number of family members

	Valid N	Mean	Minimum	Maximum	Std.Dev.
family members	200	6,3	2,0	22,0	3,692907

Figure 13a: Average number of family members

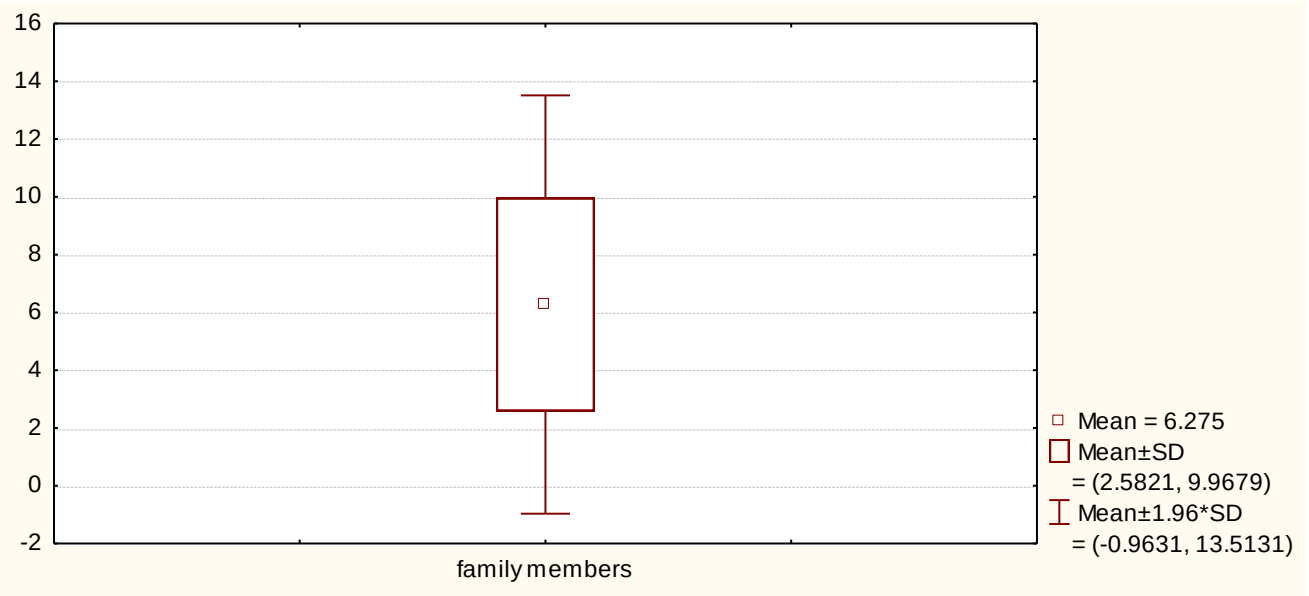
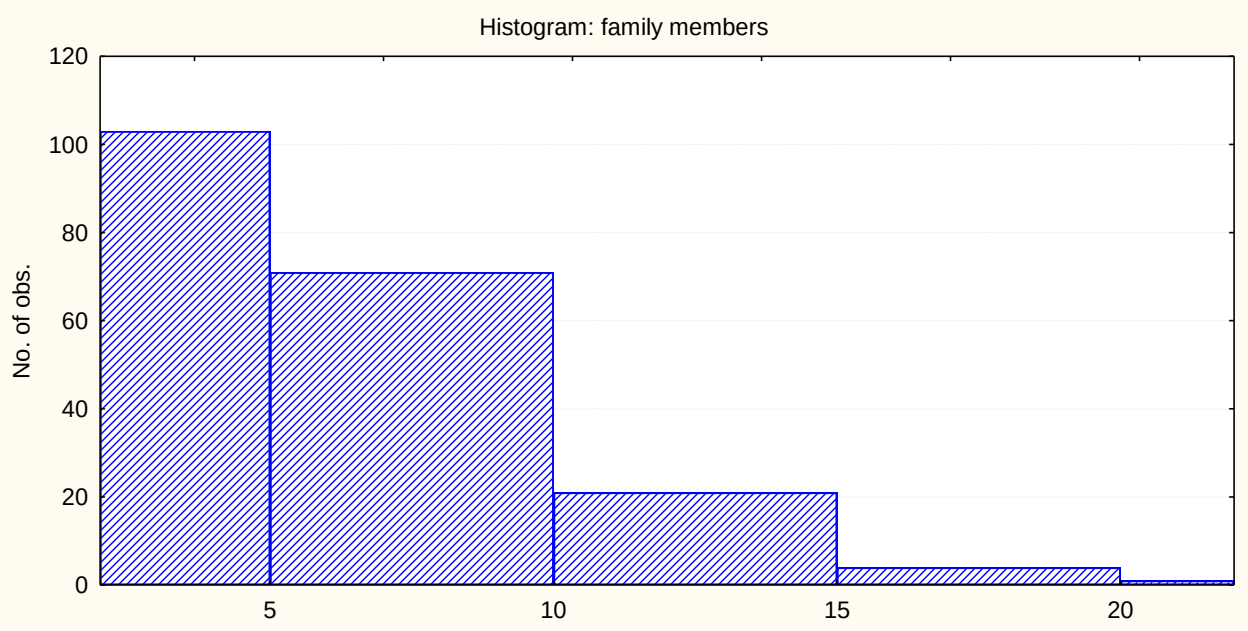


Figure 13b: Distribution according to number of family members



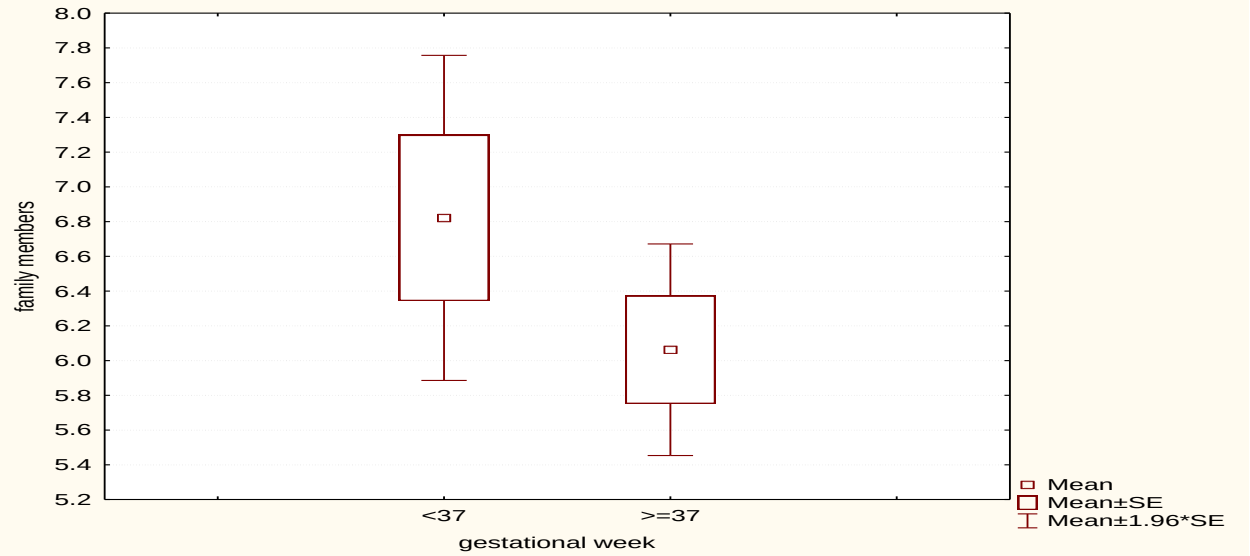
The average number of family members of patients with preterm-PROM was 6.8±3.6, and of patients with term-PROM 6.1±3.7 (Table 14 and Figure 14).

There was no statistically significant difference (p>0.05) between the average number of family members and gestational week when analyzed with the t-test (p=0.192637) (Table 14).

Table 14: Average number of family members according to registered preterm PROM and term Prom

Gestation week/	Mean <37	Mean >=37	t-value	p	Valid N	Valid N	Std.Dev.	Std.Dev.
family members	6.8	6.1	1.30726	0.192637	56	144	3.573168	3.728962

Figure 14: Average number of family members according to registered preterm PROM and term Prom



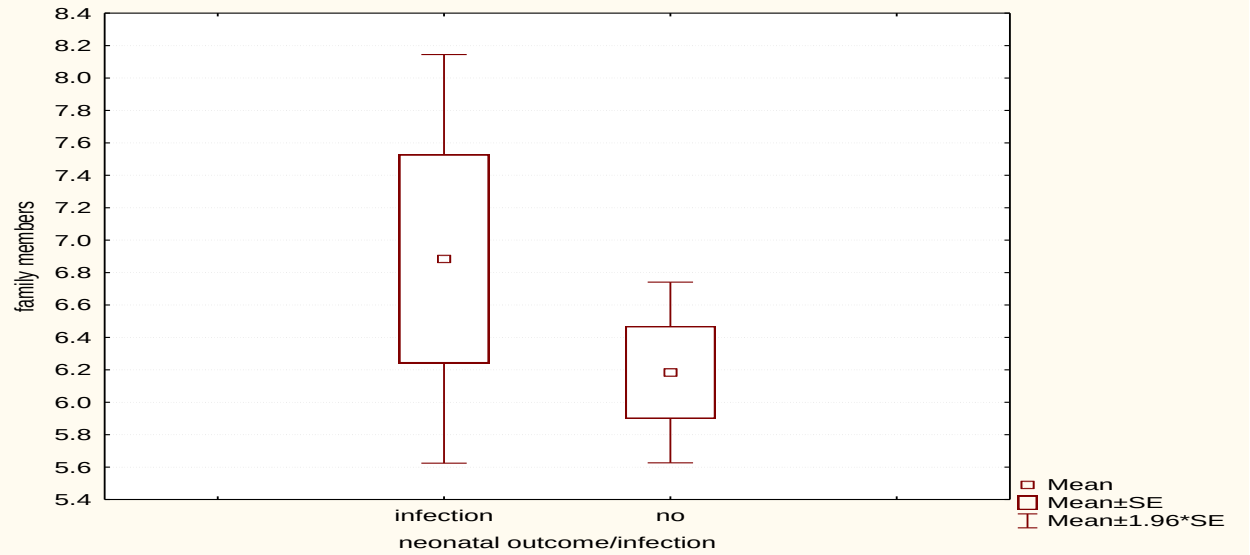
The average number of family members of patients with PROM who were registered to have neonatal infection was 6.9±3.3, and of patients with PROM who were without neonatal infection was 6.2±3.8 (Table 15 and Figure 15).

The t-test showed a statistically not significant difference (p>0.05) between the average number of family members and neonatal infection (p=0.368148) (Table 15).

Table 15: Average number of family members according to registered neonatal infection

Neonatal infection/ family members	Mean yes	Mean no	t-value	p	Valid N	Valid N	Std.Dev.	Std.Dev.
	6.9	6.2	0.90201	0.368148	26	174	3.278133	3.751004

Figure 15: Average number of family members according to registered neonatal infection



8.3. Risk factors for prelabour rupture of membranes of the patients presenting with PROM

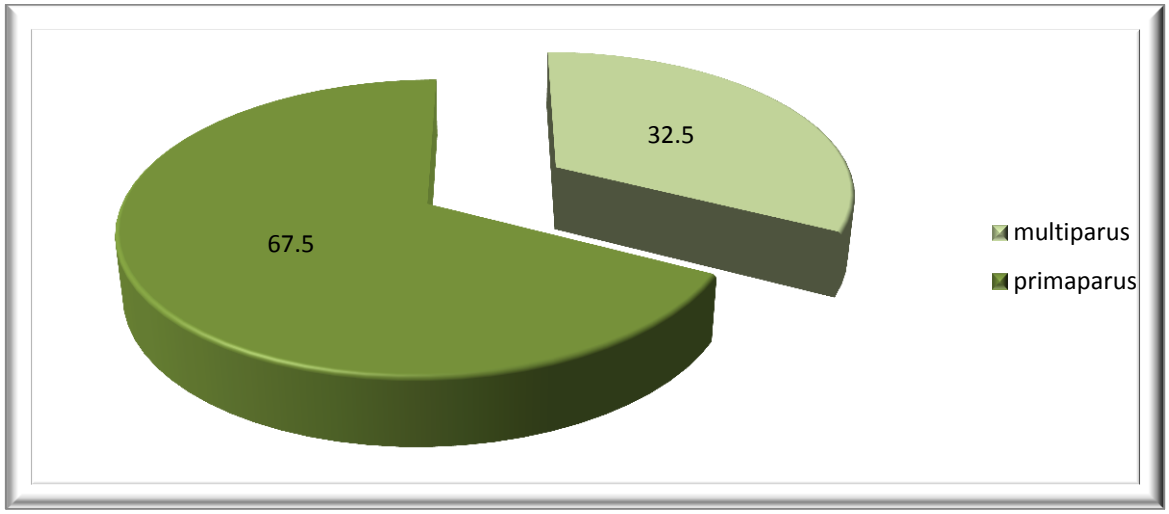
Out of 200 patients enrolled in this study, 67.5% were primiparous and 32.5% were multiparous (Table 16 and Figure 16).

Difference test showed that percentage difference registered between parity of patients with PROM primiparous and multiparous was statistically significant ($p<0.05$) ($p=0.0000$).

Table 16: Distribution of patients with PROM according to parity

parity	Count	Percent
multiparous	65	32,5
primiparous	135	67,5
total	**200**	**100.0**

Figure 16: Distribution of patients with PROM according to parity



The highest percentage (57.1%) of patients with PROM-preterm (<37gw) were primiparous, and 42.9% were multiparous (Table 17 and Figure 17).

Higher percentage (71.5%) of patients with PROM-term (>=37gw) were primiparous, and 28.5% were multiparous (Table 17 and Figure 17).

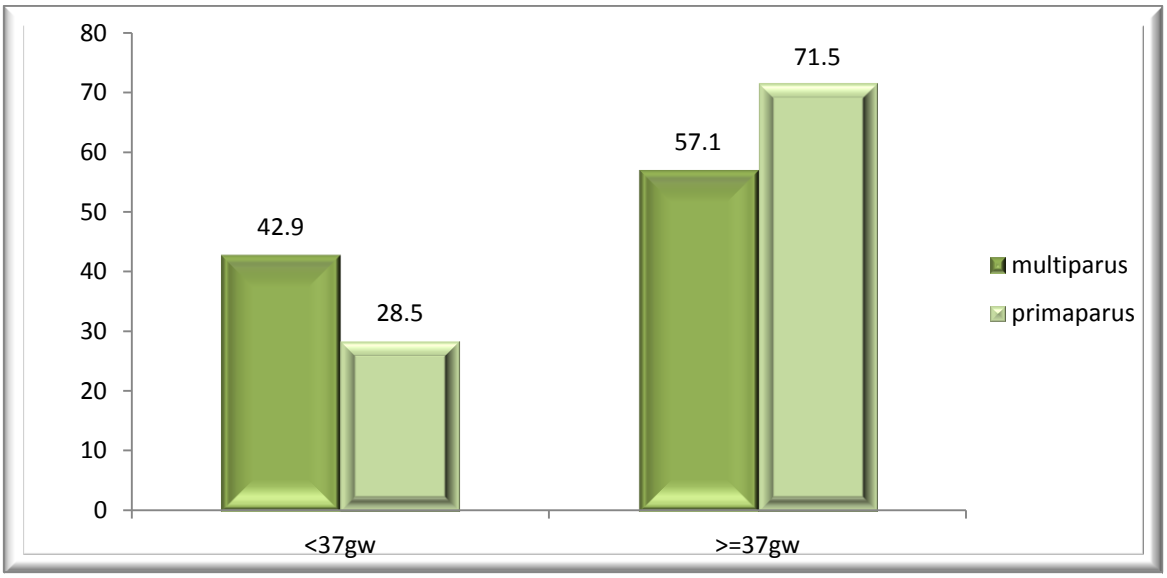
The percentage difference observed between the two groups was statistically significant for $p>0.05$ ($p=0.0524$).

No statistically significant association was registered between the modalities of parity in patients with preterm- and term-PROM (p>0.05) (Pearson Chi-square: 3.80319, df=1, p=0.051158).

Table 17: Distribution of patients according to parity and gestational age

parity	gestational age <37gw		gestational age >=37gw	
	Count	Percent	Count	Percent
multiparous	24	42.9	41	28.5
primiparous	32	57.1	103	71.5
total	**56**	**100.0**	**144**	**100.0**

Figure 17: Distribution of patients according to parity and gestational age



Neonatal infection was registered in 57.7% of patients with PROM who were multiparous and in 42.3% of primiparous patients (Table 18 and Figure 18).

Neonatal infection was not registered in 28.7% of patients with PROM who were multiparous and in 71.3% of primiparous patients (Table 18 and Figure 18).

The registered percentage difference between the two groups was statistically significant (p<0.05) (p=0.0036).

A statistically significant association was registered between the modalities of parity in patients with PROM and registered neonatal infection (p<0.05) (Pearson Chi-square: 8.64575, df=1, p=0.003279).

Table 18: Distribution of patients according to parity and registered neonatal infection

parity	neonatal outcome/infection yes		neonatal outcome/infection no	
	Count	Percent	Count	Percent
multiparous	15	57.7	50	28.7
primiparous	11	42.3	124	71.3
total	**26**	**100.0**	**174**	**100.0**

Figure 18: Distribution of patients according to parity and registered neonatal infection

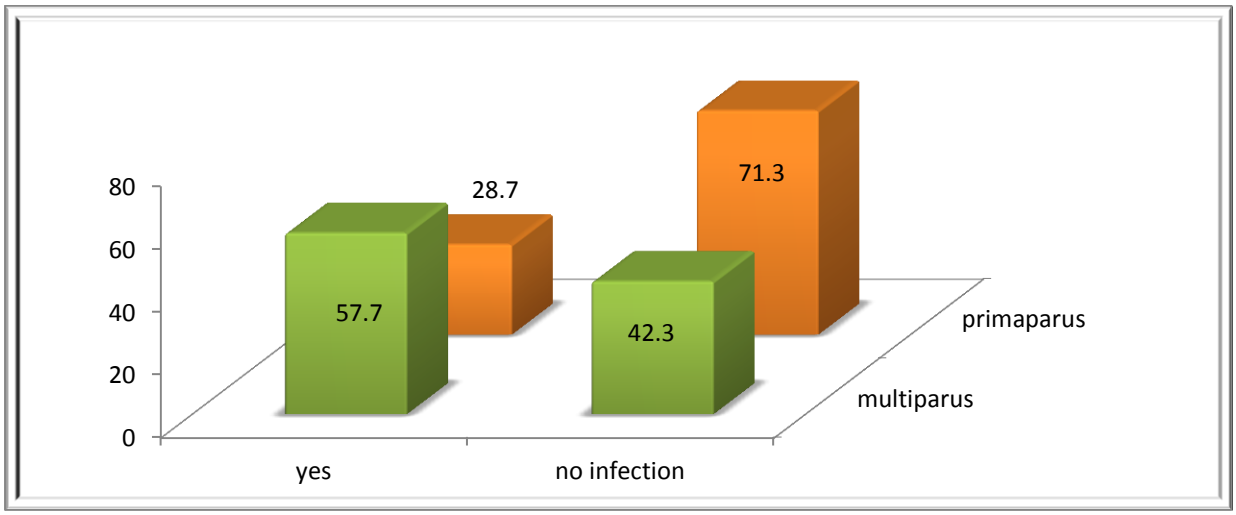
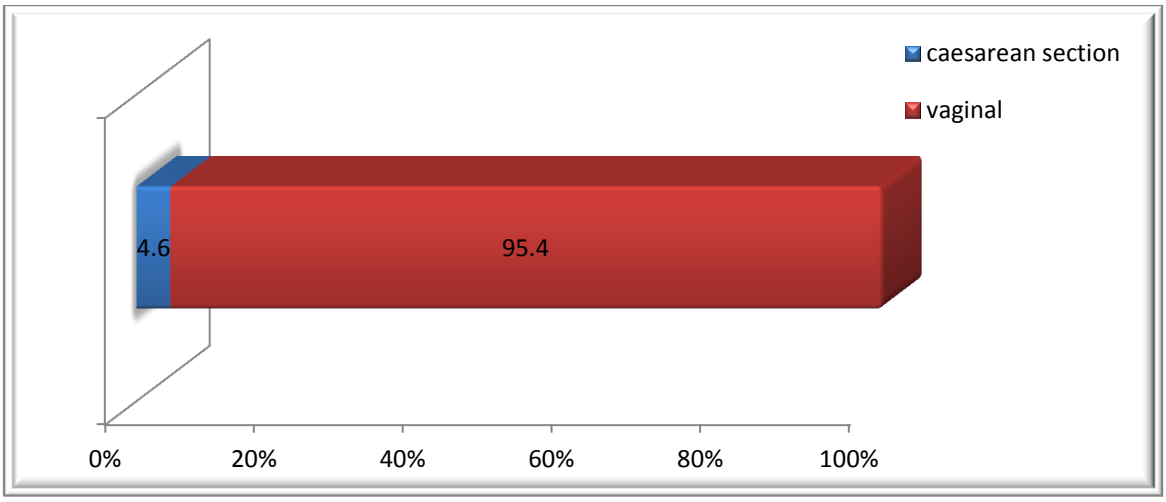


Table 19: Distribution of patients according to previous mode of delivery

previous mode of delivery	Count	Percent
vaginal	62	95.4
caesarean section	3	4.6
total	65	100.0

Figure 19: Distribution of patients according to previous mode of delivery



The previous mode of delivery was registered in 65 patients, of whom in 95.4% it was vaginal (Table 19 and Figure 19).

In patients with preterm-PROM (<37gw) the previous vaginal mode of delivery was registered in 95.8%, and only in one patient the previous mode of delivery was Caesarean section. In patients with term-PROM (>=37gw) the previous mode of delivery-vaginal was registered in 95.1%, and only in two patients the previous mode of delivery was caesarean section.

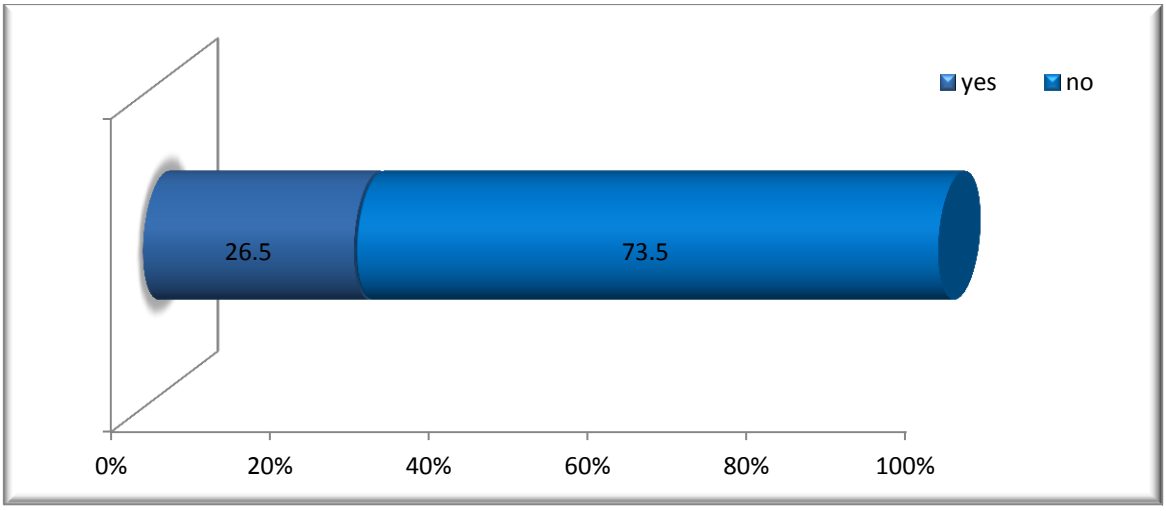
Difference test showed that percentage difference was statistically not significant (p>0.05) concerning the previous mode of delivery-vaginal between the two groups (p=0.5840).

