5.1. Preterm birth and low birth weight:

Preterm birth was reported to be responsible for mortality among at least 28% of the early neonatal deaths (within 7 days after birth) (Lawn JE et al 2006). Complications of preterm birth are attributed as “the single largest cause of neonatal deaths, responsible for 35% of the world’s 3.1 million deaths a year, and the second most common cause of under-5 deaths after pneumonia” (Lawn JE et al 2009). The increasing incidence of preterm births in the last two decades has been reported as a global concern (Beck S et al 2010). The World Health Organization Global Survey on Maternal and Perinatal Health (WHO-GS) had reported the rates of preterm births among low and middle income countries compiling the data obtained during 2007-2008 from 22 countries of Latin America, Africa and Asia. The overall rate of preterm births from these 22 countries was 8.2%. The preterm birth rate from India was 15% placing the sub-continent at the top of the list (Vogel JP et al 2014). Low birth weight (LBW) defined by the WHO as “birth weight less than 2500 gms or 5.5 pounds” (WHO report 1992) is reported as one of the most important cause of perinatal mortality and morbidity. Low birth weight in majority of the cases is a consequence of preterm birth or intrauterine growth restriction (IUGR) (Wilcox AJ 2001). The primary reason for low birth weight in developing countries was reported to be IUGR (Ugbona & Onyearugha 2013). Increased susceptibilities to infections during childhood, impaired neurological development, low IQ levels and diabetes mellitus (Olsen J et al 2001 and Thomas N et al 2013) were most commonly reported consequences of LBW. Intrauterine infections during pregnancy were associated with low birth weight. Among the babies born to women having intrauterine infections during their pregnancy, lesions of the cerebral white matter of the foetus, and cerebral palsy were reported due to the exaggerated maternal inflammatory responses (Murphy DJ et al 1995 and Verma U et al 1997). The global incidence of low birth weight was reported as 15.5% with rates varying among the more developed (7%), less developed countries (16.5%) and least developed countries (18.6%). India was ranked on the top of the list with close to 8 million babies born with low birth weight at a rate of 30% (UNICEF -WHO global estimates of LBW 2004). Low birth weight was considered as a preventable adverse outcome of pregnancy if, effective interventions were employed to prevent preterm birth and IUGR (Institute of Medicine; 1985).

Preterm birth rate among the present study population (7.6%) is lower compared to the rates reported by the WHO for developing countries (9-16%) as well as the previously
reported estimate of 15% among Indian women (Vogel JP et al 2014). Further, reduced rate of low birth weight (11.4%) was observed in the present study population compared to the previous estimate of 30%. The primary objective of this present study was to determine the association of maternal genitourinary and periodontal infections with adverse pregnancy outcomes among a cohort of pregnant women. Considering this objective, women with known confounders for preterm birth and low birth weight such as HIV, diabetes mellitus, hypertension, thyroid abnormalities, and history of smoking, alcohol consumption were excluded from the study. In addition, women with obstetric complications like placenta previa, cervical encirclage and multiple pregnancies (>3 prior pregnancies) were also excluded from the study. Primarily, our study population consisted of young women (mean age of 27.18±3.5yrs), well educated (>66.7% completed more than 12 years of their schooling and, >68% with monthly household incomes of >10,000INR). The present study was carried out at Udupi taluk, which is considered as one of the most developed taluk in Karnataka, having high literacy rate, good health care facilities (Nair NS et al 2000). Thus, the overall selection criteria (exclusion of women with known confounders) of the study population could be the reason for low preterm birth and low birth weight rates compared to the higher rates reported from other parts of India (Avachat et al 2013, Thomre PS et al 2012, Mumbare SS et al 2012 and Vogel JP et al 2014).

Though the exact reasons that lead to preterm birth are not understood, studies suggest 40-45% of the preterm births are due to spontaneous preterm labor; 30-35% is medically induced or elective deliveries and 20-25% due to preterm premature rupture of membranes (PPROM) (Conde-Agudelo et al 2011). Among our study population, medically induced preterm labor (elective LSCS) was observed among 12.9% of the women, PPROM was seen among 18.5% and spontaneous preterm birth rate was 68.6%. Recent report from lower and middle income countries state that the rate of spontaneous preterm birth occurs in approximately 75% of the total preterm births reported (Vogel JP et al 2014). This could be due to the inadequate antenatal care (<3 visits) which is considered as a major risk factor for adverse pregnancy outcomes among Indian women (Nair NS et al 2000, Thomre PS et al 2012). Lower rate of medically induced preterm labor in our study can be attributed to the timely and prompt antenatal care provided to all the study population and more importantly, all the women visited the obstetrician for at least three antenatal care check-ups during their pregnancy.
Spontaneous preterm birth is a multifactorial process with its complete etiology still unclear. From a teaching hospital of north India the preterm birth rate of 15% was reported recently among rural population. (Avachat et al 2013). However, the preterm birth rate among our study population was lesser in comparison to this study. The reason for the lower rate of preterm birth among our study population can be due to dissimilarity in the study population between the studies. Majority of our study population was primigravida (58.3%) while those studied by Avachat et al were multigravida women, which is a risk factor for preterm birth. Though we did not find a statistically significant association with parity in the present study population (p=0.098), higher rate of preterm birth (14.8%) was observed among third gravida women in comparison with primigravida women (7.4%). Maternal demographic features like religion, occupation, age and monthly household incomes did not show any statistically significant association (Table 4.3.2a) with preterm birth and low birth weight in the present study. From a multinational study (22 low and middle income countries), maternal age less than 18 years, women being illiterate, and women belonging to lower socio-economic status were reported as risk factors for spontaneous preterm birth (Vogel JP et al 2014). Since the study populations in the present study were all above 18 years of age, only two women were illiterate and majority (>68%) of the women belonged to middle income group (monthly household income >10,000 INR), lack of an association of maternal age, socio-economic status and level of educations with preterm birth and low birth weight was not surprising.

In the present study, 329 women had previous pregnancies, of which 13.9% had a history of previous abortion. Of the 219 women who had live birth as an outcome in their previous pregnancy, 13 (5.9%) of them had preterm labor. Of these 13 women, only eight women completed their study tenure under our supervision while the other five women did not turn up for follow up visits. Even with this small number of cases, previous history of preterm labor was found to be a statistically significant risk factor (on Chi-square analysis) for both preterm birth (Risk ratio: 5.1 (2.01-12.98), p=0.01) and low birth weight (RR: 4.49 (2.17-9.27), p= 0.008). However, we did not observe previous history of preterm labor as an independent risk factor (on multivariate logistic regression) among our study population. Similar association of previous preterm labor as a risk factor for preterm birth was reported previously from various countries (Olugbenga A et al 2010, Zhang Y-P et al 2012, Avachat et al 2013, and Vogel JP et al 2014). In the advent of these reports suggesting the role of genetic background as a risk factor for preterm birth among few
women (Svensson AC et al 2009, Karajalainen MK et al 2012), our findings of recurrent spontaneous preterm labor in a woman warrant the need for further studies to find the association between genetic factors and preterm birth in Indian women.

Maternal physical factors like weight, height and BMI were reported to have a significant association with preterm birth and low birth weight. Maternal obesity and overweight were reported as significant risk factors from a meta-analysis recently (McDonald SD et al 2011). In the present study, we did not find a statistically significant association of maternal weight, BMI with preterm birth and low birth weights. Similar lack of association between maternal BMI (pre-pregnancy and during first antenatal visit) with preterm birth and low birth weights was also reported among Thai women (Liabsuetrakul T 2011). However, maternal height <150cm was found to have a statistically significant association with preterm birth (Risk ratio: 1.86 (1.20-2.83), p=0.008) and low birth weight (RR: 2.38 (1.70-3.33), p= <0.01). These observations are in agreement with the previously reported research investigations in which association of lower maternal height (<145 cm) with preterm birth and low birth weight among Asian women was reported (Vogel JP et al 2014 and Rao CR et al 2014). While pre-pregnancy maternal weight was not found to have an association with the pregnancy outcomes in the present study, poor maternal weight gain (<6kgs) as determined by the difference between weights during third trimester (POG: >32 weeks) and weight during first visit (12-16weeks of POG) was found to have a statistically significant association with low birth weight (RR: 1.93 (1.39-2.69), p = <0.01). Similar association of poor maternal weight gain during pregnancy and low birth weight of the infants was reported previously by Schieve LA et al 2000 and WY Hsu et al 2013.

Association of maternal intrauterine infections with adverse pregnancy outcomes like PTB and LBW has been proposed in the late 1970’s (Bobitt JR et al 1977, Russell P 1979). Since then, numerous studies have provided evidence supporting the role of the subclinical intrauterine infections and the inflammatory responses in the causation of preterm birth (Hauth JC 1998, Goldenberg RL 2000, Sadowsky DW et al 2003 and Ilievski V et al 2010). In this context, microbiological screening of the urogenital tract infections mainly the urinary tract (Romero R 1989) and the lower genital tract infections (Gravett MG 1986, Holst E 1994) evolved as good predictors for the diagnosis of intrauterine infections. Various research investigations suggesting the role of ascent of vaginal flora in microbial invasion of the amniotic cavity (MIAC) among 12-22% of the women with
Discussion

preterm labor supported the need for screening of lower genital tract infections among pregnant women (Romero R 2007, Agarwal A 2012). Despite significant research in understanding the pathogenesis of preterm birth and low birth weight due to lower genital tract infections, the exact mechanism still remain unclear. Meanwhile, the associations of extra uterine infections like periodontitis and pyelonephritis have been proposed in the causation of preterm birth (Fan YD 1987, Offenbacher S 1996).