No significant association was found between the previous mode of delivery of patients with preterm- and term-PROM (p>0.05) (Pearson Chi-square: 0.017402, df=1, p=0.895050).

Table 20: Distribution of patients according to previous abortion

previous abortions	Count	Percent
no	147	73,5
yes	53	26,5
total	200	100.0

Figure 20: Distribution of patients according to previous abortion



Previous abortion was registered in 53 (26.5%) patients (Table 20 and Figure 20); percentage difference was not statistically significant (p<0.05) between patients with PROM with or without abortion (p=0.0000).

In 28.6% of patients with preterm-PROM (<37gw) previous abortions were registered, and in 71.4% they were not registered (Table 21 and Figure 21).

In 25.7% of patients with term-PROM (>=37gw) previous abortions were registered, and in 74.3% they were not registered (Table 21 and Figure 21).

Difference test showed that percentage difference between the two groups was not statistically significant (p>0.05) concerning the previous abortions in relation to gestational weeks (p=0.6770).

No significant association was found concerning previous abortions in patients with preterm- and term-PROM (p>0.05) (Pearson Chi-square: 171341, df=1, p=0.678923).

Table 21: Distribution of patients according to previous abortions and gestational weeks

previous abortions	gestational week <37		gestational week >=37	
	Count	Percent	Count	Percent
no	40	71.4	107	74.3
yes	16	28.6	37	25.7
total	56	100.0	144	100.0

Figure 21: Distribution of patients according to previous abortions and gestational weeks

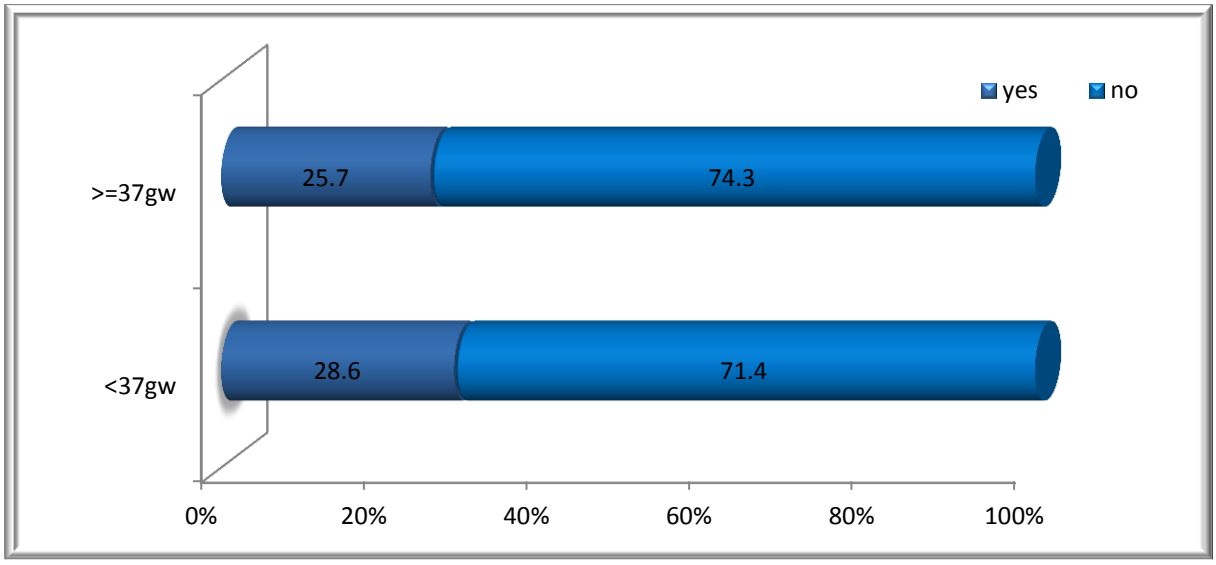
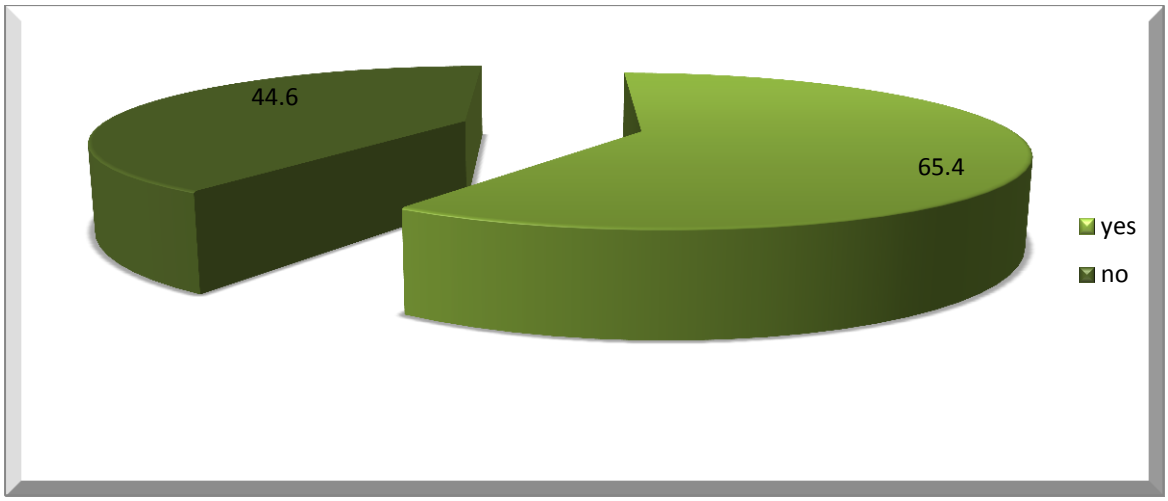


Table 22: Distribution of patients according to previous PROM

previous PROM	Count	Percent
yes	36	65.4
no	29	44.6
total	65	100.0

Figure 22: Distribution of patients according to previous PROM



Previous PROM was registered in 36 (65.4%) patients (Table 22 and Figure 22); percentage difference was statistically significant (p<0.05) between patients with PROM who were or were not previously found to have PROM (p=0.0186).

In 54.2% of patients with preterm-PROM (<37gw) previous PROM was registered, and in 45.8% of patients it was not registered (Table 23 and Figure 23).

In 56.1% of patients with term-PROM (>=37gw) previous PROM was registered, and in 43.9% of patients it was not registered (Table 23 and Figure 23).

Difference test showed that percentage difference between the two groups was not statistically significant (p>0.05) concerning previous PROM in relation to gestational weeks (p=0.8822).

No significant association was found concerning previous PROM in patients with preterm- and term-PROM (p>0.05) (Pearson Chi-square: 0.022842, df=1, p=0.879870).

Table 23: Distribution of patients according to previous PROM and gestational week

previous PROM	gestational week <37		gestational week >=37	
	Count	Percent	Count	Percent
yes	13	54.2	23	56.1
no	11	45.8	18	43.9
total	24	100.0	41	100.0

Figure 23: Distribution of patients according to previous PROM and gestational week

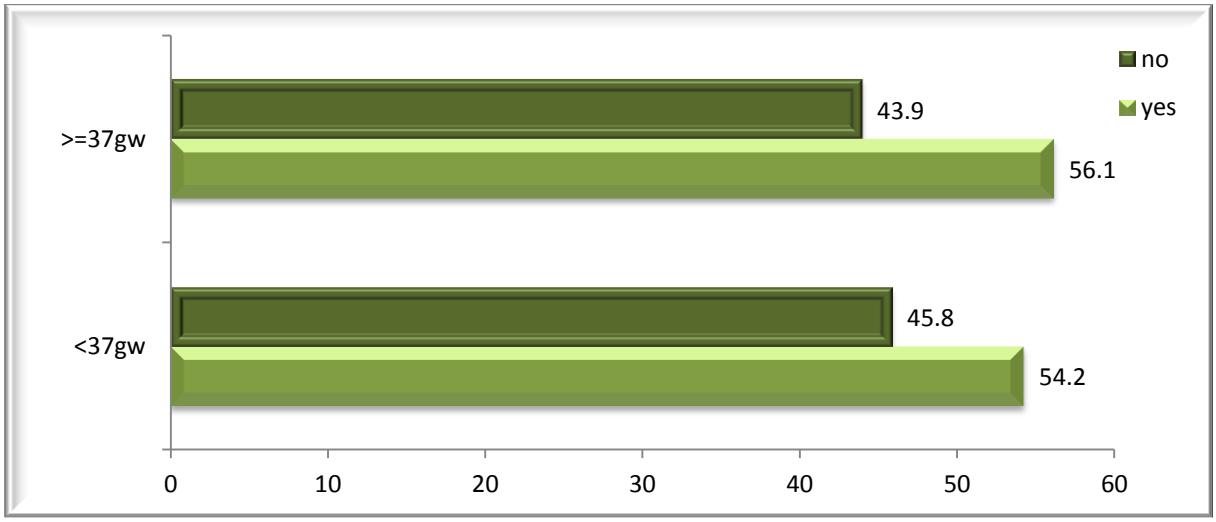
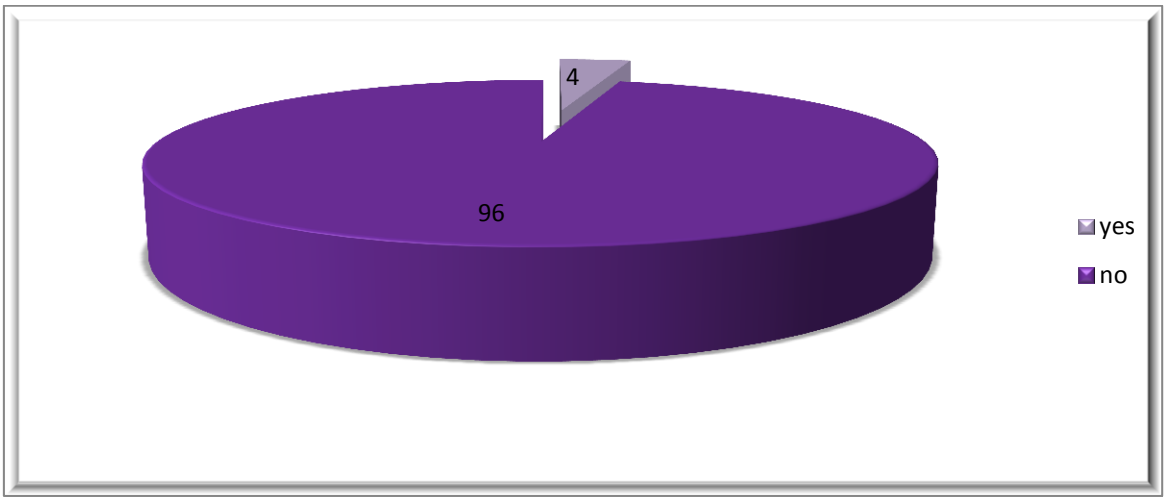


Table 24: Distribution of patients according to previous intervention in uterus

previous intervention in uterus	Count	Percent
no	192	96,0
yes	8	4,0
total	200	100.0

Figure 24: Distribution of patients according to previous intervention in uterus



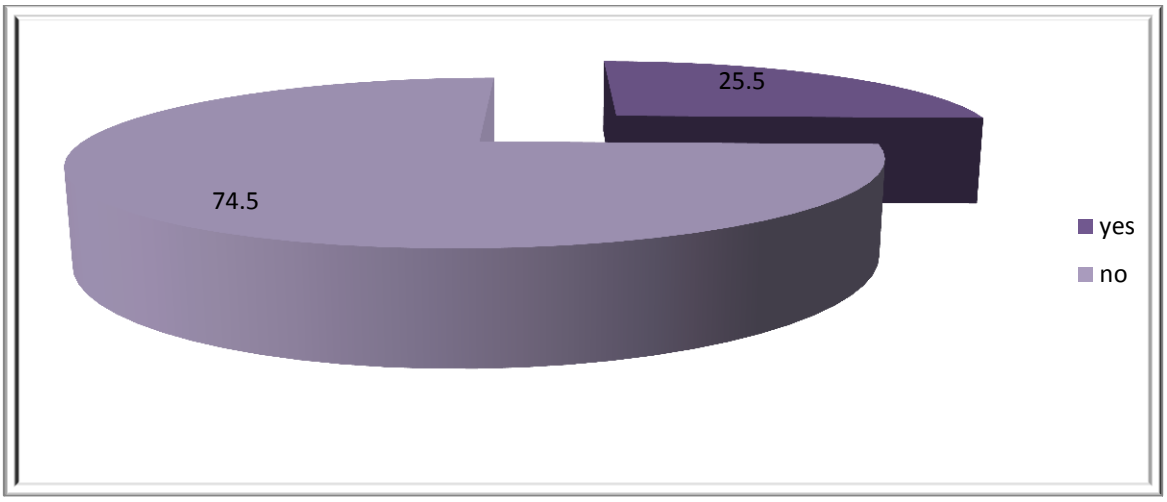
Eight (4.0%) patients had previous intervention in uterus, and 96.0% of patients did not undergo such intervention (Table 24 and Figure 24); percentage difference between those patients with PROM who had previous intervention in uterus and those who had no such intervention was statistically significant ($p<0.05$) ($p=0.00000$).

Previous intervention in uterus had 2 (3.6%) patients with preterm-PROM (<37gw) and 6 (4.2%) patients with term-PROM (>=37gw), respectively.

Table 25: Distribution of patients with PROM in relation to smoking

smoking	Count	Percent
yes	51	25,5
no	149	74,5
total	200	100.0

Figure 25 : Distribution of patients with PROM in relation to smoking



25.5% of patients with PROM were smokers and 74.5% were non-smokers (Table 25 and Figure 25); percentage difference between patients with PROM who smoke and those who did not smoke ($p<0.05$) ($p=0.0000$) was statistically significant.

21.4% of patients with preterm-PROM (<37gw) were smokers, and 79.6% were non-smokers (Table 26 and Figure 26).

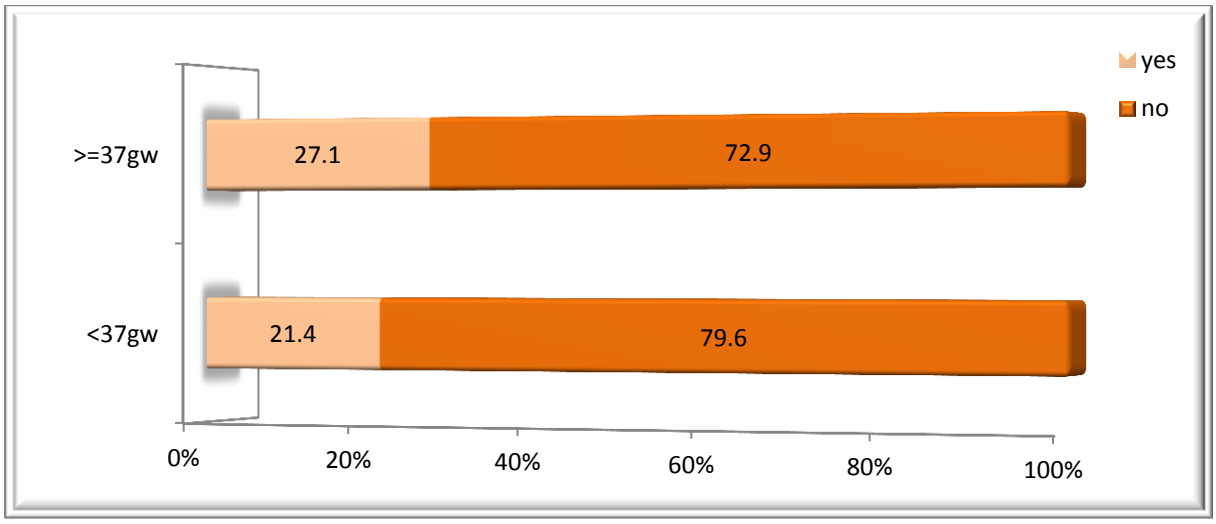
27.1% of patients with term-PROM (>=37gw) were smokers, and 72.9% were non-smokers (Table 26 and Figure 26).

Difference test showed that percentage difference between the two groups was not statistically significant ($p>0.05$) concerning smoking in relation to gestational week ($p=0.4073$). No significant association ($p>0.05$) was found concerning smoking habits in patients with preterm- and term-PROM (Pearson Chi-square: 0 .678661, df=1, p=0.410050).

Table 26: Distribution of patients according to smoking and gestational week

smoking	gestational week <37		gestational week >=37	
	Count	Percent	Count	Percent
yes	12	21.4	39	27.1
no	44	79.6	105	72.9
total	56	100.0	144	100.0

Figure 26: Distribution of patients according to smoking and gestational week



8.4. Antenatal care practices

The average number of ultrasound visits of patients with PROM was 7±2.0, minimum 2 and maximum 14 visits (Table 27 and Figure 27a).

The highest percentage of 44.0% patients had from 6 to 8 ultrasound examinations. (Figure 27 b).

Table 27: Average number of ultrasound scans of patients with PROM

	Valid N	Mean	Minimum	Maximum	Std.Dev.
no of ultrasound visits	200	7,02	2,00	14,0	1,982233

Figure 27a: Average number of ultrasound scans of patients with PROM.

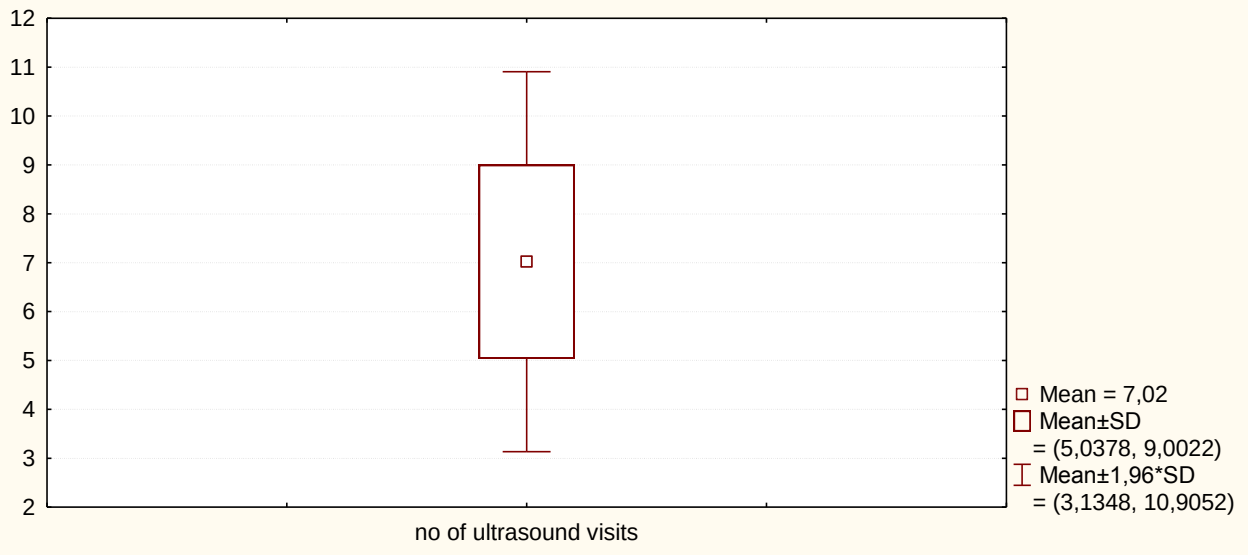


Figure 27b: Number of ultrasound scans of patients with PROM

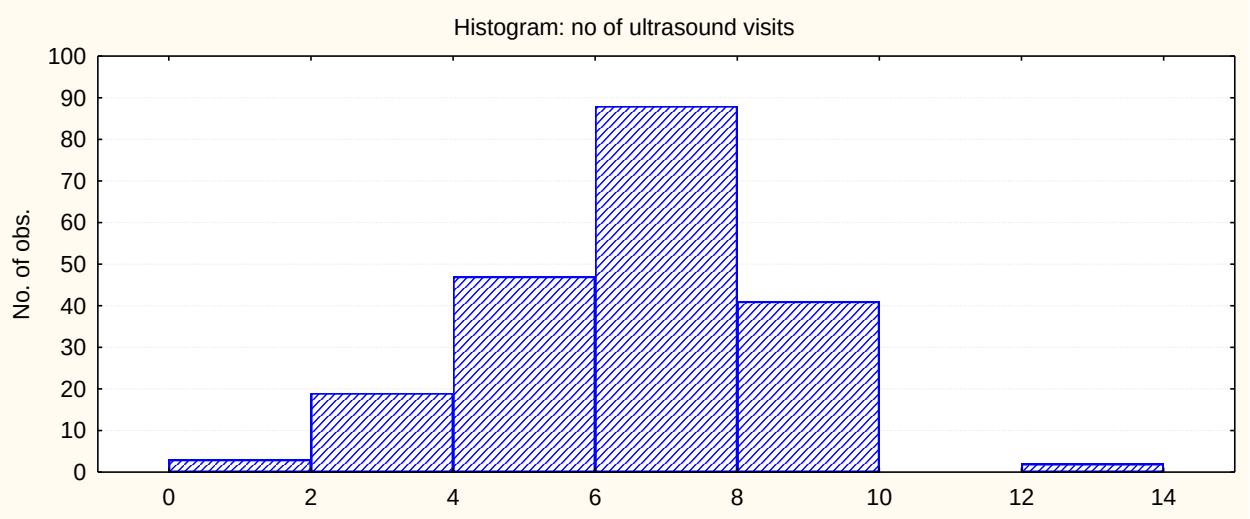
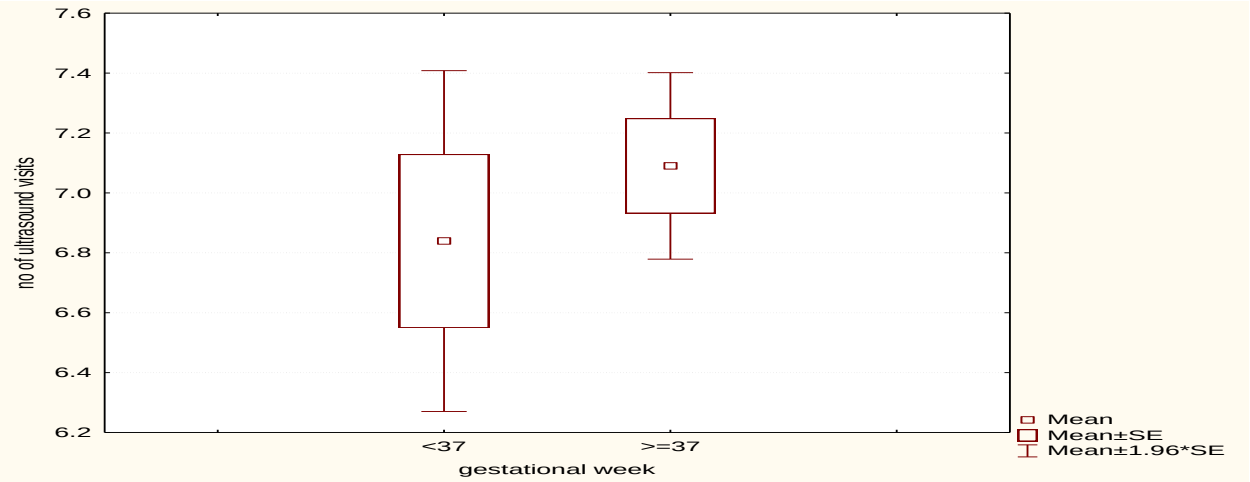


Table 28: Average number of ultrasound scans of patients with PROM according to gestational age

	Mean <37	Mean >=37	t-value	p	Valid N	Valid N	Std.Dev.	Std.Dev
Gestation weeks/ no of ultrasound visits	6.8	7.1	-0.803301	0.422764	56	144	2.17236	1.90660

Figure 28: Average number of ultrasound scans of patients with PROM according to gestational age



The average number of ultrasound examinations of patients with PROM according to gestational week <37 was 6.8±2.1, and of patients at gestational week >=37 it was 7.1±1.9; t-

test analysis showed no statistically significant difference ($p>0.05$) ($p=0.422764$) (Table 28 and Figure 28).

Table 29: Average number of gynaecological exams of patients with PROM

	Valid N	Mean	Minimum	Maximum	Std.Dev.
no of gynaecological exams	200	1,6	0,0	5,0	1,223719

Figure 29a: Average number of gynaecological exams of patients with PROM

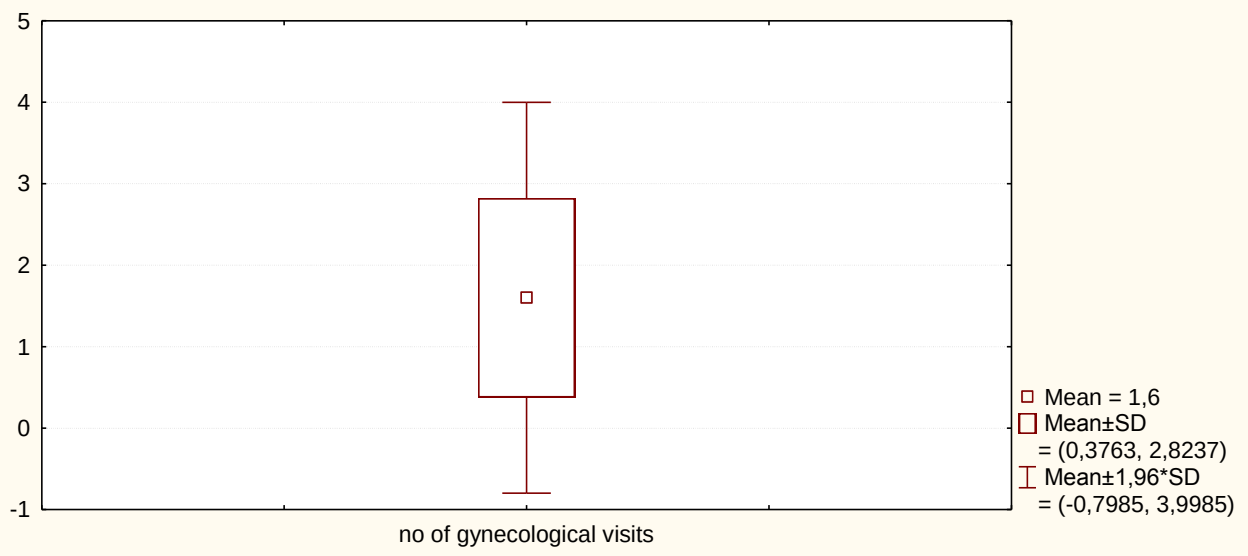
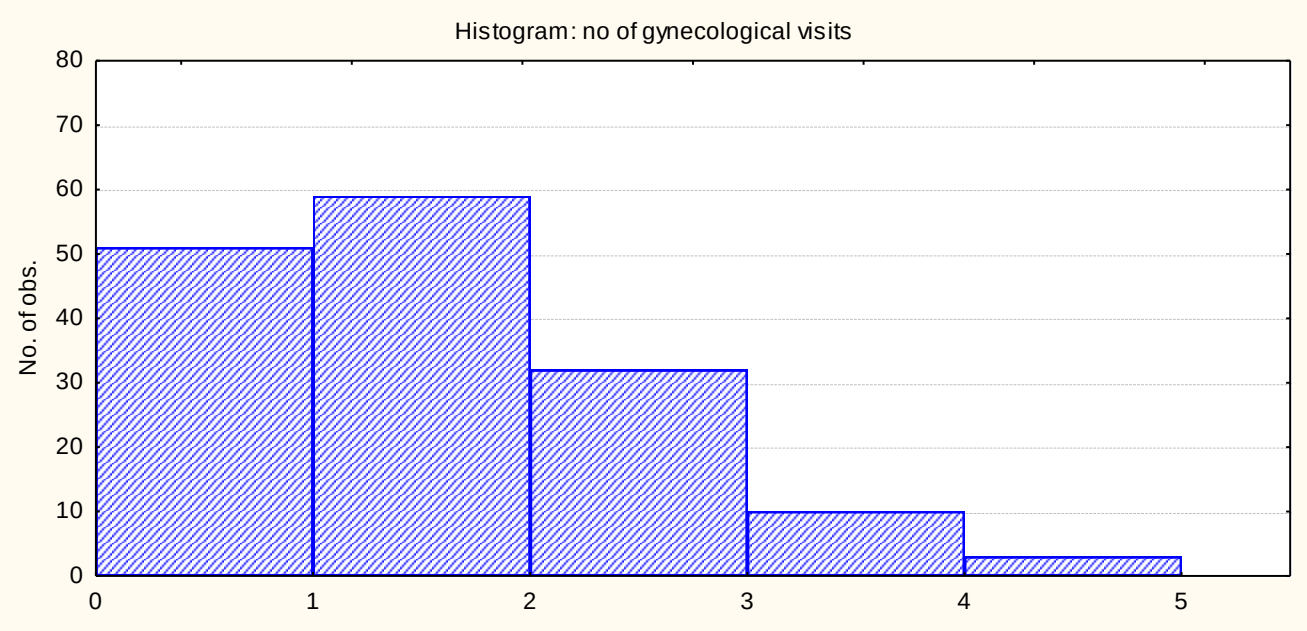


Figure 29b: Average number of gynaecological exams of patients with PROM



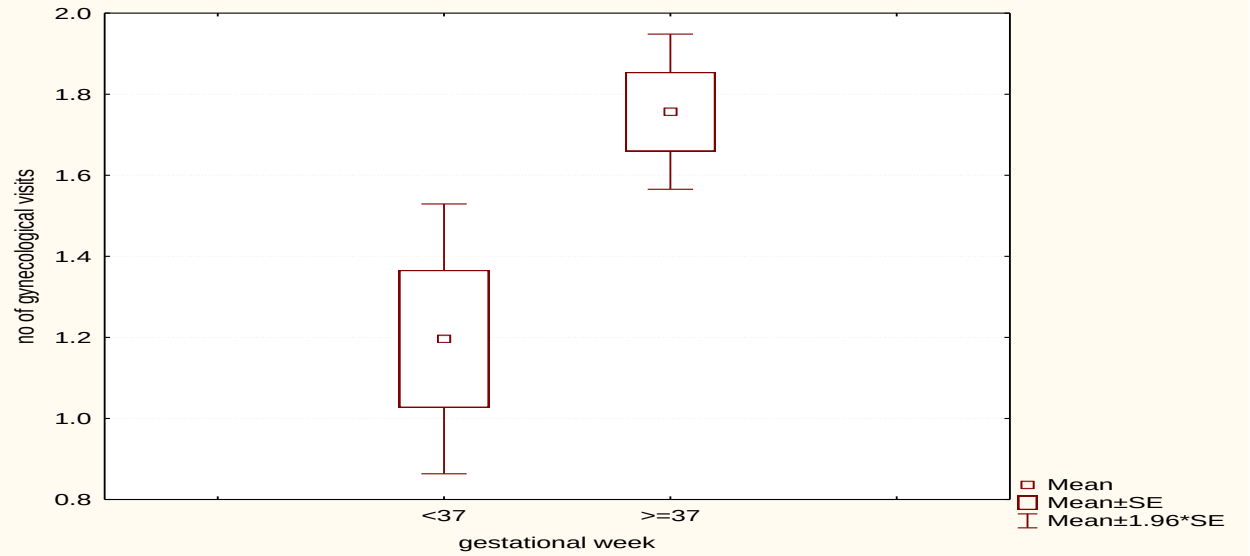
The average number of gynaecological exams of patients with PROM was 7±2.0, minimum 0, and maximum 5 visits (Table 29 and Figure 29a).

One to two gynaecological exams accounted for the registered highest percentage (29.5%), followed by one (25.5%) exam, then 22.5% accounted for no vaginal exams etc. (Figure 29b).

Table 30: Average number of gynaecological exams of patients with PROM according to gestational age

Gestation weeks/ no of gynecolog. exams	Mean <37	Mean >=37	t-value	p	Valid N	Valid N	Std.Dev.	Std.Dev
	1.2	1.8	-2.96487	0.003400	56	144	1.270929	1.17221

Figure 30: Average number of gynaecological exams of patients with PROM according to gestational age

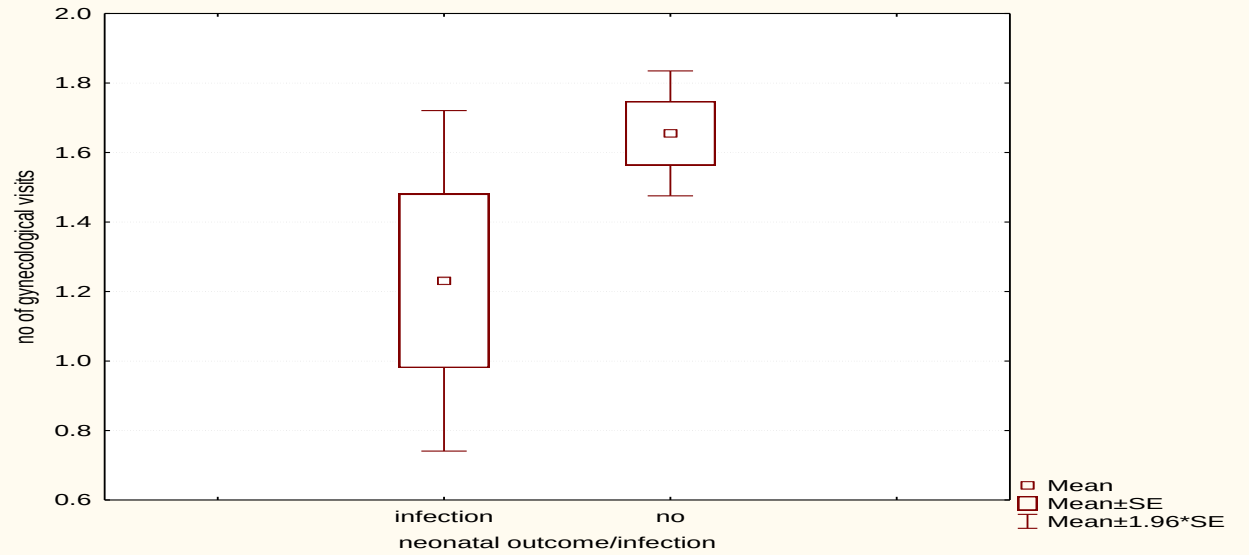


The average number of gynaecological exams of patients with PROM according to gestational week <37 was 1.2±1.3, whereas the average number of gynaecological visits was higher in patients at >=37 gestational week (1.8±1.2); t-test analysis showed a statistically significant difference between the two groups ($p<0.05$) ($p=0.003400$) (Table 30 and Figure 30).

Table 31: Average number of gynaecological exams of patients with PROM according to neonatal infection

Neonatal / no of gynaecolo. exams	Mean infection	Mean no	t-value	p	Valid N	Valid N	Std.Dev.	Std.Dev.
	1.2	1.6	-1.65668	0.099168	26	174	1.274604	1.210050

Figure 31: Average number of gynaecological exams of patients with PROM according to neonatal infection

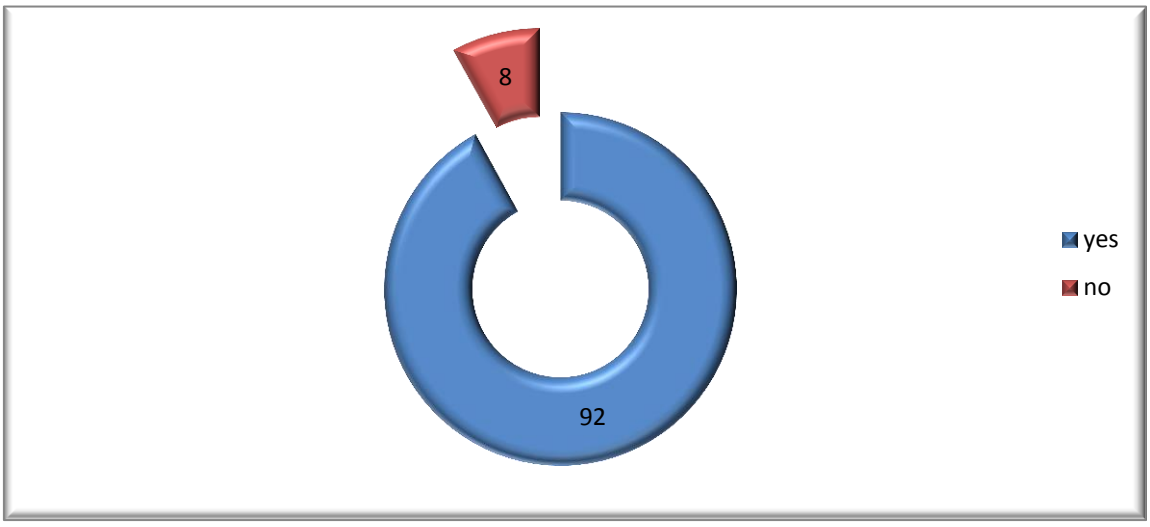


The average number of gynaecological exams of patients with PROM according to registered neonatal infection was 1.2±1.2, whereas in patients with no neonatal infection the number was higher (1.6±1.2); t-test analysis showed a statistically not significant difference between the two groups (p>0.05) (p=0.099168) (Table 31 and Figure 31).

Table 32: Distribution of patients with PROM in relation to intake of folic acid supplement

Folic acid supplementation	Count	Percent
yes	184	92,00000
no	16	8,00000
total	**200**	**100.0**

Figure 32: Distribution of patients with PROM in relation to intake of folic acid supplement

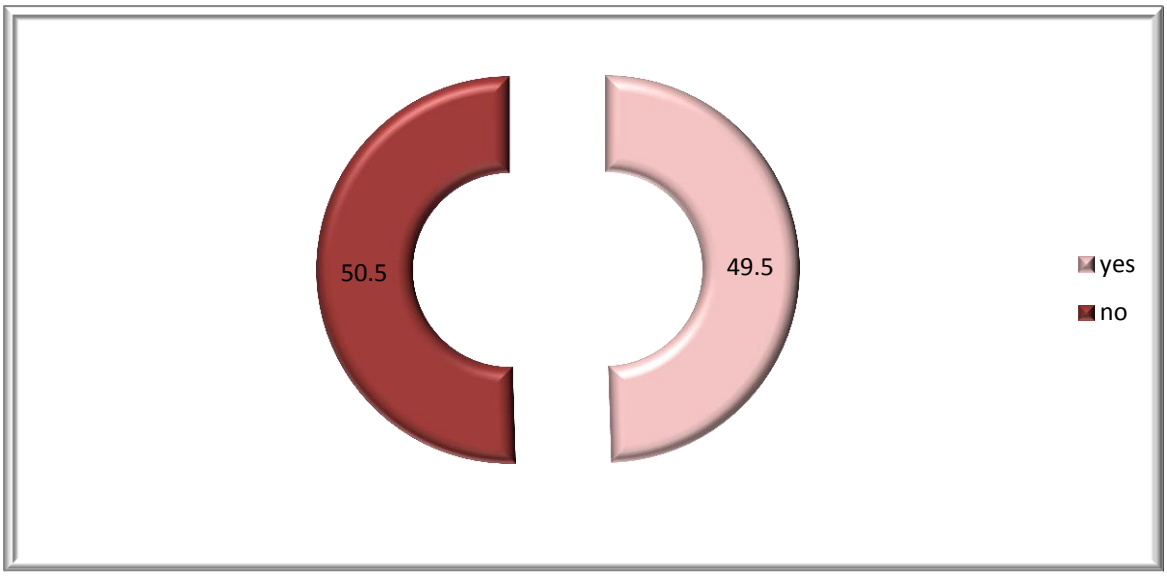


Folic acid supplement was taken by 92.0% of patients with PROM, and only 8.0% did not take it (Table 32 and Figure 32).

Table 33: Distribution of patients with PROM with regard to genital infection screening

genital infection screening	Count	Percent
yes	99	
no	101	50,5
total	**200**	**100.0**

Figure 33: Distribution of patients with PROM with regard to genital infection screening



Genital infection screening was performed in 49.5% of patients with PROM, and 50.5% of patients did not undergo this screening (Table 33 and Figure 33).

Genital infection screening was performed in 32.1% of patients with preterm-PROM, and in 56.3% of patients with term-PROM; there was a statistically significant percentage difference ($p<0.05$) ($p=0.0024$).

A statistically significant association was found between the performed genital infection screening and weeks of gestation (Pearson Chi-square: 9.37379, df=1, $p=0.002202$).

Genital infection screening was performed in 30.8% of patients with registered neonatal infection, and in 56.3% of patients without neonatal infection; the percentage difference was statistically significant ($p<0.05$) ($p=0.00424$).

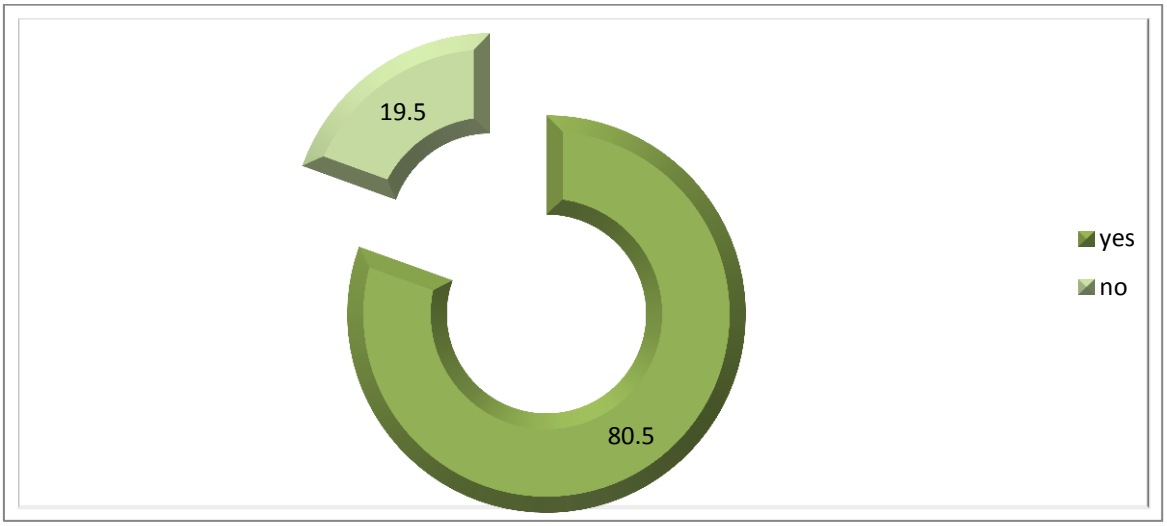
A statistically significant association was found between the performed genital infection screening and registered neonatal infection (Pearson Chi-square: 4.19439, df=1, $p=0.040561$).

Anemia screening was made in 80.5% of patients with PROM, and 19.5% of patients did not undergo this screening (Table 34 and Figure 34).

Table 34: Distribution of patients with PROM in relation to aneemia screening

anaemia screening	Count	Percent
yes	161	80,5
no	39	19.5
total	**200**	**100.0**

Figure 34: Distribution of patients with PROM in relation to anemia screening



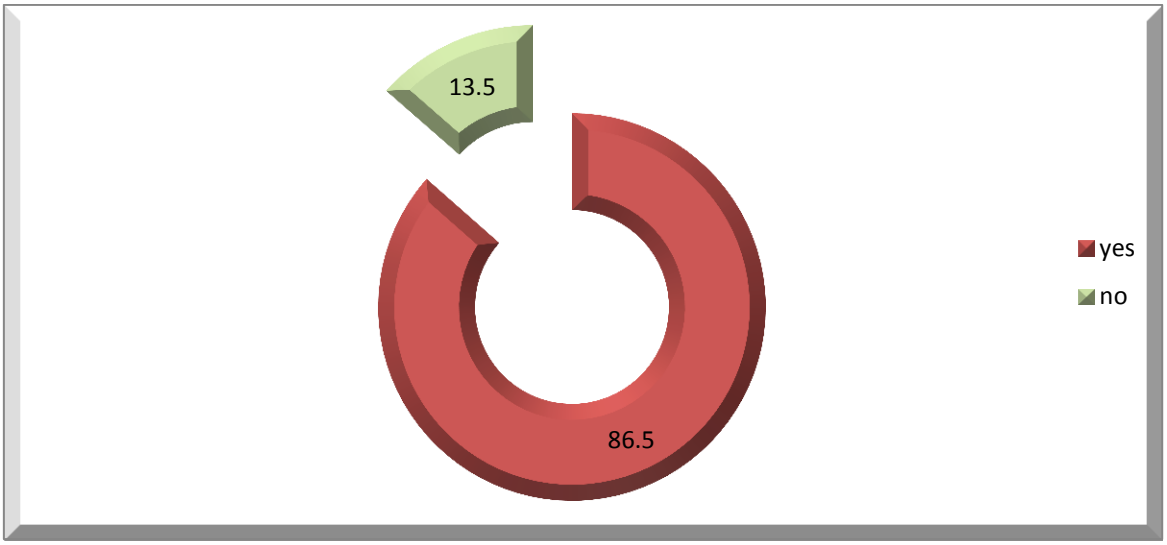
Follow-up of the blood pressure was made in 86.5% of patients with PROM, and there was not such follow-up in 13.5% of patients (Table 35 and Figure 35).

Follow-up of the blood pressure was made in 82.1% of patients with preterm-PROM, and in 88.2% of patients with term-PROM; the registered percentage difference was not statistically significant (p>0.05) (p=0.2585).