Considering the previously reported association of maternal genitourinary and periodontal infections among pregnant women with adverse pregnancy outcomes from various parts of the world, and relative paucity of data from Indian women, a prospective hospital based cohort study was undertaken to find the proportion of women having these infections during their early pregnancy (8-24 weeks of gestation) and their association with adverse pregnancy outcomes like preterm birth and low birth weight.

5.2. Bacterial Vaginosis:

In the present study, Clinical diagnosis of BV was carried out using Amsel’s criteria whereas the laboratory diagnosis of BV was carried out using Nugent’s scoring system. Nugent’s scoring system (NSS) was considered as the reference method to evaluate the efficacy of Amsel’s criteria in diagnosis of BV. Amsel’s method positivity for BV (when ≥3 of the 4 criteria were positive) was 76.2% sensitive and 98.8% specific when compared with NSS. Schwebke JR et al 1997 reported 70% sensitivity and 94% specificity of Amsel criteria among non-pregnant women for the diagnosis of BV in comparison with NSS. Our findings regarding the specificity of Amsel' method in diagnosis of BV among pregnant women are in concordance with previously reported studies (Tam MT et al 1998, Gratacos E et al 1999 and Sha et al 2005), which indicated high specificities of 95% to 99%, of the Amsel criteria in comparison with NSS. However, the sensitivity of Amsel’s method in our study was higher (76.2%) in comparison with the sensitivities reported in the same studies (35% to 46 %). The measure of agreement between Amsel’s method and NSS was found to be 0.73 indicating the reliability of Amsel’s criteria for the diagnosis of BV.

We further studied the sensitivity and specificity of each of the four Amsel’s criteria in the diagnosis of BV and found that the presence of clue cells (Sensitivity: 100%, Specificity: 100%) as the most reliable diagnostic criterion for the BV whereas the presence of thin homogenous vaginal discharge was found to have the least sensitivity (64.5%) and specificity (82.6%). Positive whiff test and vaginal pH >4.5 showed good specificities
(96.4% and 87.6% respectively) but had poor positive predictive values (41.6% and 15.6% respectively). These results indicate that the presence of clue cells and positive whiff test were more specific in the diagnosis of BV. Similar findings recommending the use of clue cells and whiff test as diagnostic criteria among pregnant women for the diagnosis of BV was reported previously by Elyan A & Rund N 2004 and Zarakholu P et al 2004. Mittal V et al (2012) studied the efficacy of individual Amsel’s criteria in comparison with NSS among 200 antenatal women and recommended the utility of vaginal pH >4.5 and positive whiff test due to their high sensitivity and positive predictive values (PPV). The vaginal pH >4.5 had a high sensitivity (95.2%) in the present study which was similar to the sensitivity (91.7%) reported by Mittal V et al. However, the PPV for vaginal pH >4.5 was low (15.6%) as compared with the PPV reported (36.7%) by Mittal et al 2012. In view of the low PPV of elevated vaginal pH (despite the high sensitivity) observed from both studies and transient variations in the vaginal pH during pregnancy, we assume the utility of this individual criterion for the diagnosis of BV during pregnancy can lead to over diagnosis. In the present study, we also observed positivity of more than 3 Amsel’s criteria among three (4%) of 75 women diagnosed with vaginitis due to bacterial etiology. At least two Amsel’s criteria were positive among 17 (22.6%) of the women with vaginitis (Figure 4.1.1h). However, these findings could not be compared with previous reports due to the paucity of such comparison in the reported literature. Using PCR based assays Srinivasan S et al 2012 have reported the positivity of Amsel’s criteria in the women when colonized with various bacteria in the absence of frank BV (Srinivasan S et al 2012). We assume the false positivity of the Amsel’s individual criteria in women with vaginitis in the current study also might be due to the presence of bacteria like *Atapobium* spp and *M. hominis*, which could not be identified using smear microscopy. These findings emphasize the need for future studies to characterize the vaginal flora using molecular methods among women with positive Amsel’s criteria.

In the present study population, a total of 21 (2.6%) and 22 (2.7%) of the women were BV positive as diagnosed by NSS and Amsel’s method (≥3 criteria positive) respectively. It is to be noted that among the 21 women diagnosed as BV by NSS, 16 (76%) of them were also diagnosed as BV by Amsel’s method and only five women who had BV by NSS were not positive for BV using Amsel’s method. However, presence of clue cells was observed in all the women indicating its high specificity and sensitivity in diagnosis of BV. Six women who were diagnosed with BV using Amsel’s criteria had Nugent’s Grade II flora.
Of the 115 women having Nugent’s Grade II flora, 23 (20%) women were positive for whiff test, 48 (42%) had vaginal pH > 4.5 and 38 (33%) had thin homogenous vaginal discharge. In the present study, low specificity of individual Amsel’s criteria (except clue cells) was observed. Similar findings were reported by various other studies previously (Krohn MA et al 1989, Nelson DB 2002, Srinivasan S et al 2012).

Nugent’s scoring system is the gold standard method and is the most widely used for the diagnosis of BV (Mohanty A et al 2010 and Chawla R et al 2013). According to the bacteria which constitute the vaginal flora, this scoring system classifies women as those harbouring normal vaginal flora (Grade I), intermediate flora (Grade II) and abnormal flora (Grade III flora) (Nugent RP, M. A. Krohn and S. L. Hiller 1991). Interobserver measure of agreement of Nugent’s scoring system between various observers was reported to be excellent indicating its high reproducibility as a diagnostic test (Mohanty A et al 2010). In the present study, of the 790 women, 654 (82.7%) had normal vaginal flora, 115 (14.5%) of them had intermediate vaginal flora and 21 (2.6%) had abnormal or BV flora.

NSS is primarily based on the counts of bacterial morphotypes of Lactobacilli, G.vaginalis and curved Gram negative bacteria (Mobilincus forms). In the present study, presence of Mobilincus forms on smear examination or on culture was not observed. However, presence of more than 20% clue cells, >20 G.vaginalis forms /OIF and Gram negative bacilli (Bacteroides forms) were summed up to obtain a score ranging between 7-10 (indicative of BV). Thus, considering the original NSS as proposed by Nugent RP et al, the proportion of women having BV was 21 (2.6%). We report these women as those having “Full BV” as suggested by Donders G et al (2009). In our study, we noticed 27 (3.4%) women with reduced vaginal lactobacilli, presence of streaks of abnormal vaginal flora (predominately anaerobic gram negative bacilli) and occasional clue cells (<20% clue cells). Using NSS, all these women were grouped as those having intermediate vaginal flora (4-6). However, using the criteria as reported previously by Donders G et al (2002), we report these women as having “Partial BV”. Finally, the proportion of women having bacterial vaginosis (Full BV + Partial BV) in our study population was 48 (6%).

From a previous systemic review on global epidemiology of BV, the prevalence was reported ranging between 4-49% (Kenyon C et al 2013). Among pregnant women, the reported rates in other parts of the world ranged between 5.9- 21.6 % (Table 2.5.3). In India, the rates of BV reported from pregnant women varied with the demographic characteristics of the study population. From a community based study comprising 680
reproductively active women (with a subgroup of 78 pregnant women) in Karnataka, Balamurugan SS et al (2012) reported 6.4% of BV. In another study (Hospital based study from New Delhi, India) with 150 antenatal women screened during their last trimesters of pregnancy (28-35 weeks of gestation), Laxmi U et al (2012) reported an incidence of 24.3% of BV. Besides these studies, Mittal et al (2012) examined 205 pregnant women complaining of excessive vaginal discharge at a tertiary care hospital in Lucknow and reported 12% prevalence of BV among their study population. In our study population, the rate of BV (6%) is lower in comparison with the above reports probably due to multiple reasons. Prevalence of BV was reported to be high among women from low socio-economic strata (Koumans EH et al 2007), who had the habit of smoking (Larsson PG et al 2007), low levels of education and history of having multiple sex partners (Misra DP and Trabert B 2007). On contrary, our study population had women primarily belonging to the middle income groups (>68% with monthly household income of >10,000 INR), mostly literate with no history of smoking). We observed no statistically significant association of socio-economic status (0.69), levels of education (p=0.273) maternal age (p=0.817) and parity index (p= 0.835) with BV in our study population. In Indian context, very few researchers have investigated the prevalence of BV among pregnant women. Though there have been few studies that reported the prevalence of BV from various parts of the country, lack of conclusive results between these reports is evident, probably due to the lack of power in the sample size to report the exact prevalence (Balamurugan SS et al 2012, Laxmi U et al 2012 and Mittal V et al 2012). In this context, our study gains importance due to the adequate sample size, different methods used for the assessment of BV (NSS and Amsel clinical criteria) and the overall methodology employed. To the best of our knowledge, presence of partial BV among pregnant women was not reported previously in any studies involving Indian women.