Table 35: Distribution of patients with PROM in relation to blood pressure follow-up

Blood pressure follow up	Count	Percent
yes	173	86,5
no	27	13,5
total	**200**	**100.0**

Figure 35: Distribution of patients with PROM in relation to blood pressure follow-up



Weight follow-up was conducted in 48.0% of patients with PROM, and there was no such follow-up in 52.0% of patients (Table 36 and Figure 36).

Weight follow-up was made in 26.8% of patients with preterm-PROM, and in 54.9% of patients with term-PROM; the registered percentage difference was statistically significant (p<0.05) (p=0.0004)

Table 36: Distribution of patients with PROM in relation to weight follow-up

BMI (weight follow up)	Count	Percent
yes	96	48,0
no	104	52,0
total	**200**	**100.0**

Figure 36: Distribution of patients with PROM in relation to weight follow-up

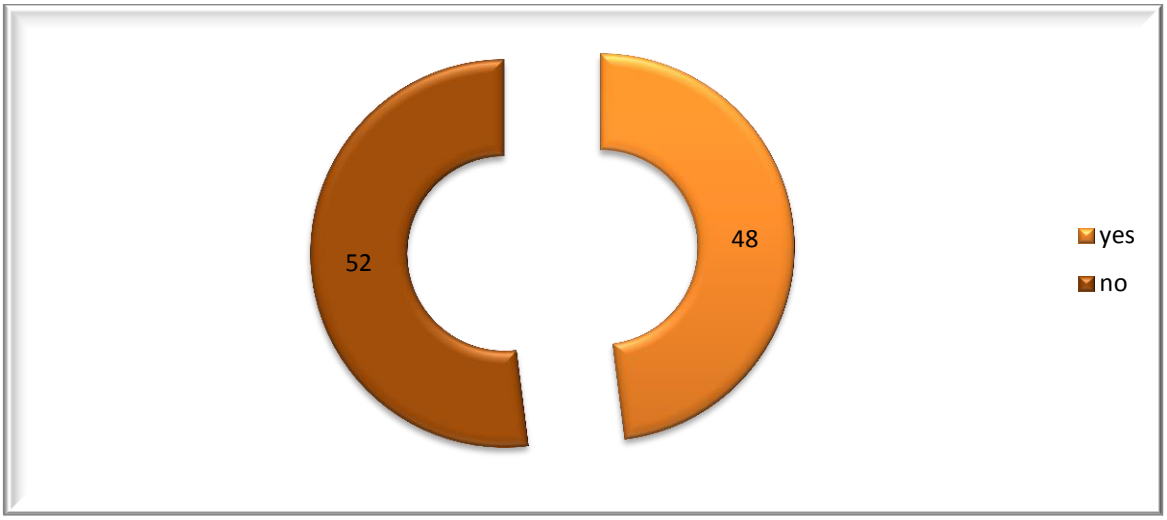
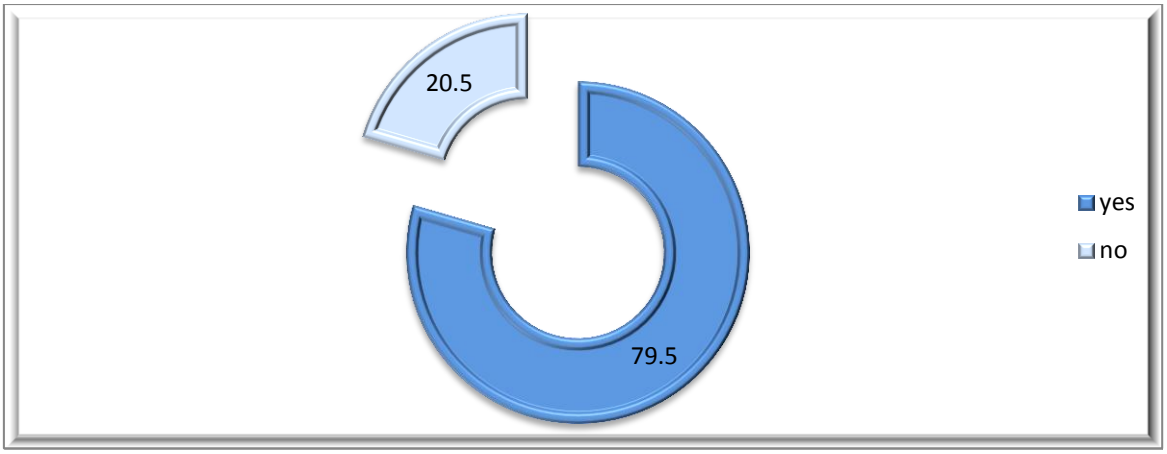


Table 37: Distribution of patients with PROM in relation to urinary infection screening

urinary infection screening	Count	Percent
yes	159	79,5
no	41	20,5
total	**200**	**100.0**

Figure 37: Distribution of patients with PROM in relation to urinary infection screening



Urinary infection screening was made in 79.5% of patients with PROM, and 20.5% of patients did not undergo this screening (Table 37 and Figure 37).

Urinary infection screening was performed in 60.7% of patients with preterm-PROM, and in 86.8% of patients with term-PROM; the registered percentage difference was statistically significant ($p<0.05$) ($p=0.0001$).

A statistically significant association was found between the performed urinary infection screening and gestational weeks (Pearson Chi-square: 16.8419, df=1, $p=0.000041$).

Urinary infection screening was made in 46.2% of patients with registered neonatal infection and in 84.5% of patients without neonatal infection; the registered percentage difference was statistically significant ($p<0.05$) ($p=0.0000$).

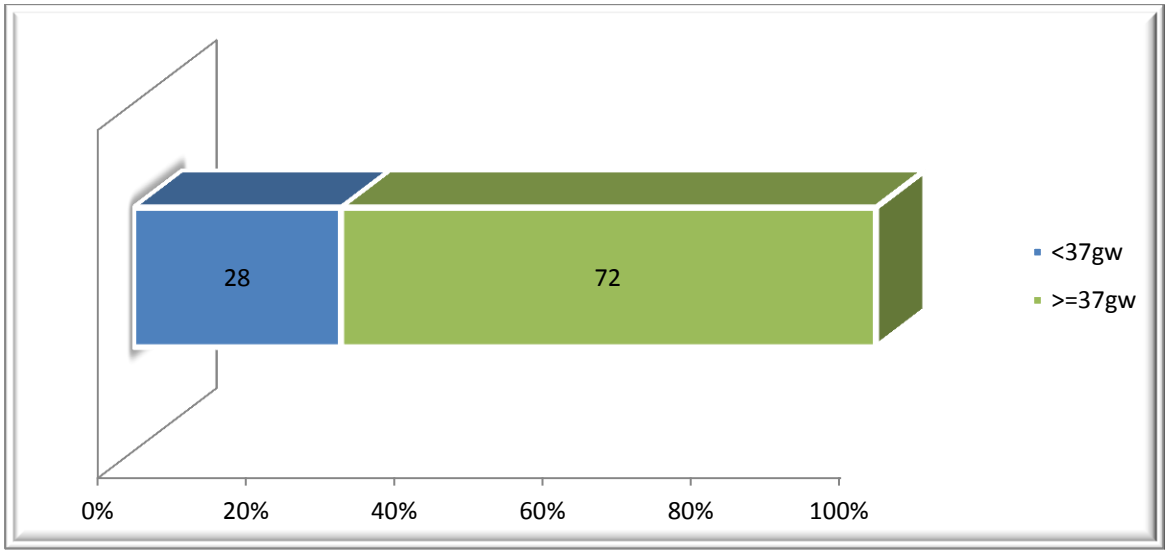
A statistically significant association was found between the performed urinary infection screening and registered neonatal infection (Pearson Chi-square: 20.3903, df=1, $p=0.000006$).

8.5. Mode of delivery of the patients presenting with PROM

Table 38: Distribution of patients with PROM in relation to gestational week

gestational week	Count	Percent
<37	56	28.0
>=37	144	72.0
total	200	100.0

Figure 38: Distribution of patients with PROM in relation to gestational week



Fifty-six of patients with PROM had preterm delivery (<37gw), and 144 of patients with PROM had term delivery (>=37gw) (Table 38 and Figure 38).

Mean interval from PROM to delivery was 24.8±14.3 hours, minimum 7, and maximum 70 hours (Table 39).

Mean interval from PROM to delivery in patients with preterm-PROM (<37gw) was 29.0±18.2 hours, and mean interval from PROM to delivery in patients with term-PROM- (>=37gw) was shorter (23.2±12.20 hours) (Table 39 and Figure 39a). According to t-test the difference was statistically significant ($p<0.05$) ($p=0.009630$) (Table 40).

Mean interval from PROM to delivery in patients with registered neonatal infection was 34.7±20.3 hours, and mean interval in the group without neonatal infection was 23.3±12.6 hours (Table 39 and Figure 39b). According to t-test the difference was statistically significant ($p<0.05$) ($p=0.000135$) (Table 40).

Table 39: Mean interval from PROM to delivery

	Valid N	Mean	Minimum	Maximum	Std.Dev.
PROM to delivery time	200	24.8	7.0	70.0	14.3
PROM to delivery time/ gestational week<37	56	29.0	8.0	70.0	18.2
PROM to delivery time/ gestational week>=37	143	23.2	7.0	66.0	12.2
PROM to delivery time/ neonatal infection	26	34.7	8.0	70.0	20.3
PROM to delivery time/ no neonatal infection	174	23.3	7.0	68.0	12.6

Figure 39a: Mean interval from PROM to delivery according to gestational week

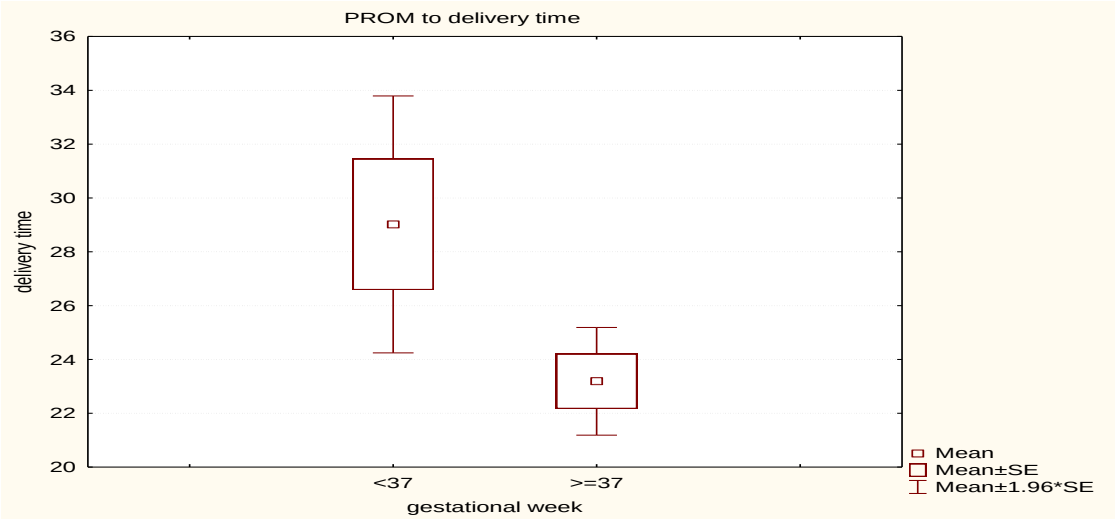


Figure 39b: Mean interval from PROM to delivery according to neonatal infection

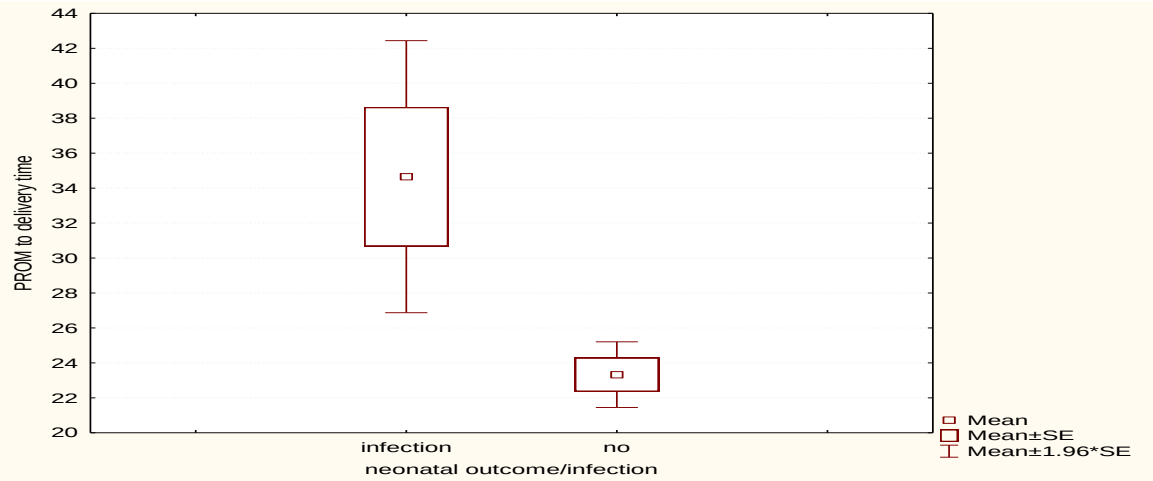


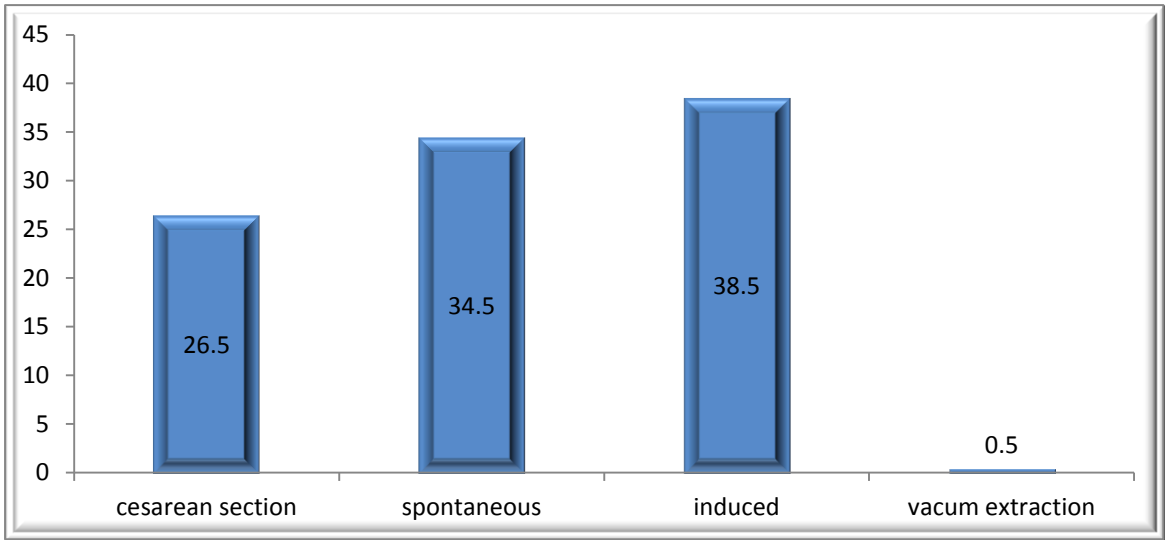
Table 40: Results of the t-test

PROM to delivery time	t-value	p
Gestation weeks	2.614382	0.009630
Neonatal infection	3.893129	0.000135

Table 41: Distribution of patients with PROM according to mode of delivery

Mode of delivery birth	Count	Percent
Cesarean Section	53	26.5
Vaginal spontaneous	69	34.5
Vaginal induced	77	38.5
Vacum extraction	1	0.5
total	200	100.0

Figure 40: Distribution of patients with PROM according to mode of delivery



The highest percentage of deliveries (34.5%) in patients with PROM was with induction, 34.5% were spontaneous deliveries, and 26.5% with Caesarean section (Table 41 and Figure 40).

Table 42 : Distribution of mode of delivery according to gestational age in patients with PROM.

Birth/>=37	Count	Percent
Cesarean Section	37	25.5
Vaginal spontaneous	42	29.0
Vaginal induced	65	44.8
Vacum extraction	1	0.7
total	145	100.0
Birth/<37	**Count**	**Percent**
Cesarean Section	16	29.1
Vaginal spontaneous	27	49.1
Vaginal induced	12	21.8
total	**55**	**100.0**

The highest percentage of deliveries (44.8%) in patients with term-PROM was by vaginal induction, and 49.1% of patients with term-PROM had vaginal spontaneous delivery (Table 42).

A statistically significant association was found between the mode of delivery and gestational week (Pearson Chi-square: 10.9533, df=3, p=0.011984).

Table 43: Distribution of mode of delivery according to neonatal infection in patients with PROM

Birth/neonatal infection yes	Count	Percent
Cesarean Section	7	26.9
Vaginal spontaneous	12	46.2
Vaginal induced	7	26.9
total	26	100.0
Birth /neonatal infection no	**Count**	**Percent**
Cesarean Section	46	26.4
Vaginal spontaneous	57	32.8
Vaginal induced	70	40.2
Vacum extraction	1	0.6
total	**174**	**100.0**

The highest percentage of deliveries (46.2%) in patients who had neonatal infection was vaginal spontaneous, and 40.2% of patients without neonatal infection had vaginal induced delivery (Table 43).

No statistically significant association was found between the mode of delivery and presence of neonatal infection (Pearson Chi-square: 2.36824, df=3, p=0.499576).

Table 44: Indications for Caesarean section according to gestational age

CS indication / ≥ 37 weeks of gestation	Count	Percent
prolonged spasmodic labour	5	13.9
fetal distress	3	8.3
breech presentation	2	5.6
previous cesarean delivery	2	5.6
failed induction	5	13.9
chorioamnionitis	4	11.1
cephalopelvic disproportion	13	36.1
presentatio recta captitis superior	1	2.8
Pelvis angusta	1	2.8
total	36	100.0
CS indication/<37 weeks of gestation	**Count**	**Percent**
prolonged spasmodic labour	4	25.0
fetal distress	5	31.25
breech presentation	1	6.25
previous cesarean delivery	1	6.25
chorioamnionitis	1	6.25
cephalopelvic disproportion	1	6.25
placental abruption	1	6.25
old primiparous (primipara vetusta)	1	6.25
total	17	100.0

The most common indication for Caesarean section in patients with term-PROM was cephalopelvic disproportion (36.1%), followed by prolonged spasmodic labour and failed induction (13.9 each), then chorioamnionitis (11.1%), fetal distress (8.3%), and breech presentation and previous Cesarean section (5.6%). The remaining indications were found in one patient each (Table 44).

The indication for Caesarean section in patients with preterm-PROM was fetal distress in the highest percentage (31.25%), followed by spasmodic prolonged labour (25.0%), and the remaining indications were found in one patient each (Table 44).

The vaginal swab result was positive in 31.0% of patients. In our culture positive patients (n=61), Staphyolococcus aureus was the most commonly cultured microorganism in 31.1 % of cases, followed by enterococcus in 22.9%, candida species in 19.7%, streptococcus in 18.0%, and Escherichia colli in 8.2% (Table 45 and Figure 41).

A statistically significant association was found between the vaginal swab result and presence of neonatal infection (Pearson Chi-square: 35.6266, df=1, p=0.000000) (Table 46).

Table 45: Distribution of patients with PROM according to vaginal swab results

Vaginal swab	Count	Percent
sterile	139	69.5
positive	61	30.5
total	200	100.0
enterococcus	14	22.9
candida species	12	19.7
escherichia coli	5	8.2
streptococcus	11	18.0
staphylococcus aureus	19	31.1
total	61	100.0

Figure 41: Distribution of patients with PROM according to vaginal swab results

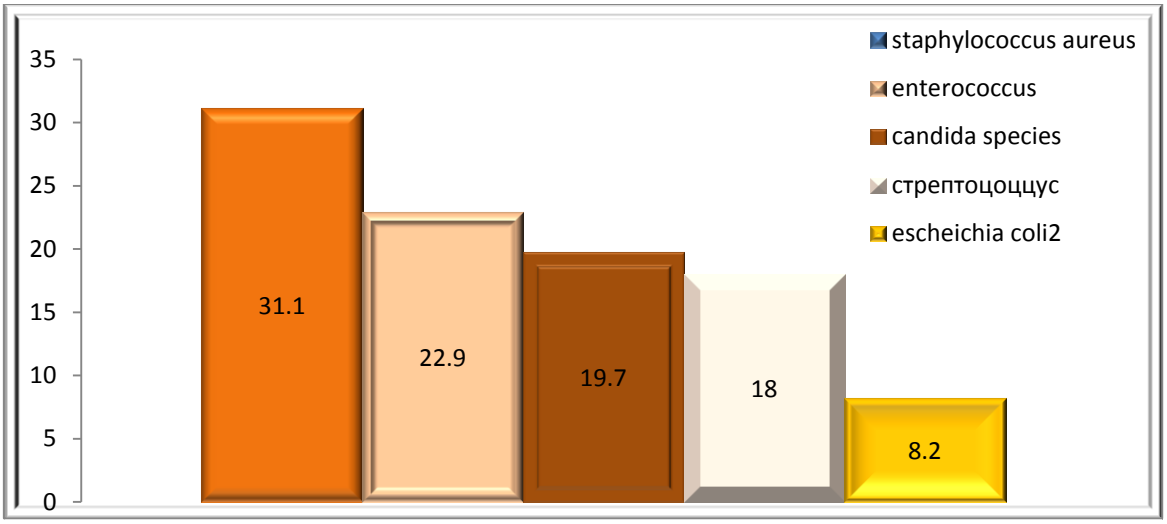


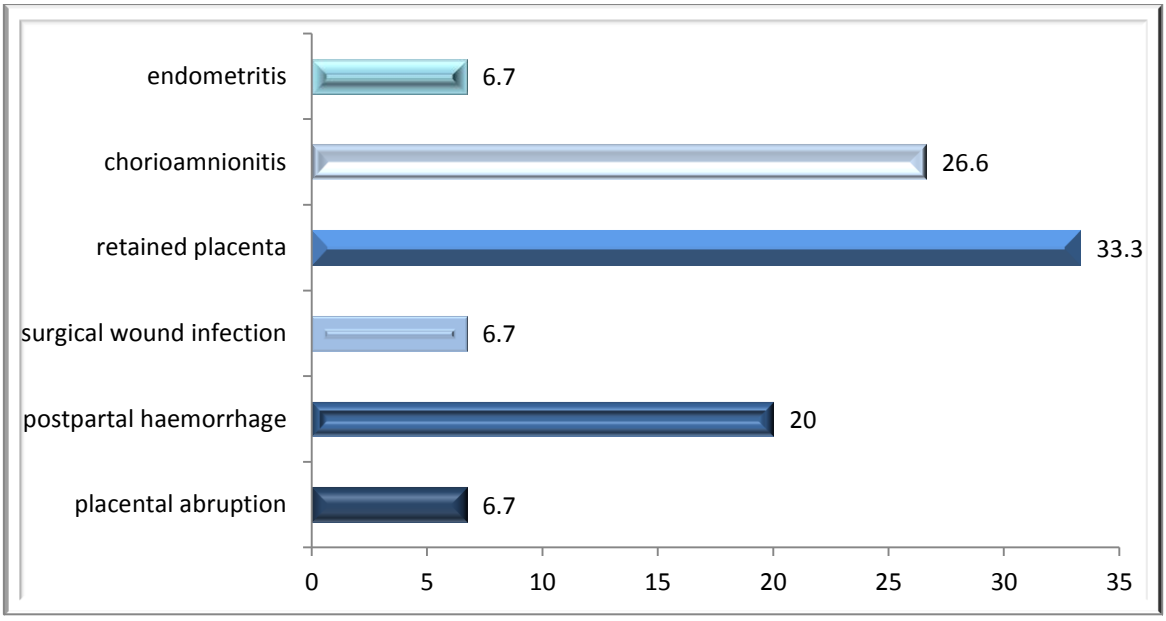
Table 46: Cross-tabulation table

vaginal swab	neonatal outcome/infection yes	neonatal outcome/infection no	total
negative	5	134	139
positive	21	40	61
total	26	174	200

Table 47: Distribution of patients with PROM according to maternal complications

maternal complication	Count	Percent
placental abruption	1	6.7
postpartal haemorrhage	3	20.0
surgical wound infection	1	6.7
retained placenta	5	33.3
chorioamnionitis	4	26.6
endometritis	1	6.7
total	15	100.0

Figure 42: Distribution of patients with PROM according to maternal complications



Maternal complications were registered in 15 (7.5%) patients with PROM, and 185 (92.5%) patients did not present with such complications. In those with maternal complications, the highest percentage accounted for retained placenta (33.3%), followed by chorioamnionitis (26.6%), postpartum haemorrhage (20.0%), and placental abruption, endometritis, and surgical wound infection found in one patient each (Table 47 and Figure 42).

In 52 (92.9%) patients with preterm-PROM no maternal complications were registered, and 4 (7.1%) had such complications.

In 133 (92.6%) patients with term-PROM no maternal complications were registered, and 11 (7.4%) had such complications.

No statistically significant association was observed between maternal complications and preterm-PROM (<37gw) and term-PROM (>=37gw) (Pearson Chi-square: 7.53745, df=7, p=0.375149).

Table 48: Average length of hospital stay of patients with PROM in relation to neonatal infection and gestational age

hospitalisation – days/ gestacion weeks	Mean <37	Mean >=37	t-value	p	Valid N	Valid N	Std.Dev.	Std.Dev.
	8.4	4.0	7.312836	0.000000	56	144	6.398458	2.387962
hospitalisation – days/ neonatal infection	Mean yes	Mean no	t-value	p	Valid N	Valid N	Std.Dev.	Std.Dev.
	11.3	4.2	8.981363	0.000000	26	174	6.473140	3.153467

Figure 43: Average length of hospital stay of patients with PROM in relation to neonatal infection.

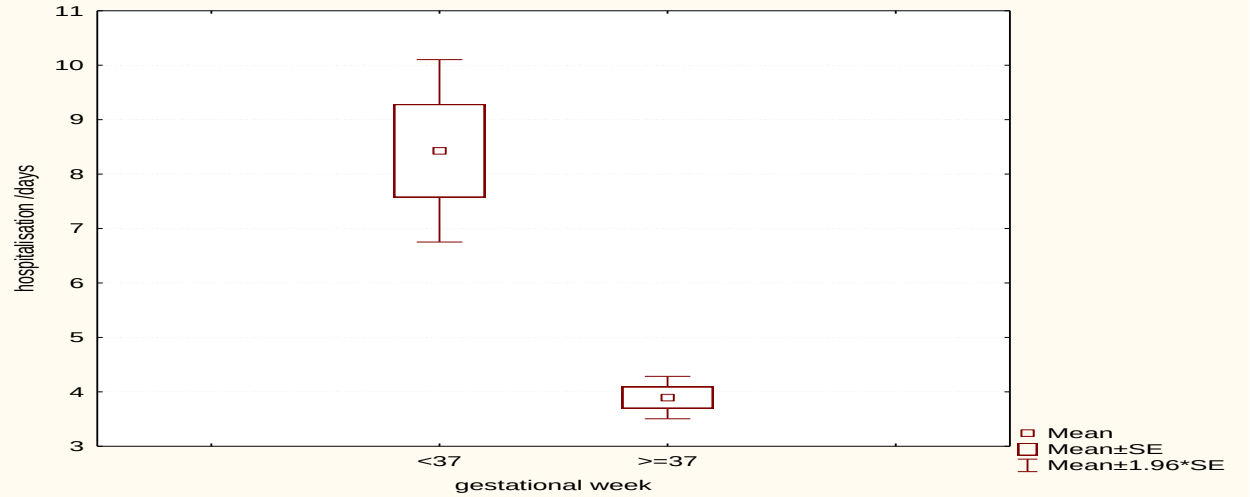
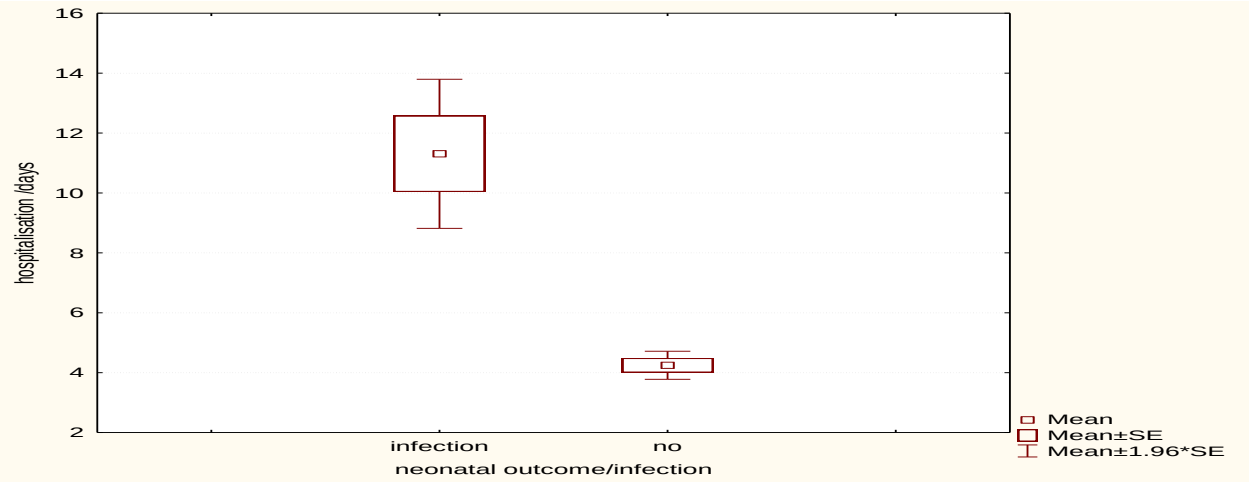


Figure 44: Average length of hospital stay of patients with PROM in relation to neonatal infection



The average length of hospital stay of patients with PROM versus preterm-PROM (<37gw) was 8.4±6.4 days, and of patients with PROM versus term-PROM (>=37gw) was 4.0±2.4 days; the difference between the average values was statistically significant ($p<0.05$) ($p=0.000000$) (Table 48 and Figure 43).

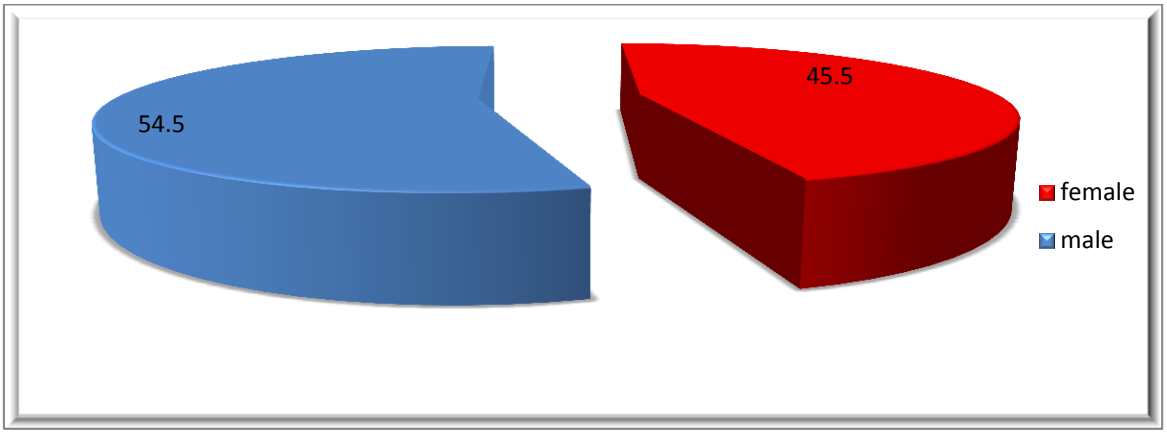
The average length of hospital stay of patients with PROM in relation to registered neonatal infection was 11.3±6.5 days, and in patients without neonatal infection 4.0±3.2 days; the difference between the average values was statistically significant ($p<0.05$) ($p=0.000000$) (Table 48 and Figure 44).

8.6. Neonatal characteristics

Table 49: Distribution of newborns according to gender

gender	Count	Percent
female	91	45,5
male	109	54,5
total	200	100.0

Figure 45: Distribution of newborns according to gender



According to gender the higher percentage of newborns were boys - 54.5% and 45.5% were girls (Table 49 and Figure 45).

Neonatal infection was equally registered in both genders (50.0%/50.0%).

No association was found between gender and neonatal infection ($p>0.05$) (Pearson Chi-square: 0.244046, df=1, p=0.621300).

Table 50: Average weight of newborns

	Valid N	Mean	Minimum	Maximum	Std.Dev.
weight	200	3090,100	1070,000	4700,000	655,4204

Figure 46 : Average weight of newborns

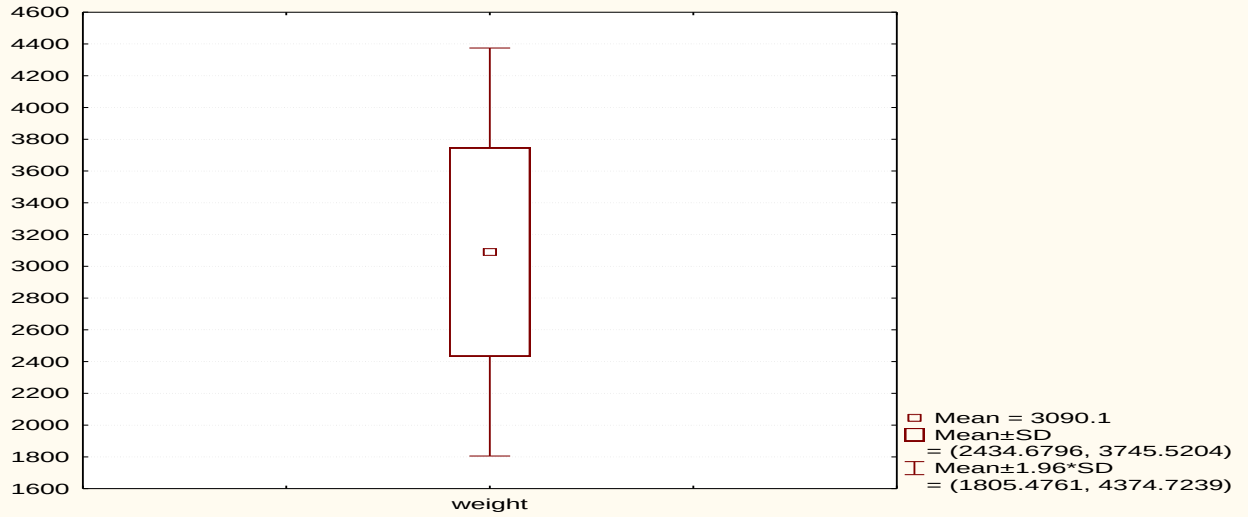
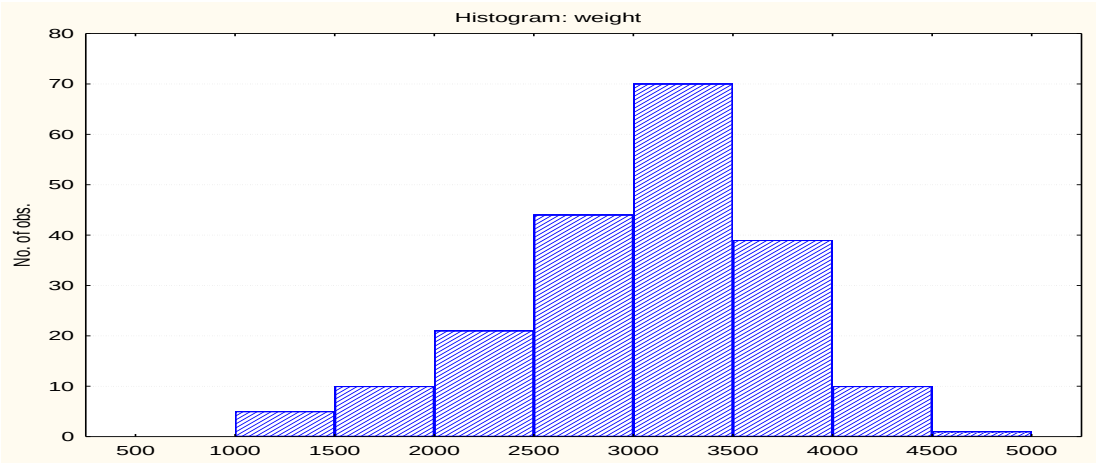


Figure 47: Weight of newborns



The average weight of newborns was 3090.1±655.4 gr, minimum 1070 gr, and maximum 4700 gr (Table 50 and Figure 46). The highest percentage of infants - 35.0% (70) were born with a weight ranging from 3000 to 3500 gr, followed by 44 (22.2%) newborns with 2000 to 2500 gr, 19.5% (39) newborns with 3500 to 4000 gr, etc. (Figure 47).

Table 51: Average weight of newborns according to gestational week and neonatal infection

Gestacion weeks/ weght	Mean <37	Mean >=37	t-value	p	Valid N	Valid N	Std.Dev.	Std.Dev.
	2335,2	3380,8	-14,5418	0,00000	56	143	551,507	413,3018
Neonatal infection/ weight	**Mean yes**	**Mean no**	**t-value**	**p**	**Valid N**	**Valid N**	**Std.Dev.**	**Std.Dev.**
	2466,9	3183,2	-5,57710	0,000000	26	174	853,729	567,2079

Figure 47a: Average weight of infants according to gestational week

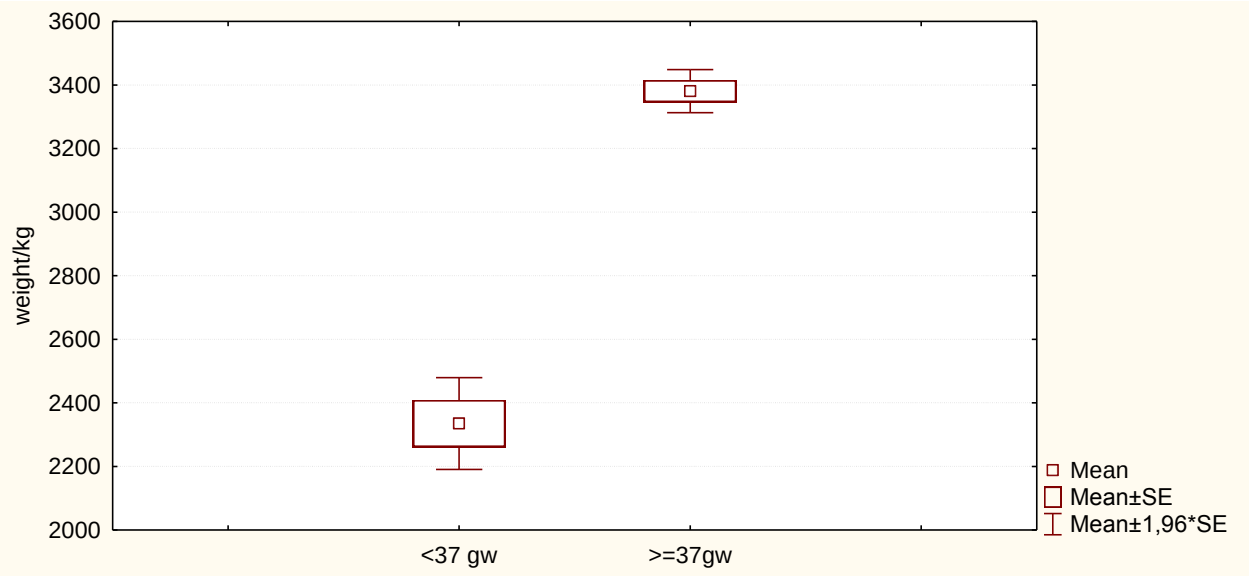
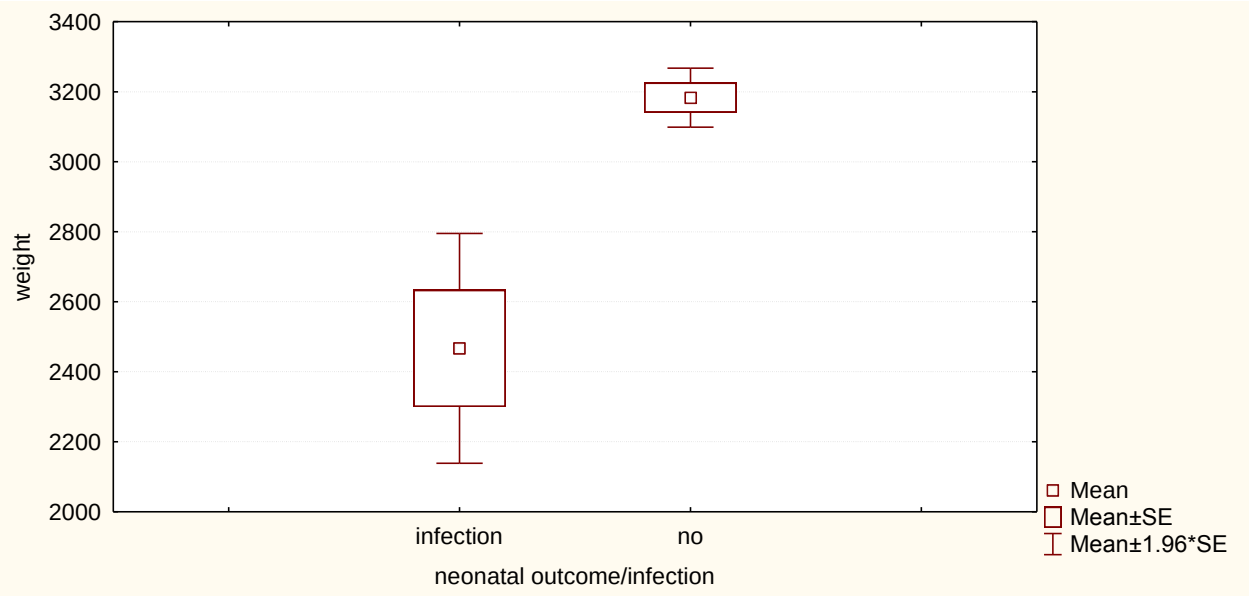


Figure 48b: Average weight of infants according to neonatal infection



The average weight of newborns with regard to preterm-PROM (<37gw) was 2335.2±551.5 gr, and with regard to term-PROM (>=37gw) it was 3380.8±413.4 gr; the difference regarding the average weight was statistically significant (p<0.05) (p=0.000000) (Table 51 and Figure 48a).

The average weight of newborns with regard to registered neonatal infection was 2466.9±853.7 gr, while in those without neonatal infection it was 3183.2±567.2 gr; the difference between the average values was statistically significant (p<0.05) (p=0.000000) (Table 51 and Figure 48b).

Table 52: Mean Apgar score at first and fifth minute in newborn infants

	Valid N	Mean	Minimum	Maximum	Std.Dev.
first minute AS	200	7,1	1,0	9,0	1,193113
5th min AS	200	8,2	2,0	10,0	1,081770

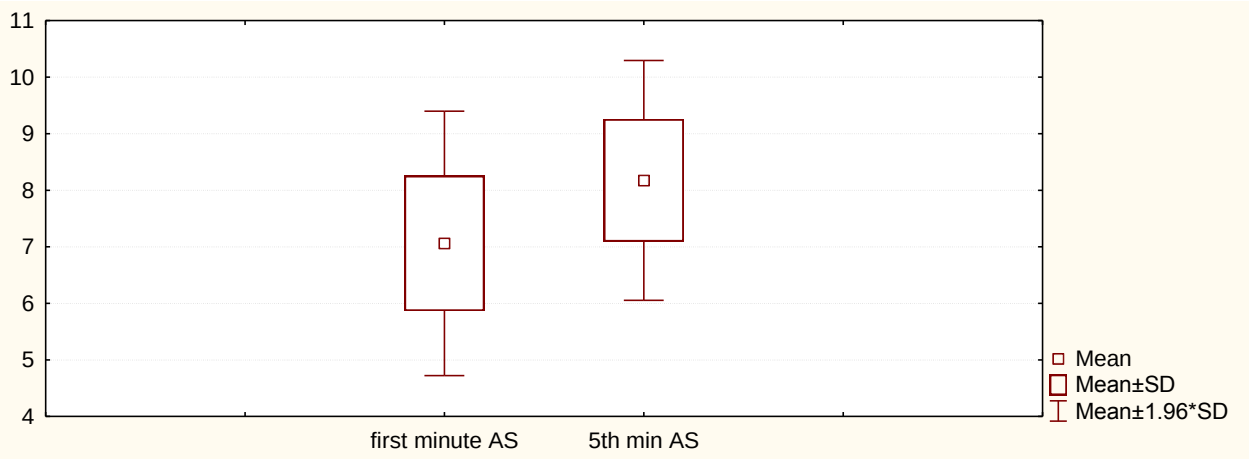
***apgar score**

>7-normal

4 to 6- fairly low

<3-critically low

Figure 48: Mean Apgar score at first and five minutes in newborn infants



The mean Apgar score at first minute after birth was normal and ranged 7.1±1.2, minimum 1, and maximum 9. The mean apgar score at five minutes after birth was normal and ranged within the reference values 8.2±1.1, minimum 2, and maximum 10 (Table 52 and Figure 49).