5.3. Vulvovaginal Candidiasis:
Global estimates suggest that at least 70% of the women in their childbearing age suffer from at least one episode of vulvovaginal candidiasis (VVC) and 8% of these women have a recurrence (Sobel JD 2007). Among pregnant women, prevalence of VVC was reported in about 44% of the women (Dias LB et al 2011). It was proposed that the confirmed diagnosis of VVC in a woman can be done only after a thorough clinical examination followed by microbiological laboratory based confirmation (Sobel JD 1998). This two
stepped diagnosis poses a challenge in large scale epidemiological studies to estimate the real incidence of VVC. Moreover, availability of anti mycotic drugs over the counter has been a major setback in estimating the real incidence (Sobel JD 2007 and Achkar JM and Fires 2010). Emphasis on laboratory based diagnosis is due to the presence of Candida in at least 20-30% healthy asymptomatic women as a part of their normal vaginal flora. So, mere culture positivity of a woman in the absence of clinical signs and symptoms was referred as colonization (Beigi RH et al 2004). Misdiagnosis of 77% of the women as VVC based on clinical findings alone, by treating physicians was reported, adding more weight to the need for microbiological confirmation of VVC (Schwiertz A et al 2006). Hence, the treatment based on clinical findings alone was not recommended considering the lack of specific pathognomic features for the diagnosis of VVC (Abbott J 1995 and Eckert LO 1998, Linhares LM et al 2001, Sujith D Rathod 2011). In the present study, presence of white curdy discharge which is a characteristic feature of VVC was observed among 131 (16.5%) of the women. Culture positivity for Candida was seen among 124 (15.6%) of the women and presence of Candida on Gram stained high-vaginal smears was observed among 98 (12.4 %) of the women. Considering the disparity in detection rates using the above three diagnostic modalities, efficacy of smear examination and presence of white curdy discharge were compared to culture positivity as a reference method (Achkar JM and Fries BC 2010). Of the 124 women who had culture positivity for Candida, smear examination was positive for 90 (72.5%) women and presence of white curdy discharge was observed in 50 (40.3%) women. The sensitivity of smear examination (78.4%) in the present study is higher than 60% as reported previously by Sobel JD 1998. As proposed by Sobel JD et al (1998), the disparities in sensitivities of smear examination for VVC in comparison with culture can be attributed to the load of yeast cells present in the specimen. In a study to estimate the prevalence of VVC among non-pregnant women, Jindal N et al (2007) reported the detection rate of Candida by culture as 23% and that of smear examination was 19%. Difference in the detection rates of Candida by culture and smear examination in the present study was 3% which is close to the value reported (4%) by Jindal N et al 2007. Similar findings indicating the superiority of microbiological culture techniques for diagnosis of VVC over clinical signs and symptoms and smear findings were reported previously (Linhares LM et al 2001). The positive predictive value (57.8%) of presence of curdy white discharge for the diagnosis of VVC in the present study is in agreement with the findings reported previously that 50% of the women with
symptoms of VVC can have other infectious causes of vaginitis other than Candida (Mendling W et al 2012).

Of the 124 women who had cultures of the high-vaginal swabs positive for Candida, presence of pus cells (>5 cells/OIF) suggestive of inflammation due to Candida infection was seen among 91 (11.5%) women. Thus, we report the proportion of women having VVC in our study population as 11.5%. In order to avoid reporting of the mere colonizers, which as discussed previously can occur in 20-30% of the healthy women, combination of smear microscopy (presence of pus cells) along with culture positivity was employed in our study. Supporting our hypothesis, we found 33 (26%) of the study population had, Candida grown in culture but their vaginal smears showed no signs of inflammation. Recently, a community based study among south Indian women to evaluate the risk factors for VVC revealed that factors such as age at initiation of sexual activity, previous diagnosis of BV and absence of lactobacilli on vaginal smears were significantly associated (Rathod SD et al 2012). In the present study, no statistically significant association of VVC with maternal age (p=0.105), level of education (p=0.148), occupation (p=0.06), monthly household income (p=0.69) and parity index (p=0.619) was observed.

5.4. Trichomoniasis:
Trichomoniasis is considered as one of the most prevalent, non-viral sexually transmitted infections, most commonly seen among reproductively active age women (Abdurehman Eshete 2013). The most common presentation among women is vaginitis. However, it remains asymptomatic in at least 50% of the women. Among the women who have symptoms of greenish yellow frothy discharge, vaginal inflammation, vaginal soreness and strawberry cervix were the other pathognomic features reported (Petrin D et al 1998 and Swygard H et al 2004). In the present study, yellowish frothy to purulent discharge was seen among 220 (28%) of the women. Among these 220 women, trichomoniasis was observed among 44 (20%) women. In a study among non-pregnant women, frothy discharge was reported to be present only among 12% of the women with trichomoniasis (Anorlu RI et al 2001). In the present study, vaginal pH >4.5 was observed among 24 (25.5%) of the women with trichomoniasis (92, 11%). Similar observation about high vaginal pH (>4.5) was reported among women with trichomoniasis (Cotch MF et al 1997). However, the elevated vaginal pH was also reported to be observed in women with BV (Franklin TL and Monif GR 2000). Like the other causes of vaginitis (either due to VVC
and BV), diagnosis of trichomoniasis based on clinical findings can always be misleading in 80% of the women (Schwiertz A et al 2006) as the clinical signs like vaginal discharge and inflammation are not specific for trichomoniasis alone. Misdiagnosis of trichomoniasis based on clinical findings alone among pregnant women has been reported in the literature (Simoes JA et al 1998 and Perazzi BE et al 2010). Presence of motile trophozoites of *T. vaginalis* in the presence of leucorrhoea was reported as the diagnostic feature. Physiological saline mount of the vaginal secretions was useful in visualizing the motile trophozoites (tumbling motility) immediately after the collection (Kingston MA et al 2003). The sensitivity of direct wet mount examination as reported in the literature had a broad range of 35-80% (Radonjic IV et al 2006, Perrazi BE et al 2010). The “gold standard” or the “reference standard test” for the diagnosis of trichomoniasis has been the culture of *T. vaginalis* from the vaginal secretions using selective media (Sobel JD 1997, Petrin D et al 1998, Patel SR et al 2000). In the present study, we used saline wet mount at the patient bed side as the key diagnostic test for trichomoniasis. The use of smear microscopy instead of culture techniques in low resource settings was recommended previously after evaluating the efficacy of smear microscopy in comparison with culture test (Sivaranjini R et al 2013). Similar recommendations were made considering the cost effectiveness and rapidity of smear microscopy in comparison with culture test by Madhumathi J et al (2012). Considering these recommendations, we restricted our diagnosis based on smear microscopy alone. Motile trophozoites with the characteristic motility and presence of pus cells (>5 cells/HPF) was considered as the diagnostic criteria in the present study. Proportion of women having trichomoniasis in our study population was 92 (11.6%). The rate of trichomoniasis in the present study is comparable with the reports from other Indian studies. In a community based study, 854 women from Karnataka with in the age group of 15-30 years were screened for *T. vaginalis* using smear and In Pouch TV cultures and reported a rate of 8.5 % (Madhivanan P et al 2009). In another study comprising of 156 reproductively active Indian women, smear examination using wet mount was used as the diagnostic test and reported 12% trichomoniasis (Fule SR et al 2012). In the present study, we did not find statistically significant association between trichomoniasis with maternal age (p=0.920), religion (p=0.612), level of education (p=0.718), occupation (p= 0.66) and parity index (0.084). In a previous study among Indian women, the association between the use of oral contraceptive pills before pregnancy and trichomoniasis was reported (Madhivanan P et al 2009). However, very
few (11, 1.3%) study participants in the present study reported a history of using OCP before pregnancy, thus limiting the power of the sample size for finding such an association in our study. Presence of abnormal flora (Nugent’s Grade III flora) was found to be a risk factor for trichomoniasis among Indian women (Sujit D Rathod et al 2011). However, in the present study none of the women with trichomoniasis had coinfection with BV. Centre for Disease Control and prevention (CDC) has recommended the screening for trichomoniasis among women with history of having multiple sex partners, previous STIs and drug abuse (Workowski KA, Berman S 2010). In the present study, none of the women had the above mentioned risk factors as proposed by the CDC.

In the present study, Nugent’s Grade II (Intermediate) vaginal flora was seen among 115 (14.5%) of the women. Presence of intermediate flora among Indian women was reported at various rates previously (Bhalla et al 2007, Hemalatha R et al 2013). Presence of intermediate flora was reported among those who are recovering from an episode of BV or those progressing towards BV. Emphasis on the presence of intermediate flora gained importance after finding their association with an increased susceptibility to HIV and other STI among women (Sewenkambo N et al 1997 and Bhalla et al 2007). Among pregnant women, the association of intermediate flora with adverse pregnancy outcomes was proposed by various studies (Carey JC and Klebanoff MA 2005, Donders GG et al 2009). Considering the importance of this intermediate flora, we further grouped these 115 women based on the presence of leukocytes in high vaginal smears. Among the 115 women, 75 (9.5%) of the women had intermediate vaginal flora with the presence of >5 pus cells/ OIF, suggestive of vaginitis due to bacterial etiology (Donders GG et al 2008). Interestingly, positivity for BV using Amsel’s criteria was observed among 3 (4%) of the women with vaginitis indicating the false positivity of vaginal pH (>4.5), amine test and whiff test (Table 4.1.1h) due to the proliferation of anaerobic bacteria and other intermediate vaginal flora. Similar findings regarding the false positivity of Amsel’s criteria were reported previously among women with intermediate vaginal flora (Srinivasan S et al 2012, Hemalatha R et al 2013). In the present study, we did not observe any statistically significant association of maternal age, religion, level of education, occupation, economic status and parity index with vaginitis due to bacterial etiology. The decreased number of Lactobacillary forms (>10 LBF/OIF) and predominance of Gram negative bacilli (>10 bacilli/OIF) was a common feature among all these women with vaginitis due to bacterial etiology. In the present study, the proportion of women having
intermediate vaginal flora was 14.5% which is similar to 16.5% reported by Hemalatha R et al 2013. However, the latter study included a broader group of study participants (reproductively active women between 18–40 years of age). Our results cannot be compared with any other studies previously reported elsewhere in India due to the unavailability of data reported among pregnant women. Donders GG et al 2009, reported the prevalence of intermediate vaginal flora among 84 (11%) of the 759 pregnant women examined during their first trimester of pregnancies. However, in their study population, vaginitis due to aerobic Gram negative bacilli (aerobic vaginitis) and Gram positive cocci (coccoid vaginitis) were the commonest etiologies unlike the predominance of anaerobic Gram negative bacilli belonging to the genus Prevotella, Porphyromonas and Bacteroides in the present study.

5.5. Genital Chlamydiasis:
In the present study, all the endocervical swabs obtained from 790 women were subjected to PCR (Nucleic acid amplification test, NAAT) for the detection of *C. trachomatis*. The lab diagnosis of genital chlamydiasis comprises of diagnostic tests like culture, serological or enzyme immuno assays mediated diagnosis and nucleic acid amplification tests. However, utility of NAAT was recommended for the diagnosis of genital chlamydiasis among both symptomatic and asymptomatic women due to their high sensitivity and specificity in diagnosis, applicability using multiple samples like urine, high vaginal and cervical swabs (Marrazzo JM et al 2005, Hobbs MM et al 2008 and Geisler WM STD guidelines 2011). The overall specificity of NAAT for diagnosis of *C. trachomatis* causing genitourinary infections has been reported to be more than 95% (Black CM et al 2002 and Gaydos CA 2004). High sensitivity of NAAT was attributed to the ability of these tests to detect the presence of even one copy of *C. trachomatis* gene in the specimen (Mahony JB et al 1993 and Schachter J et al 2005). Prevalence of *C. trachomatis* among pregnant women across the globe varied based on the type of study population and their previous history of STIs. Moreover, the diagnostic test employed for the detection of *C. trachomatis* infection played an important role in determining the burden of the disease (Table ROL). Among Indian pregnant women, prevalence of *C. trachomatis* was reported at varying rates ranging from 0.1% to 18.2% (Mayank S et al 2001, Rastogi S et al 2003 and Vidwan NK et al 2012). Serological diagnosis using ELISA (Mayank S et al 2001), Enzyme immunoassay (EIA) (Alexander R et al 1993), Direct fluorescent antibody (DFA) testing
(Rastogi S et al 2003) were more commonly used among Indian studies. In the present study, NAAT targeting the kryptic plasmid gene specific for \textit{C. trachomatis} was used as described previously (Mahony et al 1992). Interestingly, in the present study, none of the women were positive for \textit{C. trachomatis} by PCR from endocervical swabs indicating 0% proportion of women infected with \textit{C. trachomatis}. In a study among south Indian pregnant women attending the antenatal care clinic at a tertiary care hospital in Vellore, a positivity of one (0.1%) of the 799 women screened using NAAT for \textit{C. trachomatis} was reported. From the results of this study and considering the low prevalence of \textit{C. trachomatis} among asymptomatic pregnant women, the need for routine screening of \textit{C. trachomatis} among south Indian pregnant women was questioned (Vidwan NK et al 2012). Low prevalence of 1% was previously reported from a population based study comprising of healthy women in TamilNadu, where NAAT from urine specimens was done (Joyee AG et al 2004). Considering the high specificity of NAAT for detection of \textit{C. trachomatis}, it is understood that the rates of infection was over reported previously among studies using serological tests alone like ELISA and EIA. This assumption gets support from the previous reports that the prevalence of genital chlamydiasis can vary based on the diagnostic test used for the detection of \textit{C. trachomatis} (Watson EJ et al 2002). Considering the 0% proportion of women infected with \textit{C. trachomatis} among our study population, we assume that the inclusion of \textit{C. trachomatis} screening using NAAT as a part of routine antenatal care may not be a cost effective intervention in settings with low prevalence of the infection.

5.6. Asymptomatic bacteriuria:

Asymptomatic bacteriuria (AB) refers to a condition where urine culture is positive from two consecutive clean catch mid-stream urine specimens (significant counts of >10^5 CFU/ml) in the absence of any clinical symptoms of UTI (Macejko AM and Schaeffer AJ 2007). Asymptomatic bacteriuria occurs in at least 2-10% of the women with a similar incidence rate in both pregnant and non-pregnant women (Patterson TF and Andriole VT 1997 and Nicolle LE et al 2005). From a Cochrane review, it was reported that untreated AB could progress to pyelonephritis in 2.5-36% of the cases (Smaill F and Vazquez JC 2007). Nulliparity, pre-existing diabetes mellitus, smoking and age >20 years were reported as a risk factors among pregnant women to develop pyelonephritis (Wing DA et al 2014). The complications due to untreated AB were more frequently reported in pregnant women. Anatomical changes i.e., change in size and position of the uterus as the pregnancy progresses, increased smooth muscle relaxations and ureteral dilatations
associated with normal pregnancy were reported to facilitate the bacteria to ascend up the urinary tract and reach kidneys (Macejko AM and Schaeffer AJ 2007). From a large scale retrospective study done in the US singleton pregnant involving 50,000 women, the authors reported pyelonephritis as a risk factor (OR 1.3, 95% CI 1.2-1.5) for preterm birth (Wing DA et al 2014). Early diagnosis and treatment of AB and UTI during pregnancy was reported to reduce the complications associated like pyelonephritis and adverse pregnancy outcomes (Mittendorf R et al 1992, Rouse DJ et al 1995, and Villar J et al 1998). Utility of dipstick, enzymatic detection of infections using strip based kits from urinary specimens for diagnosis of AB were evaluated. These methods showed poor positive and negative predictive values in diagnosis of AB in comparison with culture (Miller L et al 2000, Mc Nair et al 2000 and Shelton SD et al 2001). In the present study, clean catch mid-stream urine specimens were collected from all the 790 women and were subjected to semi quantitative culture using Cysteine Lysine Electrolyte Deficient (CLED) media. Of the 790 women screened, positive urine cultures were observed among 57 (7.2%) of the women. Asymptomatic Bacteriuria was observed among 20 (2.5%) of the women, while symptomatic UTI was seen among 37 (4.6%) of the women during their first visit for antenatal care (early pregnancy). The rate of asymptomatic bacteriuria in the present study is lower than the rates reported from other Indian studies. Jain V et al (2013) reported a high occurrence of AB (17%) among women attending the antenatal clinic of a tertiary care hospital in Lucknow. Few other studies among Indian women reported an occurrence rate of 9% (Enayat K et al 2008, Gayathree I et al 2010 and Kerure SB et al 2013). No statistically significant association with maternal factors like age (p=0.866), religion (p=0.825), level of education (p=0.317), occupation (p= 0.857), monthly income (p=0.07) and parity index (p=0.102) with the occurrence of UTI among our study population was observed. In the present study, we did not observe any women with recurrent episodes of UTI during their pregnancy. Screening during 12-16 weeks of gestation using microbiological culture was reported to predict at least 80% of the women who would develop AB even later in their pregnancy. It was also reported that women who were negative on screening for bacteriuria during 12-16 weeks of gestation had only 1-2% of chances of developing or UTI later in their pregnancies (Stenqvist K et al 1989). In the present study, 24 (3.3%) of the women who did not have UTI or asymptomatic bacteriuria (negative urine cultures) during their first visit were diagnosed with UTI during their follow up visits (24-32 weeks of POG). However, proportion of women who had AB
during their follow up visits could not be estimated in the present study. Urine culture during the follow up visits was only performed for those women who presented with symptoms of UTI. Thus, the rate of AB reported in the present study must be considered as the rate of AB during early pregnancy (12-16 weeks of gestation) alone. In the advent of reports suggesting increasing rates of AB with advancing gestational age, under the effect of gravid uterus and the anatomical changes that predispose the ascent of bacteria, we assume screening pregnant women during later stages of gestation would have helped us in detecting few more cases of AB. Acute pyelonephritis which was reported previously as the most common complication of AB was not observed in any of the study subjects. This reemphasizes the importance of early diagnosis and prompt treatment in reducing the complications associated with untreated AB. Similar findings emphasizing the role of early diagnosis and treatment were reported previously by various authors (Nicolle LE et al 2005 and Kazemier et al 2012). While the inclusion of AB in routine antenatal care using microbiological culture can be debated in low occurrence settings (<2%), in terms of cost effectiveness as suggested by Wadland WC & Plante DA (1989), recent reports suggesting the consequences to the infant due to inappropriate antibiotic administration during pregnancy advocates the antibiotic prescription for treating AB in pregnancy, be justified and based on evidence obtained from microbiological culture and antimicrobial susceptibility testing reports (Ashkenazi- Hoffneng L et al 2011).