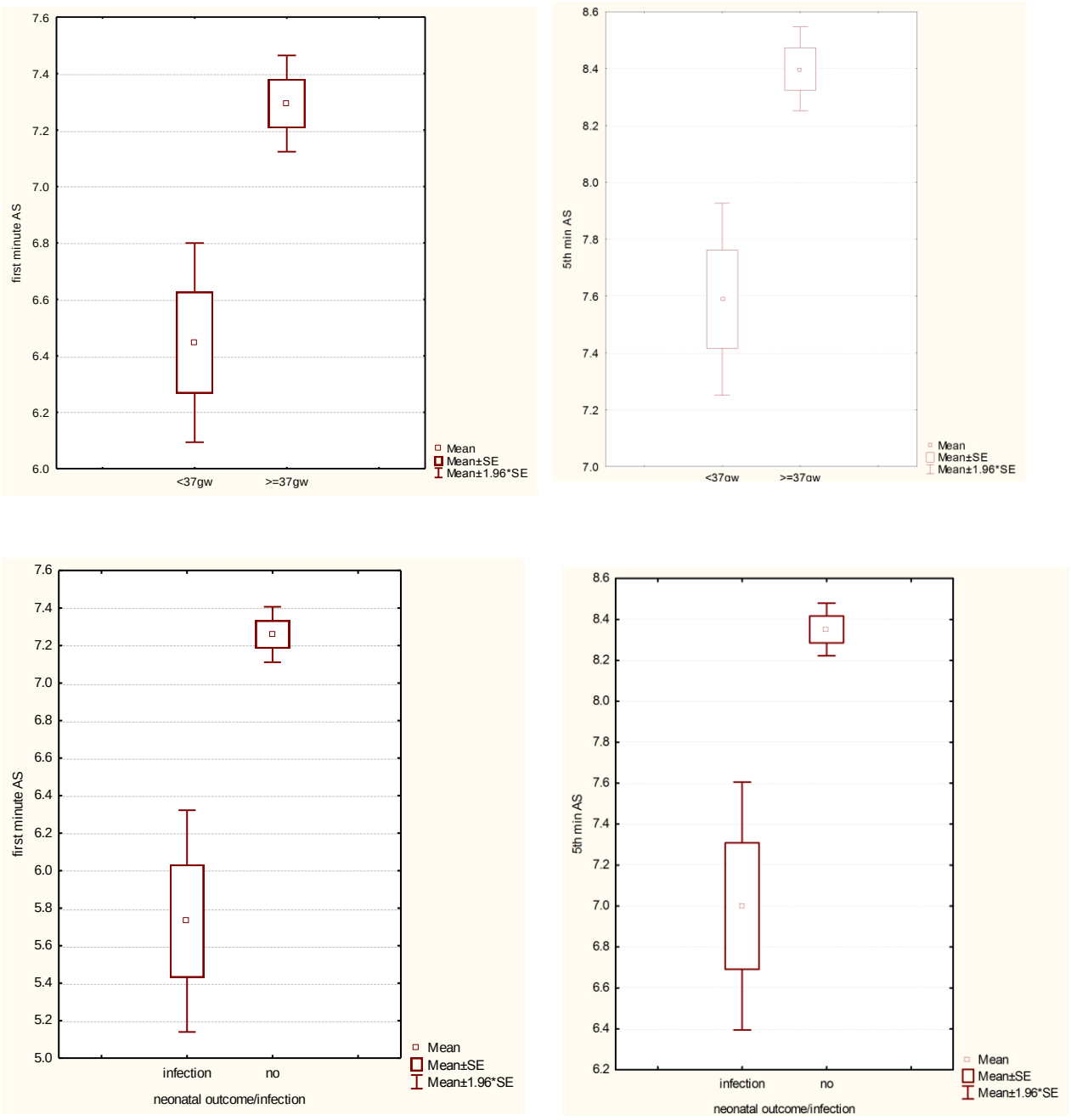
The highest percentage of infants (54.5%) had Apgar score between 4 and 6 at first minute after birth, followed by 40.5% of infants with Apgar score above 7, and 5.0% below 3 (Figure 49).

The highest percentage of infants (84.5%) had Apgar score above 7 at five minutes after birth, followed by 14.5% of infants with apgar score between 4 and 6, and 1.0% below 3 (Figure 49).

Table 53: Mean Apgar score of infants at first and five minutes according to gestational age and neonatal infection

Gestation weeks	Mean <37g w	Mean >=37g w	t-value	p	Valid N	Valid N	Std.Dev.	Std.Dev.
first minute AS	6,4	7,3	-4,73808	0,000004	56	143	1,347316	1,040261
5th min AS	7,6	8,4	-5,02260	0,000001	56	143	1,290198	0,897099
Neonatal infection	**Mean yes**	**Mean no**	**t-value**	**p**	**Valid N**	**Valid N**	**Std.Dev.**	**Std.Dev.**
first minute AS	5,7	7,3	-6,73513	0,000000	26	174	1,537731	0,995255
5th min AS	7,0	8,4	-6,52954	0,000000	26	174	1,574802	0,865575

Figure 49: Mean Apgar score of infants at first and five minutes according to gestational age and neonatal infection



The mean Apgar score of infants at first minute after birth with regard to preterm-PROM (<37gw) was 6.4±1.3, and with regard to term-PROM (>=37gw) it was 7.3±1.0; the difference between the mean values was statistically significant ($p<0.05$) ($p=0.000004$).

The mean Apgar score of infants at five minutes after birth with regard to preterm-PROM (<37gw) was 7.6±1.3, and with regard to term-PROM (>=37gw) it was 8.4±0.9; the

difference between the mean values was statistically significant (p<0.05) (p=0.000001) (Table 53 and Figure 50)

The mean Apgar score of infants at first minute after birth with regard to registered neonatal infection was 5.7±1.5, and with regard to no registered neonatal infection it was 7.3±1.0; the difference between the mean values was statistically significant (p<0.05) (p=0.000000).

The mean Apgar score of infants at five minutes after birth with regard to registered neonatal infection was 7.0±1.5, and with regard to no registered neonatal infection it was 8.4±0.9; the difference between the mean values was statistically significant (p<0.05) (p=0.000000) (Table 53 and Figure 50).

Table 54: Cross-tabulation table

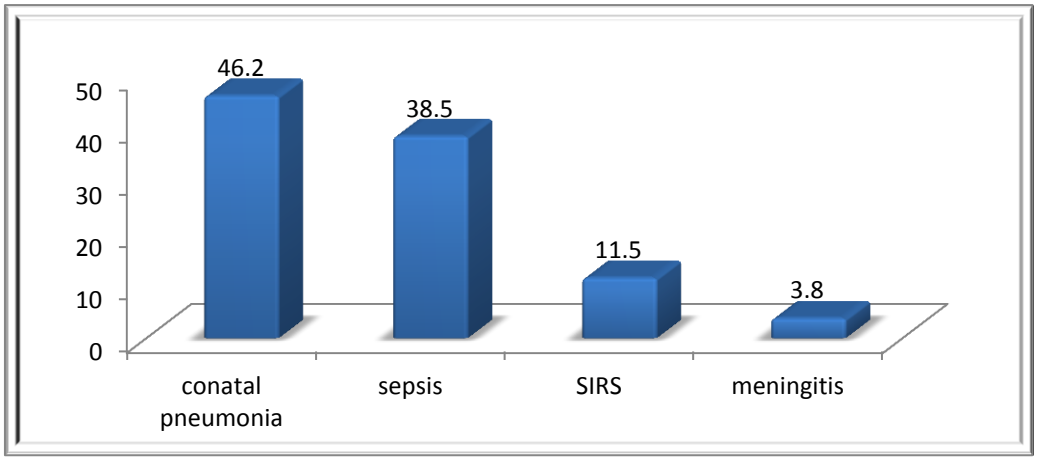
neonatal outcome/infection	gestational week <37	gestational week >=37	total
infection	**19**	**7**	**26**
no	**37**	**137**	**174**
total	**56**	**144**	**200**

A statistically significant association was found regarding neonatal infection versus preterm-PROM (<37gw) and term-PROM (>=37gw) for p <0.05 (Pearson Chi-square: 30.1212, df=1, p=0.000000) (Table 54).

Table 55: Distribution of newborns according to type of neonatal infection

Type of neonatal infection	Count	Percent
pneumonia	12	46.2
sepsis	10	38.5
SIRS	3	11.5
meningitis	1	3.8
total	**26**	**100.0**

Figure 50: Distribution of newborns according to type of neonatal infection



Out of 200 newborns, 87.0% (174) did not have neonatal infection whereas 13 % (26) had neonatal infection. Of the 26 infants with neonatal infection the highest percentage had **pneumonia** -46.2%, followed by **sepsis** - 38.5%, **SIRS** - 11.5% and 1 infant had **meningitis** (Table 55 and Figure 51).

Exitus letalis was registered in two infants (1.0%). These were babies of mothers with PROM in less than 37 weeks of gestation and who were found to have neonatal sepsis.

8.7. Markers for prediction of EONI

Table 56: Mean values of CRP mg/l in relation to neonatal infection

no	Valid N	Mean	Minimum	Maximum	Std.Dev.
CRP mg/l	174	7,6	0,4	69,3	8,113909
infection	**Valid N**	**Mean**	**Minimum**	**Maximum**	**Std.Dev.**
CRP mg/l	26	26,7	2,2	86,0	23,47342

Figure 51: Mean values of CRP mg/l in relation to neonatal infection

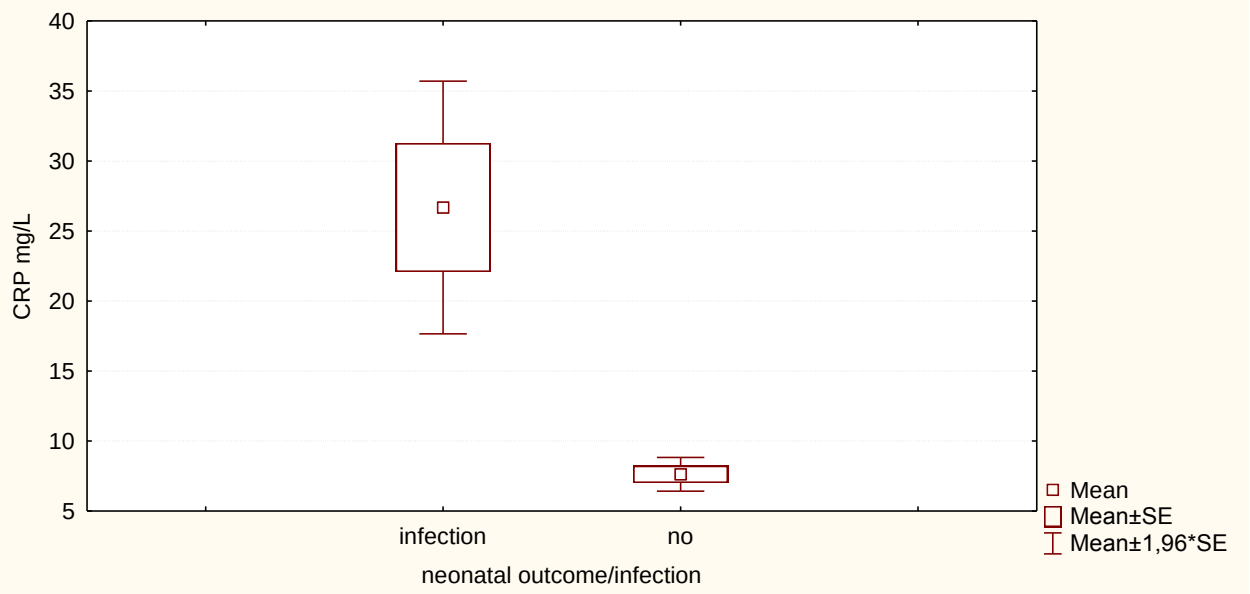


Table 57: Results of t-test

neonatal outcome-infection/CRP mg/l	t-value	p
	8,038672	0,000000

The mean value of CRP mg/l in patients who delivered newborns with neonatal infection was 26.7±23.5, minimum 2.2, and maximum 86. The mean value of CRP mg/l in those without neonatal infection was 7.6±8.1, minimum 0.4, and maximum 69.3 (Table 56 and Figure 52). There was a statistically significant difference ($p<0.05$) ($p=0.000000$) (Table 57).

Table 58: Mean values of Le x 109 in relation to neonatal infection

infection	Valid N	Mean	Minimum	Maximum	Std.Dev.
Le x 10^9	26	14,3	8,9	20,7	3,612764
no	**Valid N**	**Mean**	**Minimum**	**Maximum**	**Std.Dev.**
Le x 10^9	174	12,9	4,1	33,9	4,012919

Figure 52: Mean values of Le x 109 in relation to neonatal infection

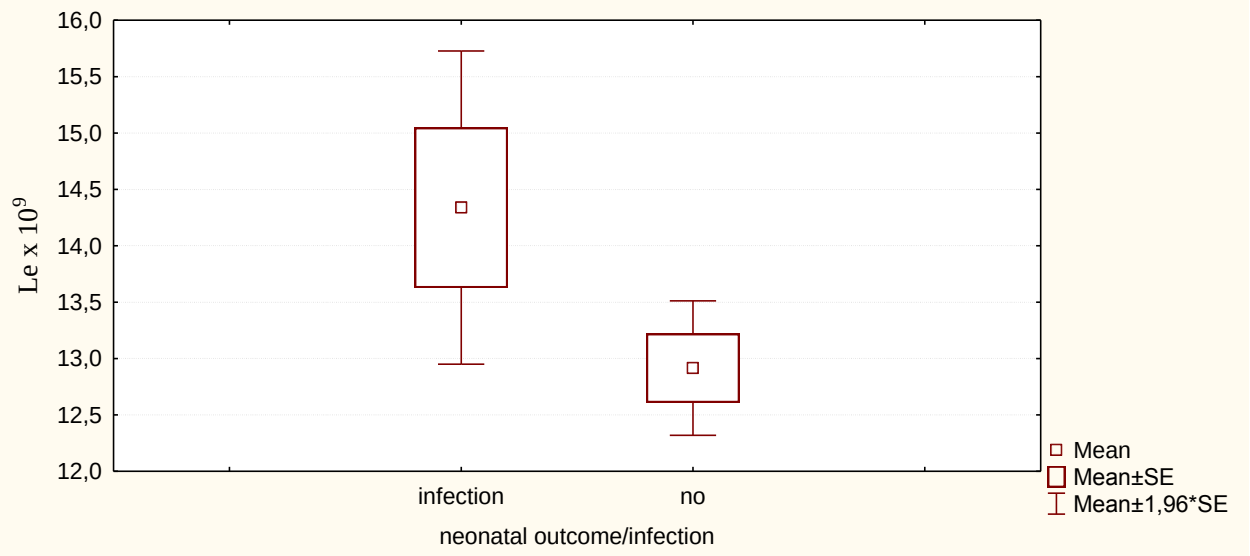


Table 59: Results of t-test

neonatal outcome-infection/Le x 10^9	t-value	p
	1,706995	0,089391

The mean value of Le x 10^9 in patients who delivered newborns with neonatal infection was 14.3±3.6, minimum 8.9, and maximum 20.7. The mean value of Le x 10^9 in those without neonatal infection was 12.9±4.0, minimum 4.1, and maximum 33.9 (Table 58 and Figure 53). The found difference was not statistically significant (p>0.05) (p=0.089391) (Table 59).

Table 60: Mean values of glucose mmol/l in relation to neonatal infection

no	Valid N	Mean	Minimum	Maximum	Std.Dev.
glucose mmol/l	174	0,6	0,009	4,6	0,563542
infection	**Valid N**	**Mean**	**Minimum**	**Maximum**	**Std.Dev.**
glucose mmol/l	26	0,7	0,009	1,8	0,552691

Figure 53: Mean values of glucose mmol/l in relation to neonatal infection

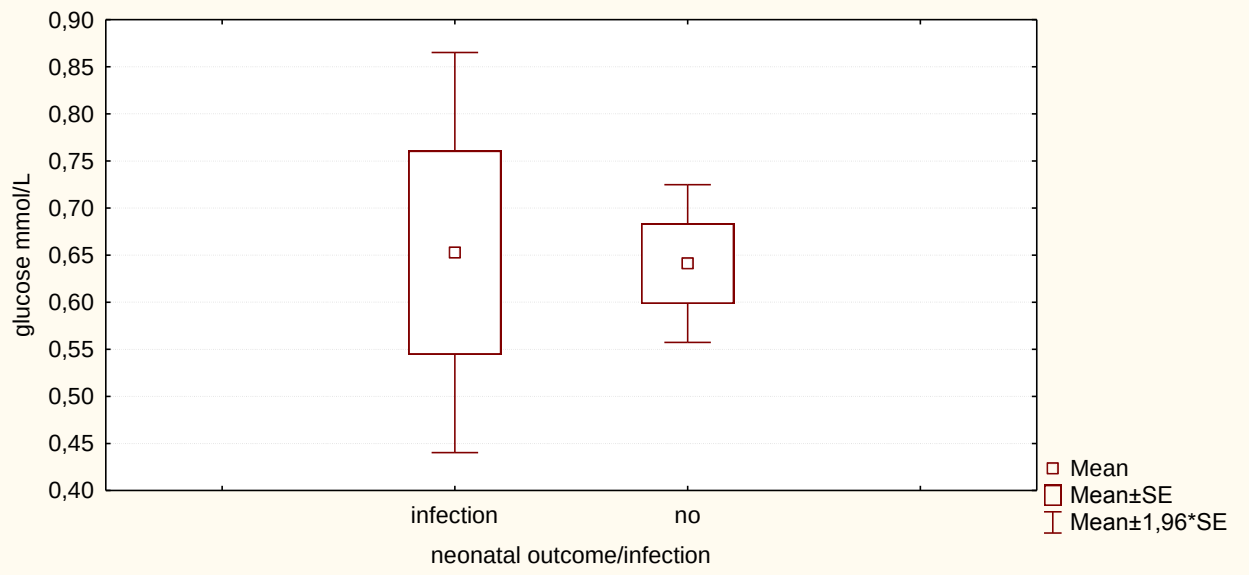


Table 61: Results of the t-test

	t-value	p
glucose mmol/l	0,098950	0,921278

The mean value of glucose mmol/l in patients who delivered newborns with neonatal infection was 0.7±0.6, minimum 0.009, and maximum 4.6. The mean value of glucose mmol/l in those without neonatal infection was 0.6±0.6, minimum 0.009, and maximum 1.8 (Table 60 and Figure 54). The observed difference was not statistically significant (p>0.05) (p=0.921278) (Table 61).

The accuracy of CRP mg/l as a diagnostic test for prediction of early onset neonatal infection in PROM is presented in Table 62. Of the 200 patients with PROM, true positive were 22 and false positive 66; there were 110 negative findings, of which 108 were true negative and 2 false negative.

Table 62: The relationship between CRP and neonatal infection

CRP mg/l	Neonatal infection		total
	present	absent	
>6 +	24	66	90
<=6 -	2	108	110
total	26	174	200

Table 63: Results of the test

CRP	Estimated Value	95% Confidence Interval	
		Lower Limit	Upper Limit
Prevalence	0.13	0.088198	0.18648
Sensitivity	0.923077	0.734003	0.986563
Specificity	0.609195	0.532184	0.681305
For any particular test result, the probability that it will be:			
Positive	0.46	0.389906	0.531656
Negative	0.54	0.468344	0.610094
For any particular positive test result, the probability that it is:			
True Positive (Positive Predictive Value)	0.26087	0.177379	0.36466
False Positive	0.73913	0.63534	0.822621
For any particular negative test result, the probability that it is:			
True Negative (Negative Predictive Value)	0.981481	0.928148	0.996786
False Negative	0.018519	0.003214	0.071852

The evaluated CRP accuracy showed sensitivity of 92.3%, specificity of 60.9%, positive predictive value of 26.1%, and negative predictive value of 98.1%. The global accuracy was 66.0% (Table 63).

Figure 54: ROC curve of CRP as predictor for neonatal infection

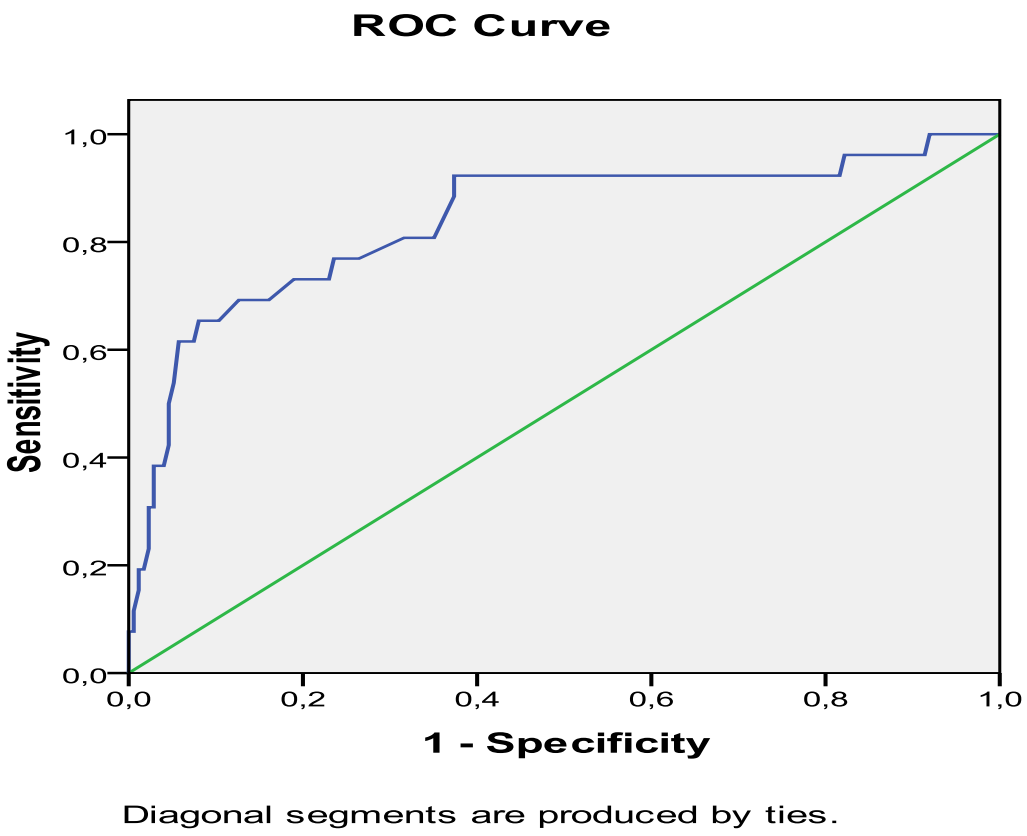


Table 64: Test Result Variable: CRP

Area Under the Curve

Test Result Variable(s):CRP1

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
,840	,048	,000	,745	,934

ROC analysis showed that CRP has a predictability of 84.0% (p=0.000) for diagnosis of early onset neonatal infection. This obtained result indicates that CRP is an excellent predictor close to the ideal value of 1.0 (Figure 55 and Table 64)

The accuracy of WBC x 10^9 as a diagnostic test for prediction of early neonatal infection in PROM is presented in Table 65.

Of the 200 patients with PROM, true positive were 15 and false positive were 55; there were 110 negative cases, of which 119 were true negative and 11 false negative.

Table 65: The relationship between WBC and neonatal infection

WBC x 10^9	Neonatal infection		total
	present	absent	
+	15	55	70
-	11	119	110
Total	26	174	200

Table 66: Results of the test

WBC	Estimated Value	95% Confidence Interval	
		Lower Limit	Upper Limit
Prevalence	0.13	0.088198	0.18648
Sensitivity	0.576923	0.371915	0.760256
Specificity	0.683908	0.608513	0.751032
For any particular test result, the probability that it will be:			
Positive	0.35	0.284942	0.420923
Negative	0.65	0.579077	0.715058
For any particular positive test result, the probability that it is:			
True Positive (Positive Predictive Value)	0.214286	0.128697	0.331726
False Positive	0.785714	0.668274	0.871303
For any particular negative test result, the probability that it is:			
True Negative (Negative Predictive Value)	0.915385	0.85019	0.954903
False Negative	0.084615	0.045097	0.14981

The evaluated accuracy of WBC showed sensitivity of 57.6%, specificity of 68.3%, positive predictive value of 21.4%, and negative predictive value of 91.5%. The global accuracy was 67.0% (Table 66).