5.7. Periodontal disease:
Periodontal diseases are defined as chronic, progressive, inflammatory diseases of the teeth and the surrounding supportive tissues. Based on the severity of the inflammation and the extent of tissue destruction, periodontal diseases can be subdivided into gingivitis and periodontitis (Pihlstrom BL et al 2005). Inflammation of superficial soft tissues around the teeth due to poor oral hygiene practices is considered as gingivitis whereas involvement of inflammation to cause a destruction of the soft tissue along with the periodontal ligament and alveolar bone is considered as periodontitis (Kinane DF 2002). While numerous factors were reported as risk factors for periodontal disease, pregnancy under the influence of hormones like estrogen and progesterone was reported as a significant risk factor (Offenbacher S et al 2006). The overall prevalence of periodontal diseases in adult population ranges between 10-60% (Huck O et al 2013). The prevalence of periodontitis among Indian population was reported as 14-18% (Murthy S et al 2012).
The prevalence of gingivitis was reported to be ranging between 30-100% among women of child bearing age (Adams D et al 1974 and Lieff S et al 2004). Since the endorsement of Community Periodontal Index (CPI) scoring system for assessing the periodontal health status by the WHO, many studies have used this method (Oral health surveys: basic methods WHO 1997). In the present study, diagnosis of periodontal disease was done based on the CPI scoring system. Numerous studies have reported varying rates of prevalence from different parts of the globe based on various other screening methods (Dye BA 2012). Among the 790 women recruited for the present study, results regarding the periodontal health status and microbiological profiles of sub gingival specimens were available for 775 (98%) of the women. Gingivitis was observed among 429 (55.3%) of the women and periodontitis was observed among 90 (11.6%) of the women. Among the women with gingivitis, mild to moderate forms of gingivitis was seen among 282/429 (65.7%) and severe gingivitis was seen among 147/429 (34.2%) of the women. Proportion of periodontitis among the present study population (11.6%) is slightly lower than the overall prevalence (14-18%) of the disease reported among Indian adult population (Murthy S et al 2012). This probably could be due to the present study population comprising of women of lower age group from middle and upper middle income category, and non-smokers, thus placing them as low risk group (Kimura S et al 2002 and Vogt M et al 2012). The overall proportion (67%) of the women having periodontal disease in the study population is similar to the prevalence (10-60%) reported among adults globally (Huck O et al 2013). Inflammation of the periodontal tissue is the pathognomic presentation of the periodontal diseases. However, mere presence of inflammation cannot be used to distinguish gingivitis from periodontitis. Thus, the need for a careful clinical examination using various parameters like bleeding upon probing (BOP) and loss of attachment (CLA) were emphasized for the diagnosis of periodontal diseases (Highfield J 2009). Negative findings on clinical examination for BOP and CLA were reported to have high negative predictive value for the disease (Haffajee AD et al 1983 & Okamato H et al 1989). Nevertheless, clinical examination of the periodontal health status using periodontal parameters only depicts the presence or absence of disease on a retrospective basis. These parameters do not provide the information regarding the cause of the disease or the underlying process of the disease, thus restricting their utility in providing information regarding the appropriate therapy (Hamlet SM 2010 & Kaman WE et al 2012). Also, the key diagnostic criteria for periodontal disease like measurement of CLA,
BOP depend up on the expertise of the examiner and are subjective to interobserver variability (Highfield J 2009). Prevalence rates of gingivitis and periodontitis among pregnant women vary according to the age, ethnicity, socioeconomic state and life style habits (Vogt M et al 2012 and Shamsi M et al 2013). In the present study, we did not find a statistically significant association of maternal age (p= 0.075), religion (p=0.09), level of education (p= 0.819), occupation (p= 0.547) and parity index (p= 0.316). As discussed previously, this probably could be attributed to the homogenous study population in the present study. Mean age of the women among our study population was 27.14±3.54 yrs and the women were educated and belonged to middle income group families with none having a history of smoking or alcohol consumption.

5.8. Microbial etiology of maternal genitourinary and periodontal infections:
In his efforts to characterize the vaginal microbiota, Professor Albert Doderlein, over a century ago reported the isolation of lactic acid producing bacteria from vaginal secretions which were then named as Doderlein bacilli (Doderlein1892). In late 1920s, these bacteria were named as Lactobacillus acidophilus (Thomas S 1928). In the past, studies have demonstrated qualitatively, the presence of Lactobacillus spp along with various other bacteria that are vaginal endogenous flora and concluded the predominance of Lactobacillus spp. isolation (Mean prevalence of 60%, Range-18-90%) from the cervical or vaginal secretions of women emphasizing their role in normal vaginal flora (Galask RP et al 1976, Larsen B and Galask RP 1982). In the present study, high-vaginal swabs obtained from all the 790 women were subjected to microbiological culture techniques and the most commonly isolated bacteria were Lactobacillus spp (97%). Globally, presence of Lactobacillus spp. in abundance as the vaginal flora has been considered as “healthy” or “normal vaginal flora” (Priestley CJ et al 1997, Donders GG et al 2007). After the advent of molecular techniques in late 1980s, these bacteria were sub classified under L.acidophilus group as various species based on DNA homology (Roller C et al 1995). Over 20 species of lactobacillus have been reported from human vagina. One or two of the four most commonly reported species namely L.crispatus, L.jensenii, L.gasseri and Liners were reported to be present in healthy women as the predominant vaginal flora (Lamont RF et al 2011 & Ravel J et al 2011). Production of antibacterial substances like hydrogen peroxide and lactic acid (which in turn maintains the acidic vaginal pH) was reported to be the key mechanisms by which Lactobacillus prevent the proliferation of pathogenic
bacteria in the vaginal milieu (Aroutcheva A et al 2001). In the present study, we did not speciate Lactobacillus, however isolation rates of this bacteria was compared among women with all the three grades (Nugent’s) of vaginal flora. The isolation rates were similar in all the three groups of women having Grade I (97%), Grade II (93%) and Grade III (95%) vaginal flora. This finding is in concordance with the previous reports where Lactobacilli were the principle bacterial species qualitatively present in the vagina (Larsen B and Monif GR 2001). In a study done among Japanese pregnant women, the rates of detection of Lactobacilli using PCR among women with normal, intermediate and abnormal flora were 99%, 100% and 92% respectively (Tamrakar R et al 2007). Higher prevalence of Lactobacilli among pregnant women was attributed to high levels of circulatory estradiol previously (Gujjar P et al 1997). However, in the present study quantitative cultures for estimating the number of Lactobacilli was not performed due to the overall cost involved in using multiple culture plates for a specimen and the cumbersomeness of the procedure. If only this could be done, correlation between the number of lactobacilli in the vagina and the risk for contracting BV could have been estimated. Lower number of Lactobacilli estimated using quantitative PCR was reported among women with bacterial vaginosis (Tamrakar R et al 2007).

In the present study, BV was diagnosed among 22 (2.7%) of the women using Amsel’s criteria alone. Culture positivity for anaerobic GNB (Fig 4.2.1a) was seen among 20 (91%) of the women followed by Lactobacilli spp (19, 86%) and G.vaginalis (16, 72%). Culture positivity for G.vaginalis, Anaerobic GNB (Prevotella spp., & Porphyromonas spp.), Anaerobic GPB like Atapobium spp., and genital mycoplasmas had statistically significant association (p<0.05) with presence of clue cells. Vaginal pH >4.5 was seen more commonly among women with positive cultures for G.vaginalis, Anaerobic GNB and GPB. The culture positivity for Candida was associated with pH <4.5. This finding reemphasizes the proliferation of Candida in the presence of vaginal Lactobacilli more commonly among the pregnant women as reported previously (Gujjar P et al 1997). Among women with positive whiff test, culture positivity for G.vaginalis and anaerobic GNB (Prevotella spp. and Porphyromonas spp.) were most commonly observed. Our findings are in concordance with a previous study where pyrosequencing and phylogenetic analysis of the vaginal microbiota in comparison with clinical signs was performed and the bacterial taxa associated with the positivity of each of the clinical sign were explored (Srinivasan S et al 2012). On further analysis, to find an association of Amsel’s criteria
Discussion

positivity for BV with microbiological culture results, a statistically significant association of culture positivity for *G.vaginalis*, *M.hominis* and anaerobic GNB was observed with Amsel score ≥3 (suggestive of BV) among our study population.

Microbiological culture results were compared with the grading obtained using NSS among all the women. Among the women having Grade III vaginal flora (N=21), *G.vaginalis* (95.2%) Anaerobic GNB (85.7%) (Including any one and/or in combination of the three bacteria *Bacteroides* spp., *Prevotella* spp. and *Porphyromonas* spp.) and *M. hominis* 3(16%) were the most commonly isolated bacteria and had statistically significant association (p<0.05) with BV diagnosed by NSS. Many studies have found a positive association with the culture positivity of *G.vaginalis* and *M.hominis* with BV previously (Sha BE et al 2005 and Tamarkar R et al 2007). In the present study, *G.vaginalis* was isolated among 45 (5.6%) of the total women tested. Among these 45 women who had *G.vaginalis* grown in culture, 20 (45%) of them had Nugent’s Grade III vaginal flora, 22 (49%) of them had intermediate or Grade II flora and 3 (6%) of them Grade I (normal vaginal flora). In the advent of recent reports suggesting the presence of *G.vaginalis* among at least 70% of the healthy women (Hummelen et al 2010 and Srinivasan S et al 2012), our study supports the hypothesis that the mere isolation of *G.vaginalis* on culture cannot be considered diagnostic for BV.

Association of various anaerobic flora like *Prevotella* spp., *Peptostreptococcus* spp., *Fusobacterium* spp., *Mobilincus* spp. and *Veilonella* spp., with BV was proposed among pregnant women by various authors (Spiegel CA et al 1980, Piot P et al 1982). Considering these diverse anaerobic bacteria (Hiller SL et al 1992) and their individual growth requirements (Robert MC et al 1985), it was proposed that culture techniques for diagnosis of BV is cumbersome and expensive (Krohn MA et al 1989). In the present study, culture positivity of anaerobic Gram negative bacteria was observed among 85.7% of the women diagnosed with BV using NSS. Culture positivity of *Bacteroides* spp., in up to 60% of the pregnant women with BV was reported previously (Rosenstein IJ et al 1996). Culture positivity rate of 30-70 % of various anaerobic bacteria among Indian pregnant women with BV was reported previously (Aggarwal A et al 2003). Comparison between the rates of isolation of anaerobes between various studies is difficult, considering the overall isolation of anaerobes depends on multiple factors including the study population, culture media and techniques used and demands expertise in culture plate interpretation. In addition, quantitative cultures of these bacteria to prove their role in
causing the disease was emphasized due to the presence of some of these anaerobic bacteria in small numbers in healthy women as endogenous vaginal flora (Spiegel CA 2002, Money D 2005). In addition to finding an association of anaerobes with BV similar to various studies discussed above, we observed a noteworthy rate of isolation of anaerobic Gram negative bacteria (67.5%) and Gram positive bacteria (51.4%) among women with intermediate vaginal flora (Nugent’s Grade II flora, N=115). Bearing in mind the suggestions by earlier reports (Carey JC and Klebanoff MA 2005 and Donders GG et al 2009), presence of intermediate flora can either be due to previously treated BV or due to the transition from normal (Lactobacillus predominance) to abnormal (mixed anaerobic flora) vaginal flora as a result of overall dynamic nature of the vaginal microbiome during pregnancy (Verstraelen H et al 2009, Aagaard K et al 2012 and Romero et al 2014). Our study underscores the need for longitudinal studies involving sampling of women at multiple times to observe the course of disease among pregnant women with intermediate flora.

In the present study, most of the Gram positive bacteria (bacilli) isolated on anaerobic cultures could not be identified using standard biochemical testing, indicating the need for molecular techniques to identify these bacteria. Also, there have been reports suggesting the presence of metronidazole resistant gram positive bacteria like Atapobium spp., (Ferris MJ et al 2004), Megasphere spp., which are increasingly being reported as etiology of BV (Sha BE et al 2005, Fredricks DN et al 2007 and Fettweis JM et al 2012). Hence, we reemphasize on the need for culture independent diagnostic tests like PCR based assays for the diagnosis and better understanding of the etiology of BV.

In the present study, we did not find any woman positive on culture for Mobilincus spp. Similar findings indicating the absence of Mobilincus spp., among European women with BV was reported previously (Donders GG et al 2009). Mobilincus spp. has been reported as one of the most common etiology of BV by various researchers, mainly from the western parts of the world (Sha BE et al 2005, Fredricks DN et al 2007). In the last decade, studies have reported the differences in vaginal microbiome between women from different ethnicities (Zhou X et al 2004 & 2007). Hence, we could not extrapolate the absence of Mobilincus spp., to a wide population of Indian pregnant women based on the present study alone due to the small number of cases of BV in the present study and a homogenous study population. Moreover, culture positivity for Mobilincus spp., has been reported previously as extremely low (Krohn MA et al 1989). Thus, our findings need to
be further validated by studies involving women from various geographic and social background across India by use of culture independent techniques like PCR and pyrosequencing that have been reported to have higher sensitivity in detecting the presence of extremely fastidious organisms like *Mobilincus* spp., (Fredricks DN et al 2007, Srinivasan S et al 2012).

In the present study, *Candida* spp. was isolated among 124 (15.6%) of the women. Of the 124 women, who had Candida grown in culture, 92 (74.1%) women had Nugent’s Grade I flora, 31 (25%) had Grade II flora and 1 (0.8%) of them had Grade III flora. Vaginal pH was observed to be <4.5 in 93 (75%) of the women who had Candida grown in culture. In the present study, VVC was more commonly seen among women having normal vaginal pH (<4.5) and normal vaginal flora (Nugent’s Grade I flora). Similar findings from a longitudinal study among Kenyan women was reported where the risk of contracting VVC was high among women who had H$_2$O$_2$ producing lactobacilli in their vaginal secretions than women without lactobacilli (McClelland RS et al 2009).

With reports available suggesting the increasing trend of non-albicans *Candida* in VVC, and the antifungal resistance conferred by some of the non-albicans candida and *C.albicans* to the most routinely used antifungal agents, need for culture of the vaginal swabs for yeast identification up to species level and in vitro antifungal susceptibility testing is warranted (Priestley CJ et al 1997, Jindal N et al 2007, Sobel JD 2007 and Kumari V et al 2013). *C.albicans* (34, 61%) was the most common pathogenic species isolated followed by *C.glabrata* (11, 20%) among the 55 women with VVC. Among pregnant women, *C.albicans* has been reported as the primary etiology of VVC, in similar proportions with our study by various authors previously (Garcia H et al 2001, Namking LA et al, Jindal N et al 2007 and Dias LB et al 2011). Similar to our findings, *C.glabrata* has been reported as the second most common isolate from women with VVC previously (Ahmad A and A U Khan 2009, Kumari V et al 2013). Inappropriate treatment of VVC, as in case of misdiagnosed or absence of microbiologically proven VVC have led to the emergence of drug resistant strains of *C.albicans* to the most commonly used antifungal agents. Such inappropriate therapy also was proposed as the cause of emergence of non albicans *Candida* as the causal agents of VVC (Achkar JM & Fries BC 2010 and Kumari V et al 2013). In our study, 100% susceptibility towards amphotericin B and flucytosine was observed among all the species of Candida. Two (6%) of the *C.albicans* showed intermediate susceptibility to fluconazole and 1 (9%) of the *C.glabrata* showed
Discussion

intermediate susceptibility to voriconazole. While there have been reports indicating the emergence of resistance towards various antifungal agents by both *C. albicans* and non-albicans Candida (Richter SS et al 2005 and Chander J et al 2013), such resistance was not observed in our study.

In the present study, we used culture and PCR for the detection of genital mycoplasmas (*Ureaplasma urealyticum, Mycoplasma hominis*) from high-vaginal specimens. However, we considered PCR as the reference standard method for reporting the rate of detection and determining the efficacy of the culture techniques for isolation of genital mycoplasmas. *U. urealyticum* and *M. hominis* were detected among 9.1% and 0.5% by PCR and isolated on culture among 4.1% and 0.3% of the women respectively. The detection rates of genital mycoplasmas among healthy women vary across the globe. While there have been studies reporting high detection rates up to 60% (Kong F et al 2000 and Yamazaki et al 2012), there are also reports with lower rates similar to the present study: 6.1-19% among Australian women (Mclver CJ et al 2009), 8.7% among Japanese women (Kataoka S et al 2006), 4.6-10.5% among Chinese women (Cao X et al 2007) and 2.6% among women from Poland (Eikel AM et al 2009). Colonization rate of genital mycoplasmas was associated with the sexual activity (Yamazaki et al 2012) ethnicity (Zhou X et al 2007) and lifestyle factors. In a cohort study involving 300 antenatal Japanese women, Yamazaki et al 2012 indicated the presence of Urea plasmas favour further colonization and infection by *C. trachomatis* in women. Interestingly, in our study population, we noticed a low detection rate of *U. urealyticum* (9%) and 0% detection rate of *C. trachomatis*. Though our observation involves small number of women positive for genital mycoplasmas to draw any conclusion supporting the findings of Yamazaki et al (2012), it provides the baseline information about the detection rates of both *U. urealyticum* and *C. trachomatis* among a cohort of healthy south Indian pregnant women.

In a different study from south Indian pregnant women, the rates of Ureaplasma (39%) and *M. hominis* (20%) during third trimester were reported (Choudhury MR et al 1994). However, in the present study we noticed lower rates of genital mycoplasmas collected from women during their late first trimesters - early second trimesters. In a study from South African pregnant women, the detection rate of genital mycoplasmas among pregnant women during their first trimester was 13%. The study also emphasized on the colonization rate varying with maternal age (Govender S et al 2009). The lower rates of genital mycoplasmas in the present study could be due to the sampling time of gestation
Discussion

(mean POG at testing was 14± 4.2 weeks) at which the sampling was done. Although, the colonization rate is lower during first trimester of pregnancy, it was reported to be more significantly associated with adverse pregnancy outcomes (Kataoka S et al 2006, Larsen B and Hwang J 2010).