Figure 55: ROC curve of WBC as predictor of neonatal infection

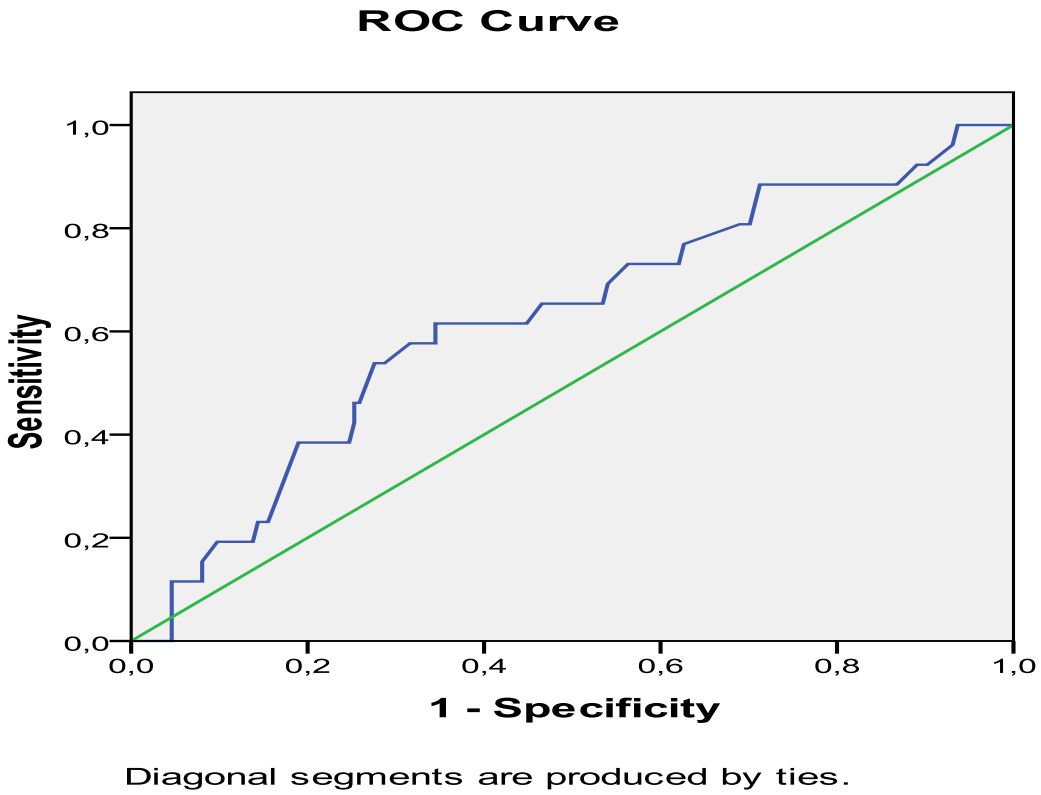


Table 67: Test Result Variable: WBC

Area Under the Curve

Test Result Variable(s):WBC1

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
,623	,059	,043	,507	,740

ROC analysis showed that WBC predictability in establishing the diagnosis of neonatal infection was 62.3% (p=0.043) (good predictor of neonatal infection) (Figure 56 and Table 67).

The accuracy of glucose mmol/l as a diagnostic test for prediction of early neonatal infection in PROM is presented in Table 68. Of the 200 patients with PROM, true positive were 15 and false positive 115; there were 70 negative findings, of which 59 were true negative and 11 false negative.

Table 68: The relationship between Glucose and neonatal infection

glucose mmol/l	Neonatal infection		total
	present	absent	
+	15	115	130
-	11	59	70
total	26	174	200

The evaluated accuracy of glucose mmol/l showed sensitivity of 57.6%, specificity of 33.9%, positive predictive value of 11.5%, and negative predictive value of 84.3%. The global accuracy was 37.0% (Table 69).

Table 69: Results of the test

glucose mmol/l	Estimated Value	95% Confidence Interval	
		Lower Limit	Upper Limit
Prevalence	0.13	0.088198	0.18648
Sensitivity	0.576923	0.371915	0.760256
Specificity	0.33908	0.270224	0.415169
For any particular test result, the probability that it will be:			
Positive	0.65	0.579077	0.715058
Negative	0.35	0.284942	0.420923
For any particular positive test result, the probability that it is:			
True Positive (Positive Predictive Value)	0.115385	0.068203	0.186137
False Positive	0.884615	0.813863	0.931797
For any particular negative test result, the probability that it is:			
True Negative (Negative Predictive Value)	0.842857	0.73195	0.915245
False Negative	0.157143	0.084755	0.268

ROC analysis showed glucose predictability of 49.7% (p=0.964); in establishing the diagnosis of neonatal infection, thus results indicate that this test had no predictive performances for neonatal infection (Figure 57 and Table 70).

Figure 56: ROC curve of Glucose as predictor of neonatal infection

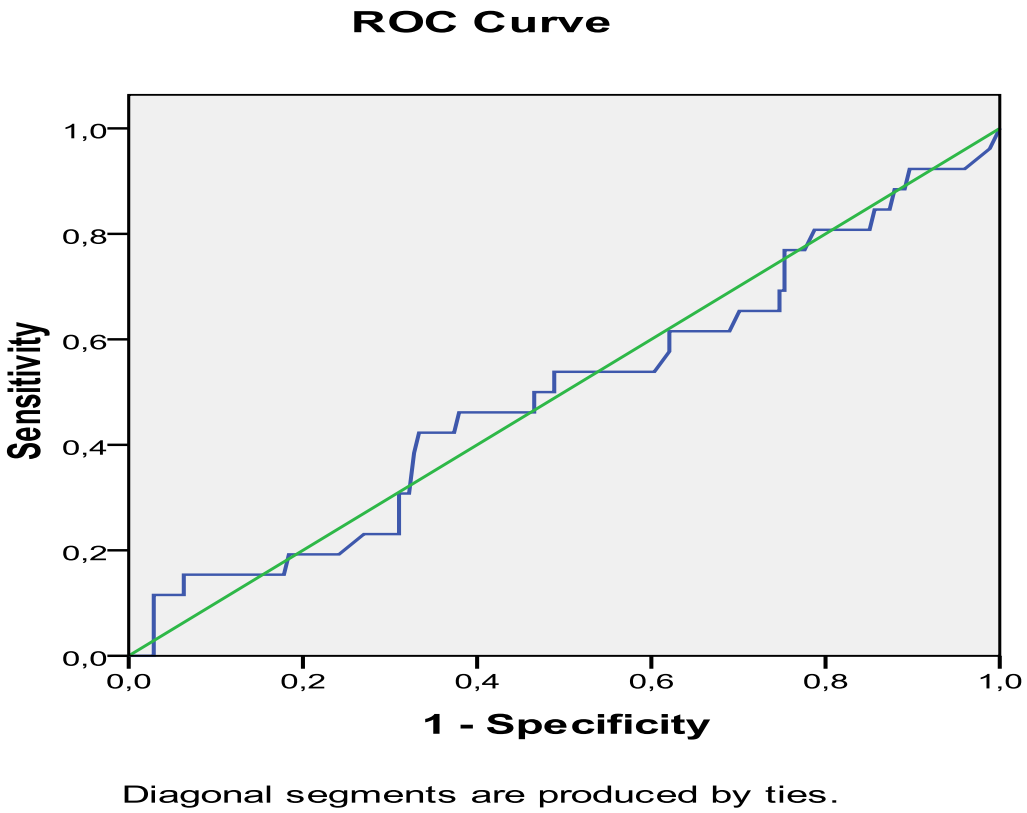


Table 70: Test Result Variable: Glucose

Area Under the Curve

Test Result Variable(s):glucose1

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
,497	,064	,964	,373	,622

9. Discussion

Prelabour rupture of membranes (PROM) is a common obstetric event in which maternal and fetal consequences may arise during pregnancy and can happen at or after birth. A serious neonatal consequence that can happen related to PROM is neonatal infection.

Accurately predicting EONI in pregnancies complicated with PROM remains a critical challenge worldwide. In developing and undeveloped countries this challenge is even greater because the availability of tests and in-country resources for application of markers of infection are not always available or the use, reliable. In Kosova, the setting for this study, complex and expensive laboratory analyses are not available and physicians are only able to implement tests that are appropriate for the local setting. Maternal serum CRP and WBC and amniotic fluid glucose are inexpensive and available tests of infection and are found in the literature to be an effective predictor of EONI. These tests are available in Kosova, but to date, have not been studied related to prediction of EONI in pregnant women with PROM in Kosova.

PROM is a common complication of pregnancy among women worldwide. The risk factors, practices of mode of the delivery and the maternal and neonatal outcome, vary from country-to-country. This type of investigation has not happened in Kosova and so the results from this study will contribute to the body of knowledge on this topic. The findings from women in Kosova will be compared to results from around the world and regionally for similarities or differences. Data analyzed includes demographic characteristics, risk factors, antenatal care utilization, mode of delivery, most common maternal complications and neonatal infection rate in pregnancies complicated with PROM. This study includes 200 pregnant patients presenting with prelabour rupture of membranes and their newborns who met the inclusive and exclusive criteria.

Maternal Age

In this study, the mean age of patients was 27.5 (±5.5 years). The majority of patients were between 20 to 30 years of age (66.0%) followed by 26,5 % in the age group above 30 years age. Seven and a half percent of the patients were 20 years old or younger.

The results from this Kosova in study revealed no statistically significant differences between term and pre term PROM groups with regard to maternal age. This indicates that age is not a risk factor for preterm PROM. This finding was also reported in a study by Clearly Goldman et al.104, where in a large prospective multicenter study reported no statistically significant association between maternal age and increased risk for preterm PROM.

It should be noted that other studies, including Amildo et al.29, did report a significant association of increasing maternal age and preterm PROM.

Socioeconomic and Employment Status

Ferguson et al.[34] reported a positive association between low socio economic status and preterm PROM. In this study, authors reported a threefold increased risk of having preterm PROM in pregnant women with low socio-economic status.

The results from this study found no significant association between socio-economic status and term PROM or preterm PROM neither between socioeconomic status and early onset neonatal infection. Results identified a higher occurrence rate of prelabour rupture of membranes in women of lower and middle educational level and the majority of them (82.5%) were unemployed. The highest PROM rate belonged to the group of middle socioeconomic status (66.5 %), followed by the group of high socioeconomic status (24,5%). The lowest (9%) was in low socioeconomic status.

Employment Status

The Kosovo Agency of Statistics in Social Statistics Report for 2014 [105] indicates a unemployment rate of 41.6 % for females in Kosova and this is in contrast with our study population data in which 82.5 % of participants were unemployment. Our data analysis also showed significant association of employment status and the development of early onset neonatal infection. These findings could reflect sedentary lifestyle, poor antenatal care, chronic stress and other adverse factors associated with the education and occupation.

Family Size

Family size or number of persons living together is another social indicator which may impact maternal fetal wellbeing. In our study, more than half (51.5 %) of patients with PROM used to live in households with 2 to 4 family members, 35.5 % with 5 to 10 and remaining 10.5 % lived in households with 10 to 15 family members. The average number of people per household revealed 6,3 ± 3,7.

These data indicate that a large proportion of the study population lives in extended families. The extended families can include parents and children as well as other kin such as grandparents, uncles and aunts. This form of extended family was found to be more present in rural residents which compromise 50 % of the study population. Although an international research [106] has pointed to family size as important influence on the health and well – being of women and their children, respectively the large number of persons in a family can have negative impact on general well-being in our data there was no significant statistical association between the family size and early onset neonatal infection .

Other Factors

Parity, previous PROM, smoking in pregnancy, previous abortion, previous interventions in the uterus are other risk factors for prelabour rupture of membranes implicated in the literature were also examined in this study.

Parity

Parity is one of the factors associated with PROM according to searched literature.

Similarly, present study results found an association of parity and prelabour rupture of membranes and a significant difference of PROM rate between primiparous and multiparous women. The results in this study indicate that PROM occurs significantly more often among primiparous than among multiparous women. Similar results are reported by other authors including Ladfors L et al.[21] conducted a study about prevalence and risk factors for prelabour rupture of membranes at or near term in Swedish population. In the analysis of 2208 pregnant patient who experienced PROM they noted that the condition occurs more commonly in primiparous woman compared with multiparous. Similar results are reported by Prechapanich J et al.[33] in a study conducted in Thailand about relationship between parity and pregnancy

outcome. The effect of parity to pregnancy outcome was analyzed among 976 singleton pregnancies. In the primiparous group there was a significant higher risk of premature rupture of membranes (8.8% vs. 5.1%; odds ratio 1.79, 95% CI 1.07-2.98).

Previous PROM

Previous PROM is one of the most frequent risk factors for prelabour rupture of membranes implicated in the searched literature.

In this study, among 65 multiparous patients, there was a significant high percentage (65.4%) of women has experienced previous PROM. The analysis of the recurrence rate between term and preterm PROM didn't show any significant difference. This is in a contrast to population study in the Washington State which reported that the recurrence rate among preterm PROM was markedly greater (62.0%) than that of case with term PROM (26.9). For more, within each PROM category (term and preterm PROM) cases were more likely to repeat the experience of their first birth ; those with preterm PROM at their first birth were subsequently more likely to have another preterm PROM than a term PROM and vice versa.[107] Lower recurrence (32.2%) of preterm PROM is reported by Asrat T et al.[22] who in contradiction with this current study finding regarding recurrence couldn't find any association between the estimated gestational age at the time of rupture in the index pregnancy and the probability of repeat preterm premature rupture of membranes in the next pregnancy.

Smoking

Previous studies has linked smoking as a risk factor for prelabour rupture of membranes. Harger et al. [28] in a cohort study conducted in six tertiary perinatal centers in the United States, the association of 41 potential risk factors with preterm PROM. After multiple logistic regression analysis they found cigarette smoking to be one out of three independent risk factors for preterm PROM. Similar results are reported by other authors.[27,29].

In the present study, using self-reported information, an overall rate for smoking in pregnancy of 25.5 % was registered and there was no significant difference in rates of smoking in pregnancy in regard to gestational age at the time of membrane rupture. Even when excluding the fact that pregnant women may conceal their smoking, the smoking rates

reported in this study in Kosova indicate a higher rate of smoking during pregnancy than what is found in other studies around the world [108,109,110].

Antenatal Care

The impact of antenatal care (ANC) in perinatal outcome is well established. Good quality antenatal care reduces maternal and neonatal morbidity and mortality thus improves health outcomes associated with pregnancy and childbirth. Quality of ANC is measured by three dimensions: number of visits, timing of initiation of care and inclusion of all the recommended components of care.[111] In 1995, World Health Organisation working group on ANC established four as the minimum number of prenatal care visits for women without identified problems.[112]

In 2008, the Society of Gynecologists and Obstetricians of Kosova in collaboration with Kosova Ministry of Health issued local guidelines for Obstetrics and Gynecology. The guideline for ANC for uncomplicated pregnancy recommends seven antenatal visits. The exams and tests recommended include collection of maternal medical history, folic acid supplementation, screening for anemia, blood pressure check up, weight gain check up, and screening for urinary infection. According to these local guidelines, antenatal visits should be provided by skilled health personnel and completed at specified times during the pregnancy. It is recommended that the first visit occurs before 12 weeks into pregnancy, the second at 15-17 weeks, the third at 20-22 weeks, the fourth at 26-28 weeks, the fifth at 32-36 weeks, the sixth at 38-40, and seventh at 41-42 weeks.[113]

In this study in Kosova, the adequacy of antenatal care was evaluated according to the adequacy of the number of antenatal visits and the adequacy of the included components of care. For almost all participants in this study, the antenatal care was provided by health care professionals, specifically by obstetricians in private clinics. The majority of women in this study had completed at least four ANC visits.

Local guidelines in Kosova recommend four ultrasound examinations per pregnancy for uncomplicated pregnancy.[123] Analysis of the study data revealed the actual number of ultrasounds was higher, including that almost half of the women (44 %), had six to eight ultrasound examinations and 20.5 % had eight to 10. Among all patients in the study, only 23.5 % had four to six ultrasound examinations, and maximum number of ultrasound

examinations during a pregnancy reported in the study population was 14. Taking into consideration that this study included pregnant women presenting with PROM without any other comorbidity, the finding regarding the number of performed ultrasound examination indicates a significantly higher number than recommended.

Routine prenatal ultrasonography is an important and useful screening tool during antenatal care, however, there is research that warns about exceeding the recommended number due to potential negative consequences. One negative outcome is reported by Dr. Cose Rogers in his 2006 study in United States that focused on a possible link to autism.[114] Previous studies have reported subtle effects of neurological damage linked to ultrasound including an increased incidence in left-handedness in boys and speech delays.[115]

Analysis of the implementation of basic recommended components of care during ANC visits revealed that almost 23 % of the patient did not have any pelvic examinations, and in 52 % of cases the weight gain was not checked. In the study, 92 % of patients received folic acid supplementation, 80.5 % were screened for anemia in pregnancy, and blood pressure check up was evident in 86.5 %. Screening for urinary infection was performed in 79.5 % of cases while screening for genital infection was performed in only 49.5 %. The numbers of antenatal visits indicate that the majority of pregnant women in Kosova are aware of and have knowledge about the importance of antenatal care. Despite knowing about the importance of antenatal care, the findings also show most women do not complete all the suggested visits and components of care. A suggestion from this study, and therefore with the hope of lowering neonatal morbidity and mortality and improve maternal and neonatal outcomes, is better adherence to recommended antenatal care

PROM to Delivery Interval

The time interval between PROM and delivery is a factor that may influence maternal and fetal wellbeing in pregnancies complicated with PROM. In the recent research, the interval from membrane rupture to delivery ranged from 7 to 70 hours with the mean of 24.8 hours. PROM to delivery interval was longer among patients with PROM prior to term than in group with PROM at term. The mean interval from membrane rupture to delivery in preterm PROM group was 29 hours, while in term PROM group was shorter, respectively 23.2 hours. The T test showed a significant difference between the two groups ($p=0.009$). This finding was expected since it is an established fact that the latency period is in adverse

relation to gestation age at membrane rupture, thus when PROM occurs at term labour starts spontaneously soon or labour is induced.

Evaluation of PROM to delivery interval in terms of neonatal infection, showed a significant longer interval of 34.7 hours in group with neonatal infection versus 23.3 hours in group without neonatal infection. Therefore, the interval from membrane rupture to delivery was significantly longer in group with neonatal infection compared to the group without neonatal infection (p=0.0001).This finding is in accordance with the findings of Herbst et al.[116] which in a study among 113.568 pregnant women investigated how the interval between membrane rupture and delivery affects the risk of neonatal infection- sepsis. They reported that the duration of membranes rupture was an independent risk factor for neonatal sepsis and this risk of neonatal sepsis increases independently and nearly linearly with duration of membrane rupture up to 36 hours, with an odds ratio of 1.29 for each 6-hour increase in membrane rupture duration. Similar results are reported by Chua et al. which in their study showed that there is an association between interval from PROM to delivery and neonatal infection respectively prolongation of PROM to delivery interval increases the incidence of neonatal infection.[117]

Mode of Delivery

In certain situations PROM and associated complications may influence the mode of delivery. In the recent research, induced vaginal delivery was the most common mode of delivery (77 cases, 38.5 %), followed by spontaneous vaginal delivery (69 cases, 34.5 %), while cesarean section rate was 26.5 % (53 cases) and instrumental delivery rate compromising of 0.5 % (1 case).

The cesarean delivery rates are increasing worldwide and becoming a global issue. The perinatal report of Kosova for 2014 reports an overall cesarean section rate of 33.5 %. [63] In the recent study the given lower (26.5 %) CS rate might be due to the fact that from the study were excluded all the pregnant women with other co-morbidities such as diabetes in pregnancy, and hypertensive disorders. Comparing the mode of delivery in terms of gestational age at birth, it is found a significant relation between mode of delivery and gestational age at birth. Induced vaginal delivery was the most common (44.8 % of cases) mode of delivery in term PROM group while the spontaneous vaginal delivery was the commonest mode of delivery in preterm PROM group (49.1 %).

Despite the fact that Kosovo health institutions do not have developed yet the guidelines and protocols for management of pregnancies with prelabour rupture of membranes, in the recent study induction of labour is found to be the most common mode of delivery in term PROM pregnancies therefore induction of labour is being applied in term PROM pregnancies as it is recommended in western guidelines.
The rates of neonatal infection were comparable between different modes of delivery without significant difference in between.

Analysis of the indications for performed cesarean delivery revealed differences in terms of term and preterm PROM groups. Among patients with term PROM, the most common indication for cesarean delivery was cephalopelvic dysproportion present in 36.1 % of cases. This was followed by spasmodic prolonged labour in 13.9 % of cases, failed induction in 11.1 %, and fetal distress as an indication for cesarean delivery present in 8.3 % of cases.

The given high rate of cephalopelvic disproportion may suggest that prelabour rupture of membranes at term often indicates cephallopelvic disproportion.

On the same line, Li et al. [118] in a large observational study found that cefalopelvic disproportion was the main reason for cesarean delivery in pregnant women diagnosed with prelabour rupture of membranes. Similarly, authors suggest that PROM occurrence may indicate early warning sign of cephalopelvic disproportion and explain it with the reason that in the cephalopelvic dispro- portion, fetal presentation and pelvic floor can not engage properly, leaving gaps between fetal presentation and pelvis. When the uterine pressure rises, increased intrauterine pressure is applied to the amniotic sac evenly through the gaps, which can lead to prelabour rupture of membrane.

Meanwhile, in the preterm PROM group the most common indications for cesarean delivery is found to be fetal distress (31.2 %).This finding suggest that pregnancies complicated with preterm PROM should be closely monitored for signs of fetal distress.

Maternal Complications

Among neonatal implications, prelabour rupture of membranes may pose risk factor for development of immediate and delayed maternal complications. The recent study results indicate that maternal complications may arise during pregnancy, at birth and following birth. In this Kosova study, the analysis showed that out of 200 patients, 15 (7.5%) had maternal complications. The most common maternal complication was retained placenta followed by chorioamnionitis and postpartal haemorrhage. In the recent study placental abruption occurred only in one case although the searched literature shows greater incidence of placental abruption in pregnancies complicated with PROM.[119] This may be explained by the differences in sample size and gestational age of study population. There was no significant difference on terms of maternal complications between term and preterm pregnancies.

Maternal Genital Tract Colonization

A number of studies highlight the association between neonatal infection in the first days of life and maternal genital tract colonisation. In the recent study among 200 participants included, the high vaginal swab resulted positive in 31 % of cases. The data analysis revealed that out of 61 colonized mothers 21 of them had newborns with infection, thus indicating significant statistical association between maternal genital tract colonization and early onset neonatal infection.