The role of bacteria in the causation of periodontitis was proposed by many authors after isolating bacteria like Porphyromonas gingivalis and Actinobacillus (Aggregatibacter) actinomycetemcomitans from diseased periodontal tissues (Allenspach-Petrzilka GE & Guggenheim B 1983, Manor A et al 1984 and Christersson LA et al 1987). However, attempts to prove the etiological role of A.actinomycetemcomitans as a periodontal pathogen using Koch’s postulates could not be validated (Christersson LA et al 1985). Polymicrobial nature of these infections then gained recognition along with the criteria for naming a microbe as periodontopathogenic bacteria (Socransky SS 1979). In a study using over 13,000 dental plaque specimens and DNA–DNA hybridization, the sub gingival microbiota was characterized to contain bacteria belonging to more than 32 taxa. Bacteria isolated were clustered as complexes namely red, orange, green, yellow and purple. Of these complexes, bacteria belonging to the red complex were considered as primary periodontopathogenic bacteria. It was also demonstrated that orange complex members (Fusobacterium nucleatum, Prevotella spp, Campylobacter rectus) pre-colonize the gingival tissue making it more favorable for the red complex members (P.gingivalis, T.forsythia and T.denticola) to colonize and cause the tissue destruction (Socransky SS et al 1998). Majority of the studies have found a positive correlation between organisms belonging to red complex and the increasing probing depth, chronic periodontitis and localized aggressive periodontitis (Socransky SS et al 1998, Kumar PS et al 2003, Nishihara T & Koseki T 2004 and Riep et al 2009). Considering the global observations of prevalence of bacteria in plaque and sub gingival specimens, the American Academy of Periodontology has declared P.gingivalis, T.forsythia and A.actinomycetemcomitans as periodontal pathogens (American academy of periodontology 2005). In addition to these three bacteria, seven more putative periodontal pathogens were also proposed namely Treponema denticola, Prevotella intermedia, Prevotella nigrescens, Eikenella corrodans, Campylobacter rectus, Peptostreptococcus micros and Eubacterium nodatum (Socransky SS & Haffajee AD 2005). Presence of Capnocytophaga ochracea as a predictor for periodontal health was reported previously (Riep et al 2009). Role of above mentioned bacteria in depicting the health and diseased status of periodontium, presence of putative
periodontal pathogens along with indicators of healthy periodontium like C.ochracea and C.sputigens had prompted us to test comprehensively the sub gingival plaque specimens in the present study. Culture techniques for the isolation of periodontal pathogens was reported to be laborious, time consuming and cumbersome in comparison with PCR based assays previously (Ready et al 2008 and Riep et al 2009).

In the present study, all the 775 women for whom the clinical periodontal examination was performed, microbiological examination for the presence of putative pathogens using multiplex PCR was also carried out according the method reported by Kimura S et al 2002. The overall detection rates of P.gingivalis (30%), C.rectus (24%), A.actinomycetemcomitans (23%), E.corrodans (21%) and T.denticola (20%) were higher than the other bacteria in the study population. Among the women with periodontitis, the rates of detection of P.gingivalis (61%), P.nigerscens (46.7%), A.actinomycetemcomitans (42.2%), T.denticola (40%) and P.intermedius (35.6%) were observed. In a study reported from south India (Krishnan Mahalakshmi et al 2012) to determine the prevalence of periodontal pathogens (using broad range PCR) among general adult patients with periodontitis (N=200), the highest rates of detection were observed for P.gingivalis (80%), T.forsythia (73.4%), T.denticola (71%), A.actinomycetemcomitans (61%). These results are in partial agreement with the results of the present study in terms of the high detection rates of bacteria belonging to “red complex”. However, the rate of detection of T.forsythia was very low (14%) in our study population that is possibly attributed to less severity of the disease in our study population. Previous reports suggested higher detection rates of T.forsythia among patients with chronic or severe form of the disease as this bacterium was detected from deeper periodontal pockets (Kasuga Y et al 2000). In another study among Indian pregnant women, microbiological culture was used as the method for isolation of periodontopathogenic bacteria and T.forsythia was reported among 14% of the women while P.gingivalis, P.intermedius and P.nigerscens were reported at higher rates (Anuradha Basavaraju et al 2012). Presence of “orange complex members” like P.intermedius and P.nigerscens in the present study population can be attributed to the transient colonization of orange complex members that can, in few cases, create an ideal niche for the red complex members to colonize and cause the disease (Socransky SS et al 1998). This is further supported by the finding that colonization of pathogenic bacteria occurs at higher rates under the influence of pregnancy related hormones as previously reported (Adrianes L et al 2009).
In the present study, presence of *P.gingivalis* (Relative risk: 2.71(1.59-4.60), *p*=0.001), *P.intermedius* (RR: 3.37(1.91-5.96), *p*= 0.001), *P.nigerscens* (RR: 3.54(2.00-6.26), *p*=0.001) and *T.denticola* (RR: 1.6(1.30-2.78), *p*= 0.04) were found to have a statistically significant association with periodontitis. Similar positive association of these four pathogens with periodontitis in general population and children was reported previously (Socransky SS et al 1998 and Kimura S et al 2001). Presence of other previously reported putative pathogens like *T.forsythia*, *E.corrodans*, *C.rectus*, *A.actinomycetemcomitans*, *C.ochracea* and *C.sputigens* had no statistically significant association (*p* <0.05) with periodontitis in the present study population. Bacteriological profiles of the periodontal tissues was reported to be a geographical phenomenon and the lower detection of some pathogens can also be due to the shallow pathological pockets (based on the severity of the disease) at which the sub gingival plaques were examined (Van Winkelhoff A et al 2002 and Riep et al 2009). Of the 775 women examined, 107 (14%) of them had more than any of the three bacterial species detected from their sub gingival plaque specimens. Using ROC curve, it was observed that the presence of any of the three among ten putative periodontal pathogens tested in the present study is diagnostic for periodontitis when compared with CPI scoring system with a sensitivity, specificity and area under curve of 65%, 76% and 76% respectively. Moreover, it was also observed that the presence of more than three bacteria is a statistically significant risk factor [Odds ratio: 6.41 (3.94-10.44), *p*=<0.01] for periodontitis. This finding from the present study supports the polymicrobial etiology of periodontitis as reported previously (Socransky SS et al 1998, Riep et al 2009). Despite reports suggesting that the worsening or the improvement of periodontitis depends on the colonizing oral microbiota, the utility of microbiological examination for the diagnosis and management of periodontitis in routine dental practice has been limited. This could possibly be attributed to the complex nature of the oral microbiome which varies with race, ethnicity, lifestyle factors, genetic composition and many other factors (Page RC et al 2000 and Nibali L et al 2007). Advent of molecular biology techniques revealed the diverse nature of oral microbiome among various populations and the paucity of standard laboratory procedures for the detection of this flora (Riep et al 2009). From the present study, we observed a positive correlation of presence of the putative bacteria among asymptomatic pregnant women with periodontitis. With the increasing evidence of systemic inflammatory responses due to the colonizing bacteria in the oral cavity leading to adverse pregnancy outcomes (Offenbacher S et al 2006 & 2009), we propose the
microbiological diagnosis among pregnant women can help in the better understanding of the pathogens associated with the disease and thus, provide useful information to explore the need for antimicrobial therapy in cases where mechanical scaling alone may not suffice as management tool.

Association of maternal genitourinary and periodontal infections with adverse pregnancy outcomes

In the present study, women with BV were stratified further as those having “full BV” and “partial BV” as discussed above. We did not observe a statistically significant association of full BV (Risk ratio: 2.32 (0.79-6.75), p= 0.143) and partial BV (RR: 2.43 (0.86-6.85), p=0.102) with preterm birth. However, full BV (RR: 2.48 (1.14-5.39), p=0.04) and partial BV (RR: 2.56 (1.05-6.30), p= 0.04) had statistically significant association with LBW in the study population. For better understanding of infection status, term “Cumulative BV” (Full + Partial BV) was employed in the present study, and it was found to have statistically significant association with both preterm birth (RR: 2.49 (1.16-5.34), p= 0.029) and low birth weight (RR: 2.70 (1.41-5.16), p= 0.05). Similar positive association of BV with preterm birth and low birth weight was reported previously among women from various ethnicities and settings (Lata I et al 2010, Simhan et al 2011, Donders GG et al 2009). While there have been numerous reports suggesting the association of Full BV with preterm birth and low birth weight (Goldenberg RL 2000, Klebanoff MA 2005) presence of partial BV during early pregnancy (intermediate flora along with streaks of BV flora) was reported as a risk factor for preterm birth by few studies outside India (Carey JC et al 2005, Donders GG et al 2009, Lamont RF et al 2010). To the best of our knowledge, the present study reports for the first time, an association of partial BV among Indian pregnant women with low birth weight. More importantly, from the study additive effect of full BV and partial BV was evident as these two entities individually did not show significant associations with PTB. However, when summed up together the cumulative BV was found to be a significant risk factor for PTB. Despite the positive association of BV with PTB and LBW using Chi square test and univariate analysis, we did not find a similar association when tested using a multivariate logistic regression model. Similar findings were reported form a large scale prospective multi centered cohort study done in Philadelphia, wherein BV diagnosed during early pregnancy was not found to be an independent risk factor for PTB in the presence of other infections like periodontitis (Harper et al 2012). However, our study differs from other studies which
have reported BV as an independent risk factor for PTB and LBW (Hillier SL et al 1995, Paige DM et al 1998, Svare JA et al 2006). With the overall low proportion of women with BV (48/790, 6%) and further loss of 6 women on follow ups, the present study had relatively a small cohort of 42 women with BV for the final outcome analysis. In this context, the finding of BV not being an independent risk factor for PTB and LBW in the present study population is not surprising. Effect of treatment for BV is yet another factor that can theoretically influence the outcome of pregnancy. In the present study, treatment for BV in the form of clindamycin vaginal pessaries was given to 15 (37%) of the women based on the clinician’s discretion. Of these 15 women, three (20%) of them had preterm labor in comparison with 4/27 (14.8%) women, who did not receive treatment. We did not observe any statistically significant difference (p=0.66) among both groups (based on the treatment) of women. Similar findings suggesting no reduction in the rate of PTB among the women who were treated for BV in comparison with those not treated was reported previously (Sangkomkamhang US et al 2008, Harper et al 2012).