Similarly, Chan G et al.[120] in a meta analysis about prevalence of early onset neonatal infection among newborns of mothers with genital tract colonization , reported that up to 7 % of newborns exposed to maternal colonization has developed neonatal infection. Another global systematic review and meta analysis of eighty-three studies, reports that newborns of mother with colonization have a 9.4 (95 % CI 3.1-28.5) times higher risk of neonatal infection than newborns of non-colonized mothers, whereas in newborns of mothers with prelabour rupture of membranes this risk increases for 2.3 times. Based on the results authors suggest the need for improving the detection of maternal colonization during the intrapartum period using new technologies and tests that are cheap, fast, highly sensitive and specific which may allow health care workers to identify at- risk newborns sooner [121] Similarly, a research conducted in a tertiary center of Saudi Arabia, has investigated the association between mother colonization and neonatal sepsis in pregnancies complicated with prelabour rupture of membranes .Analysis showed that in the study population consisting of

pregnant woman complicated with PROM , fourteen percent of the infants exposed to maternal genital colonisation were infected . [122]

The recent study findings are additional evidence that indicate a high level of early onset neonatal infection among newborns of mothers complicated with prelabour rupture of membranes and genital tract colonization. Thus, we suggest that in high risk women respectively in women complicated with prelabour rupture of membranes, a better diagnostic approach and intrapartum antibiotic prophylaxis would lead to decrease of morbidity and mortality from neonatal infection during the first week of life.

In recent study, the most common organism isolated from maternal genital tract was staphylococcus aureus followed by enterococus, candida species and SBG. Escherichia colli was less common isolated in 8.2 % of cases. Similarly, a research conducted in a tertiary center of Saudi Arabia, has investigated the association between mother colonization and neonatal sepsis in pregnancies complicated with prelabour rupture of membranes .Authors reported that in their study population consisting of pregnant woman complicated with PROM ,fourteen percent of the infants were infected. The major microorganisms involved in genital colonization of the mothers were coagulase negative Staphylococcus (24%), Klebsiella pneumoniae (13%), Pseudomonas aeruginosa (11.3%) and Enterococcus species (11.3%).[3] In agreement with our results, Kerur et al.[123] in their study among 102 newborns with early onset neonatal sepsis, found that prelabour rupture of membranes was an important risk factor for development of early onset sepsis. Maternal genital tract colonization was positive in 52 cases with neonatal sepsis. The most common organism isolated from maternal genital tract was Escherichia coli followed by Klebsiella species and Streptococcus.

The finding in terms of most common microorganisms isolated from maternal genital tract is additional evidence about existence of regional variation of genital colonization. Several previous studies indicate that PROM results in increased hospital costs and longer length of stay for both mother and infant. Data from the Washington State linked births-hospitalization database show that a term birth complicated by PROM increases average hospital costs by 40 %,while hospital costs for a preterm PROM birth are eight times that of an uncomplicated birth.[107]

Hospital Length of Stay

The average length of hospital stay in recent study was eight days in preterm PROM group and 4 days in term PROM group. In both groups the median length of hospital stay was longer in comparison with an average two day length of hospital stay of uncomplicated pregnancy as indicated in the database of the Obstetrics and Gynecology Clinic in Prishtina. Comparison of the average length of hospital stay in terms of neonatal infection showed significant statistical difference in the length of hospital stay between two groups. Average length of hospital stay in the group with neonatal infection was 11.3 days whereas in group without neonatal infection the average hospital stay was 4 days. The recent finding regarding the average length of hospital stay indicates that both prelabour rupture of membranes and neonatal infection increases the length of hospital stay and the hospital costs.

Gender and Birthweight

An association between fetal sex and pregnancy outcome has been previously reported. The present study examined the association of fetal sex with both, prelabour rupture of membranes and development of early onset neonatal infection.
In the recent study, among 200 neonates born from pregnancies complicated with PROM, 54.5 % were male infants, with no statistical difference in terms of newborns gender and PROM. Contrasting, Melamed et al. in their research about fetal gender and pregnancy outcome report that woman carrying male fetuses are at increased risk for prelabour rupture of membranes. [124] Similarly, McGregor et al.in a Cohort study conducted in North America report a higher incidence of PROM among women delivering male newborns compared with female newborns.[125]

In a number of previous studies, male sex is one among other variables linked to early onset neonatal infection and sepsis.[126] In this study, the statistical analysis of differences between fetal genders with regard to development of early onset neonatal infection revealed no significant differences. Sample size may be the explanation for the differences in between recent study results and other studies, thus we consider that or appropriate assessment of whether fetal gender is associated with increased risk of prelabour rupture of membranes or early onset neonatal infection a study with a larger sample size is needed .

Association of birthweight and neonatal outcome is well known. In the recent study the median birth weight of neonates in Term PROM and Preterm Prom group was 3380 g and 2335 g, respectively. The median birthweight of group of neonates with early onset neonatal infection was lower (2446 g) than of neonates without infection (3183 g), the difference being statistically significant ($p<0.05$). Based in recent study analysis, lower birth weight is identified as important risk factor for early onset neonatal infection in pregnancies with prelabour rupture of membranes. This higher rate of EONI among newborns with lower birth weight is expected and is explained by the immaturity of systems of organs including immune system. This is in accordance with the finding that birth weight is a significant predictor of neonatal outcome and it is inversely related to risk of early onset neonatal infection, as observed in other studies.[127]

Physical Health of Newborn

The physical condition of the newborns shortly after the birth has been evaluated by using Apgar score. Apgar scores were determined by a neonatologist in the delivery room. Out of all 200 neonates, median Apgar score ranged from 7 to 9 . Distribution of Apgar Scores depended on gestational age and early onset neonatal infection. Statistical analysis revealed that neonates born from mothers in preterm Prom group with less than 37 weeks of gestation had lower 1st and 5th minute Apgar score comparing with neonates born from Term PROM group of mothers.

The 1st minute score in preterm PROM group was 6.4±1.3 and 1st minute score in term PROM group was 7.3±1.0.5th minute Apgar score was determined as 7.6±1.3 in preterm PROM group and 8.4±0.9 in Term PROM group.

The results show a significant difference for both 1st and 5th minute Apgar score in between neonates in terms of preterm and term PROM group. This result indicates that in pregnancies complicated with PROM, Apgar score of the newborns is affected by gestational age at birth, respectively lower Apgar Scores are found at earlier gestational ages. This result is in accordance with the results of other studies. Similarly, Tanir et al.[128] and Kirmizi et al. [129] in has investigated the impact of preterm premature rupture of membranes in neonatal outcome. Authors report that Apgar score is mainly affected by prematurity rather than premature rupture of membranes.

In the recent study, evaluation of 1st and 5th minute Apgar score among neonates with and without neonatal infection showed that Apgar scores were lower in group with neonatal infection with significant difference ($p<0.05$) compared to group without neonatal infection. The recent study analysis identifies 1st minute Apgar score of 5.7±1.5 as a strong risk factor for development of early neonatal infection, thus this finding indicates observation and screening of neonate for possible early onset neonatal infection. In accordance with our results Hayun et al. [130] reported that Apgar score, gestational age and weight at birth are risk factors for EONI. In their study they found that early onset neonatal infection occurrence on newborns with 1st minute Apgar score <7, is increased for 14.05 times. Similarly, another study [131] conducted in India reported the early onset neonatal infection risk increased to 11.1 times if 1st minute Apgar score <7.

EONI in Pregnancies complicated with PROM

Early onset neonatal infection remains one of the most serious complications in pregnancies complicated with PROM and poses a major health challenge especially for developing countries with higher rates of early neonatal morbidity and mortality reported. In the current research, out of 200 newborns born following PROM, 13 % of them had early onset neonatal infection. Given incidence is comparable with the results of some other studies, but literature search has revealed a wide variation of EONI incidence in pregnancies complicated with PROM.

Recent research finding is in congruence with Asindi et al.[70] who report an incidence of 14 % of neonatal infection after PROM.[103] In contrast, Popowski et al.[132] in a study among 399 pregnant women complicated with PROM, reported that 4.3 % of newborns were diagnosed with EONI.[2] Similarly ,a lower incidence (11.5 %) of EONI and in contrast with recent research results is reported by Tiufekchieva et al. [133] Meanwhile, Wu et al.[69] in a cohort study conducted in China has reported a much greater incidence (25 %) of EONI in pregnancies complicated with PROM. The observed rate of EONI in pregnancies complicated with PROM Indicates a high neonatal infectious morbidity rate comparing with the results of more developed countries.

Pneumonia, sepsis, SIRS and meningitis were the main clinical syndromes that have been associated with early onset neonatal infection. Pneumonia was the most common clinical syndrome representing 46.2 % of cases with EONI, followed by sepsis registered in 38.5 %, SIRS in 11.5 % while cerebrospinal fluid positive culture respectively meningitis was determined in 3.8 % of cases.

Out of all 200 newborns, early onset neonatal sepsis was proven in 5 % of cases. This finding is in agreement with Alam et al.[134] conducted a cohort study over a five years period. Authors analyzed neonatal outcome among neonates who had a maternal history of PROM and they report a incidence of early neonatal sepsis to be 4 %. Meanwhile in another prospective study among 135 infants born after PROM , the reported incidence of early onset neonatal sepsis is 8.1 %.[135] Similarly Lee at al.[100] report an incidence of 6.5 % of culture of proven sepsis among neonates born from pregnancies complicated with PROM.

This greater incidence of sepsis among infants born after PROM might be due to the fact that in these previous studies are enrolled newborns born after PROM with a latency period of greater than 24 hours, whereas in our research are included newborns born after PROM in which cases the duration of latency period was restricted to a minimum of 1 hour and maximum of 72 hours.

Among neonates in term PROM group there was no case of early neonatal death whereas among infants born after preterm PROM, two cases of neonatal death were registered and both infants were previously diagnosed with confirmed sepsis. The resent research finding of the EONI rate of 13 % in pregnancies complicated with PROM, confirms that neonatal infection continues to be one of the major contributors of early neonatal morbidity and mortality of newborns in Kosova. Considering that a number of newborn deaths can be prevented with effective health measure during pregnancy at birth and during the first week of life, this research may contribute to a in decrease in early onset neonatal morbidity and mortality through early prediction of EONI in pregnancies with available markers of infection.

Prediction of EONI in pregnancies complicated with PROM

Although several researches are undertaken in order to find efficient antenatal marker for prediction of EONI, prediction of early onset neonatal infection in pregnancies with PROM remains critical challenge. This current research represents the findings about the accuracy of prediction of EONI of the maternal serum C reactive protein, maternal serum WBC and amniotic fluid glucose concentration in patients presenting with PROM.

Analysis of maternal plasma CRP revealed a significant correlation between C-reactive protein and early onset neonatal infection ($p<0.05$). Median maternal CRP in patients with PROM who delivered newborns with EONI was significantly higher than in patients who delivered newborns without neonatal infection (26.7 versus 7.6 mg/L). The cutoff value of >6 mg/L predicted early onset neonatal infection with a sensitivity of 92.3 %, a specificity of 60.9%, a PPV of 26.1 % and an NPV of 66 %. Area under receiver operating characteristics (ROC) curve for the maternal serum CRP was 0.84 (95 % CI,0.745-0.934).

Consistent with the recent study finding, Xie et al. found plasma CRP levels to be elevated in pregnancies complicated with PROM and associated with infection.They report that the concentration of CRP at admission appears to be an accurate marker for the prediction of early onset neonatal infection with a sensitivity of > 90 %. [136]

Jeon et al.[137] in their study about diagnostic performance of maternal CRP in predicting early neonatal sepsis has observed that maternal CRP was significantly higher in neonatal sepsis group than in control (3.55±2.69 vs. 0.48±0.31 mg/dL, p=0.0001). The cutoff value of >1.22 mg/dL (12.2 mg/L) predicted early onset neonatal infection with a sensitivity of 71% and specificity 84% .Their results are in same in line with the recent research when taking in consideration the cutoff value used. Based on the obtained results, authors suggest that in newborn of CRP positive mother, the clinician may be alerted to earlier evaluation for possible neonatal infection prior to development of sepsis.

Lee et al.[100] reported that maternal serum CRP level obtained up to 72 hr before delivery is an independent predictor of early-onset neonatal sepsis in women with preterm PROM. Their study showed that maternal serum CRP levels ≥ 8 mg/L predicted early onset neonatal infection with a sensitivity of 67.7%, a specificity of 63.3%, a PPV of 17.2%, and an NPV of 94.6%. Authors conclude that the serum CRP level < 8 mg/L has a good negative

predictive value in excluding early-onset neonatal infection, and may therefore be a useful non-invasive adjunct to clinical judgment to identify low-risk patients.

Torbe et al.[93] compared the prognostic value of maternal WBC, CRP and plasma procalcitonin (PCT) determinations in the prediction of early neonatal infection in 142 pregnant women complicated with PROM at different gestational ages. They found that the sensitivity, specificity , PPV and NPV of WBC was 48 %, 85 %, 63 % and 76 % respectively using cut off value of 15.0 g/L while the sensitivity, specificity, PPV and NPV of CRP was 52 %,76 %,52 % and 76 % using cut off value of 10 mg/L.

Another study conducted by Popowski et al.[132] investigated maternal markers for detecting EONI and chorioamnionitis in cases of premature rupture of membranes at or after 34 weeks of gestation. In their investigation conducted in 399 woman with PROM, 4,3 % of the newborns had an early onset neonatal infection. Authors reported that white blood cell counts and C-reactive protein concentrations were significantly associated with early-onset neonatal infection. Their study shows that a CRP concentration of 5 mg/L or higher predicts EONI with high sensitivity, thus the authors suggest a routine measuring of CRP as a useful marker for selecting a population among whom the risk of EONI is high.

The above results are consistent with our study but during literature search we also found conflicting results reported by other authors. Van der Heyden et al.[138] studied the accuracy of measuring CRP and leukocytes in maternal serum to predict neonatal infection among 299 woman with PROM. The results showed that in women with PROM, CRP and leukocytes should not be measured routinely.

Based on recent study result on terms of maternal plasma CRP usefulness as a predictor of early onset neonatal infection we suggest routine measurement of CRP as a screening tool in women presenting with prelabour rupture of membranes in order to classify high and low risk groups for early onset neonatal infection. This additional test would help obstetricians in decision making process for appropriate management of these cases.

The recent research also evaluated the usefulness of maternal leukocyte count in prediction of EONI. Leukocytosis, defined as a white blood cell count greater than $11x10^9$ /L typically reflects the normal response of bone marrow to an infection. In pregnancy occurs a physiologic increase of WBC which is attributed to physiologic stress and increased

inflammatory response associated with pregnancy. Because of this physiologic increase of leukocyte count in pregnancy, in the recent research the cutoff point used for leukocyte count was $\geq 14x10^9/L$. Analysis of our data in terms of WBC use in prediction of EONI showed a sensitivity of 57.6 %, a specificity of 68.3%, a PPV of 21.4 % and an NPV of 91.5 %.

Area under ROC curve for the maternal serum leukocyte count was 0.623 (95 % CI,0.507-0.740). The association between EONI and maternal leukocytosis alone or in combination with other markers, were widely analyzed.

Yoon et al.[139] has compared diagnostic performance of maternal blood C-reactive protein, white blood cell count and amniotic fluid WBC in the identification of the neonatal infectious morbidity. In the same line with the results of recent research, authors reported that maternal WBC showed a poor predictive value of neonatal infectious morbidity among pregnancies complicated withPPROM.

Another study conducted in Thailand evaluated the diagnostic performance of maternal WBC, maternal serum CRP and neutrophil count in the detection of infection among women with PROM. Their results are in agreement with the recent research finding[140] Meanwhile ,a study conducted in Korea indicates that maternal WBC are useful in antenatal prediction of EONI .Based on their report, elevated maternal WBC are associated with the likelihood of histologic chorioamnionitis, shorter interval to delivery, clinical chorioamnionitis, and neonatal sepsis ($P < .05$ for each).[141]

Although controversy results are found during the literature search, the recent study findings indicate a poor predictive value of maternal leukocyte count in prediction EONI thus we suggest that in pregnancies complicated with PROM, leucocytosis should be interpreted with caution and further tests are necessary to predict EONI.

Correlations between amniotic fluid glucose concentration and intra-amniotic infection have been reported previously. It has been suggested that the metabolism of glucose by microorganisms and the consumption of glucose by activated neutrophils reduce amniotic fluid glucose concentrations.[142]

According to a report of 1665 normal pregnancies by Weiss et al.[98] the mean amniotic fluid glucose concentration increased mildly between weeks 14 and 17 of gestation and decreased gradually as the gestational age increased, from 45.9mg/dl between weeks 16 and 17 to 15.8mg/dl during weeks 40 to 42.

Taking into consideration that in normal pregnancy alterations of amniotic fluid glucose concentrations depend on gestational age, studies regarding amniotic fluid glucose concentration in detecting infection of the amniotic cavity and predicting EONI have used different cutoffs of amniotic fluid glucose level depending on the gestational age of the sample studied.

Most of the previous paper, has investigated the clinical significance of amniotic fluid glucose concentration in detection of intra amniotic infection. Dildy et al.[99] evaluated the value of glucose in amniotic fluid to detect microbial invasion of the amniotic cavity in patients with PROM. At a cut-off value of 15 mg/mL (0.832 mmol/L), glucose reached a sensitivity of 73.3 % ,a specificity of 88.1 % and positive predictive value of 68.8 %.Similar results are reported by Buchimski et al. [103] in their study about the use of vaginal amniotic fluid glucose measurements in predicting infection of the amniotic fluid.

To our knowledge this is the first study evaluating the value of vaginal amniotic fluid glucose concentration in prediction of EONI. The cut-off value of glucose, < 0.777 mmol/L (< 14 mg/dl), seemed to be a poor predictor for EONI, with a sensitivity of 57.6 %, specificity of 33.9%, PPV of 11.5%, and NPV of 84.3 %. The AUC was also low, which confirms this marker as poor predictor of early onset neonatal infection. Despite the fact that amniotic fluid glucose determination is rapid, inexpensive and simple test, we suggest that this test it is not useful in prediction of early onset neonatal infection in pregnancies complicated with PROM.

In summary, prediction of EONI in pregnancies with PROM is difficult especially in cases with silent infection. Markers of infection such as procalcitonin, fibronectin and cytokines such as TNF, IL8, IL6 although found to be accurate in other settings, are not available for use in Kosova. The tests are not available for routine use and in uncommon events such as PROM. Physicians must rely in available markers of infection. The recent research found that maternal plasma CRP > 6 mg/L has 84 % predictability of early onset neonatal infection support the recommendation in this study to conduct measurement of

maternal plasma C - reactive protein in every women presenting with PROM. Meanwhile maternal plasma WBC and amniotic fluid glucose concentration has poor predictive value in prediction of early onset neonatal infection, thus we do not recommend routine measurement of these markers but we suggest that maternal plasma WBC and amniotic fluid glucose may be used only as additional tests in combination with CRP for prediction of EONI in pregnancies complicated with PROM.

10. Conclusions

This doctoral dissertation represents the first research of its type conducted on a population of pregnant women in Kosova. Among determining the accuracy of the CRP, WBC and glucose in prediction of EONI in pregnancies complicated with PROM, the research answered to different aspects of pregnancies complicated with prelabour rupture of membranes including the most common risk factors associated with PROM, early onset neonatal infection rate, associated risk factors for development of early onset neonatal infection. Additionally, the study revealed the most common maternal complications, mode of delivery and the most common indications for cesarean delivery in term and preterm PROM,

Based on the results of this research the following can be concluded:

- Primiparity, previous PROM, low socioeconomic status, and maternal genital tract colonization are the identified risk factors for prelabour rupture of membranes.
- Prelabour rupture of membranes at term may indicate early sign of cephalo pelvic disproportion while preterm prelabour rupture of membranes requires close monitoring because of common fetal distress.
- Retained placenta, chorioamnionitis and postpartum haemorrhage are the most common maternal complications in pregnancies with prelabour rupture of membranes
- The majority of pregnant women in Kosova- as evident by the high antenatal care service utilization rate- are aware of and have knowledge about importance of antenatal care. It is worth noting, however, that the recommended components of antenatal care are only partially being implemented. The study findings lead to a recommendation for better adherence to antenatal care, and specifically, the need to increase awareness of the importance of screening and treatment of genital infections, and to advocate for women to stop smoking, especially during pregnancy.
- The finding of Early Onset Neonatal Infection rate of 14 %, of which 5 % were confirmed Early Onset Neonatal Sepsis, means that neonatal infection is one of the major threats in pregnancies complicated with PROM.
- Associated risk factors for development of early onset neonatal infection in pregnancies complicated with PROM are: PROM- delivery interval, maternal colonization, gestational age and gestational weight at birth.

- Maternal serum CRP is the most accurate marker (predictability of 84 %) for prediction of EONI in pregnancies complicated with PROM and thus it is recommend to routinely conduct CRP measurement in every patient presenting with PROM.
- Maternal plasma WBC and amniotic fluid glucose concentration alone has poor predictive value in prediction of early onset neonatal infection, thus we suggest that maternal plasma WBC and amniotic fluid glucose may be used only as additional tests in combination with CRP for prediction of EONI in pregnancies complicated with PROM.

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ANEX

12. Survey and data collection instrument

A. Maternal Data

1.	**Age**	≤ 20	☐
		21-30	☐
		31-40	☐
		41-50	☐
2.	**Level of ecucation**	Elementary	☐
		Secondary	☐
		University	☐
3.	**Employment status**	Employed	☐
		Unemployed	☐
4.	**Economic status**	Low	☐
		Medium	☐
		High	☐
5.	**Family size/ Persons living in a family**	2-4	☐
		5-10	☐
		10-15	☐
6.	**Living area**	Rural	☐
		Urban	☐
7.	**Parity**	Primiparous	☐
		Multiparous	☐
8.	Previous deliveries, if multiparous	Vaginal	☐
		Cesarean	☐

9.	**Previous PROM**	Yes	☐
		No	☐
10.	**Previous abortions**	Yes	☐
		No	☐
11.	**Previous surgical interventions in the uterus**	Yes	☐
		No	☐
12.	**Smoking in pregnancy**	Yes	☐
		No	☐

B. Antenatal Care in current pregnancy

1.	**Number of ultrasound scans**	……	
2.	**Number of pelvic exams**	……	
3.	**Folic acid supplementation**	Yes	☐
		No	☐
4.	**Screening for anemia**	Yes	☐
		No	☐
5.	**Screening for genital infection**	Yes	☐
		No	☐
6.	**Screening for urinary infection**	Yes	☐
		No	☐
7.	**Blood pressure check up**	Yes	☐
		No	☐
8.	**Weight gain follow up**	Yes	☐
		No	☐

C.	**Markers of Infection/ Laboratory Data**	**Referent value**
1.	CRP ……	> 6 mg/ L

2.	WBC …….	≥ 14 x 10 ^{9} / L
3.	Glucose …….	≤ 0.777 mmol/L.
4.	Vaginal swab result	…….

D. Birth data

1.	Interval Period in Hours	……
2.	Mode of delivery	Spontaneous ☐
		Induced ☐
		Cesarean ☐
3.	Indications , if Cesarean Delivery	……

E Newborns Data

1.	Gestational week at birth	…….
2.	Gender	Male ☐
		Female ☐
3.	Weight / grams	…….
4.	Apgar Score 1st and 5th minute	……. ……..
5.	Neonatal infection	Yes ☐
		No ☐
6.	Type of Neonatal Infection	……
7.	Hemoculture result if taken	……
8.	Lumbal puncture result,if taken	……
9.	Early Neonatal Death	Yes ☐
		No ☐

F. Other Maternal / Neonatal Data

1. Maternal complications …….

2. Length of hospital stay/ Days …….