Among the microbiological etiology associated with BV, culture detection of G. vaginalis (Risk ratio: 2.3 (1.06-5.09), p=0.04), mixed anaerobic Gram negative bacteria (RR: 2.8 (1.64-4.9), p=0.001) and presence of M. hominis (RR: 8.9 (3.85-20.92), p= 0.017) were found to have statistically significant association with preterm birth. Culture positivity for mixed anaerobic gram negative bacteria (RR: 1.86 (1.15-3.03), p= 0.019) and M. hominis (RR: 5.92 (2.58-13.58), p=0.036) were found to have statistically significant association with low birth weight. Similar findings suggesting the positive association of M. hominis (Goldenberg RL et al 2008, Capoccia R et al 2013), G. vaginalis (McDonald H.M. et al 1997, Gergova RT 2013) and anaerobic Gram negative bacteria like Prevotella spp., Porphyromonas spp., and Bacteroides spp., (Hillier SL 1993, Carey JC et al 2000, Onderdonk AB et al 2003) with adverse pregnancy outcomes have been reported in the literature. A positive association of presence of U. urealyticum (Risk ratio: 5.2 (1.38-19.58), p=0.025) with pPROM was observed in the present study as reported earlier (Mc Donald HM et al 1992, Goldenberg RL 2008). We did not find positive association of presence of U. urealyticum with BV and preterm birth. Presence of U. urealyticum along with a disturbed normal vaginal flora (as in the case of BV) was reported to have more significant association with preterm birth than in women who merely had colonization (in the absence of BV) of U. urealyticum (Carey JC et al 199, Capoccia R et al 2013). We attribute the lack of association of U. urealyticum with PTB in the present study.
population, as the presence of *U. urealyticum* was not restricted to the women with BV alone (Table 4.1.2b).

In the present study, other forms of vaginal infections like VVC and Trichomoniasis were more commonly observed than BV. However, both VVC and Trichomoniasis did not have any statistically significant association with either preterm birth or low birth weight (Table 4.3.3). These findings are similar with those reported by Cotch MF et al (1997 & 1998), Klebanoff MA et al (1995 & 2001), and Paulo César Giraldo et al 2012. While there have been few studies reporting a positive association of *T. vaginalis* with LBW and PTB (Kigozi GG et al 2003), guidelines provided for the management of STI among pregnant women by CDC, recommend the treatment for trichomoniasis only among symptomatic women and contraindicate the treatment among asymptomatic women (Klein & Gibbs 2005). Trichomoniasis was found to have a statistically significant association (Risk ratio: 5.8 (1.12-30.42), p=0.05) with preterm premature rupture of membranes (pPROM) in the present study. Though, association of trichomoniasis with premature rupture of membranes was reported previously (Young F 2006), in the present study, we did not find such association of Trichomoniasis with PROM.

The presence of intermediate vaginal flora (Nugent’s Grade II flora) and their association with adverse pregnancy outcomes has been increasingly emphasized in the past two decades (Donders et al 1993, McDonald et al 1997, Oakeshott P et al 2002). Presence of aerobic bacteria like *E. coli* and *S. aureus* during early pregnancy was reported to have a positive association with preterm birth (Carey JC et al 2005). Association of the presence of vaginitis causing flora during early pregnancy with chorioamnionitis was reported more recently (Rezeberga et al 2008), reemphasizing the ascent of the abnormal vaginal flora during pregnancy to cause intrauterine infections which in turn could lead to preterm birth. In the present study, vaginitis caused due to anaerobic bacteria (Risk Ratio: 2.3 (1.22-4.42), p=0.02) and presence of intermediate vaginal flora (RR: 2.07 (1.15-3.74), p=0.02) were associated with preterm birth. Similar association of vaginitis with preterm birth was reported previously (Lamont RF et al 2003, Donders GG et al 2009, and Donati L et al 2010). Change in the vaginal flora was also previously reported as a risk factor for preterm birth (Carel JK & Klebanoff MA 2005, Donders GG et al 2010). While the exact mechanism by which the presence of intermediate vaginal flora (anaerobic Gram negative bacteria, aerobic bacteria and low numbers of lactobacilli) can cause preterm birth is unknown, inflammatory responses primarily the abundance of IL1, IL6 (reported to be
present in five to eight fold higher volumes than in women with BV) were reported to mediate the production of prostaglandins and cause preterm uterine contractions (Romero R et al 1993, Donati L et al 2010). In a study comprising of 500 antenatal women within 20-28 weeks of gestation from North India, 52 (10%) women who had intermediate flora on NSS did not result in preterm birth (Gupta A et al 2013). On the contrary, presence of intermediate vaginal flora was found to be an independent risk factor for both preterm birth (Relative risk: 3.1 (1.44-6.85), p=0.004) and low birth weight (Relative risk: 2.3 (1.18-4.74), p= 0.015) in the present study. To the best of our knowledge, the present study reports, for the first time, a positive association of intermediate vaginal flora and vaginitis due to anaerobic bacteria with preterm birth among Indian pregnant women.

With the global incidence ranging between 2-10% among pregnant women, the association of asymptomatic bacteriuria (AB) with preterm birth and low birth weight has been controversial (Smaill F, Vazquez JC 2007). In the present study, the proportion of women with asymptomatic bacteriuria (AB) was 2.5% and we did not observe a statistically significant association with preterm birth (Risk ratio: 0.83 (0.27-2.57), p=0.751) and low birth weight (RR: 1.0 (0.49-2.36), p= 0.84). Similar lack of association between AB and spontaneous preterm birth was also reported form a huge population based survey previously (Meis PJ et al 1995). In the present study, UTI during the first visit was seen among 37/790 (4.6%) of the women. Of the total 57 (7.1%) women diagnosed with AB and UTI during their first visit, 55(97%) of them received specific antibiotic therapy and none of them developed complications like pyelonephritis. Previously from a meta-analysis, it was reported that treatment of AB and UTI among pregnant women was a useful intervention to avoid complications like pyelonephritis and its associated adverse pregnancy outcomes like preterm birth and low birth weight (Smaill F, Vazquez JC 2007). In a prospective study from North Indian pregnant women, no association between AB diagnosed early during pregnancy with adverse pregnancy outcome was reported but the AB detected late during the pregnancy was found to be a significant risk factor for preterm birth and intrauterine growth retardation (Jain et al 2013). On the contrary, our study did not find a positive association of UTI diagnosed during the later stages of pregnancy (24-33 weeks of gestation) with preterm birth (Risk ratio: 1.7 (0.59-5.21), p=0.409) and low birth weight (RR: 0.37 (0.54-2.55), p=0.50). We attribute the lack of association of AB and UTI with PTB and LBW in the present study, to the early diagnosis and effective antibiotic treatment. Based on the current study
findings, we can thus, recommend the routine screening of pregnant women for the presence of AB during their early pregnancies as a cost-effective intervention to avoid further complications, as previously suggested by Adam T et al (2005).

Since the first proposal of an association of periodontitis with preterm birth was made (Offenbacher S et al 1996), numerous studies have reported similar findings suggesting the role of periodontitis with adverse pregnancy outcomes (Offenbacher S et al 2006, Scannapieco FA et al 2010). However, there were also studies that reported no association of periodontal diseases during pregnancy with adverse pregnancy outcomes on case-control comparisons (Davenport CS et al 2002, Moore S et al 2004, Noack B et al 2005 and Nabet C et al 2010). The exact mechanisms by which these subtle infections lead to preterm birth are still unclear. However, presence of periodontopathogenic bacteria in the amniotic fluids of women was reported by many authors (Gonclaves LF et al 2002, Barak S et al 2007 and Kotz J 2009). Proliferation of these bacteria in the intrauterine cavity causes a localized immune response and production of prostaglandins that could cause intrauterine contractions (Loos BG 2005 and Fardini Y et al 2010). Increased amounts of inflammatory cytokines (TNF-α, IL-6 and IL-1β) and prostaglandins in amniotic fluid and cervical secretions of women at preterm labor were reported from various studies among women with periodontitis and deeper pathological periodontal pockets (Loss BG 2005, Casarin RCV et al 2010, Pressman EK et al 2011 and Andrukhov O et al 2011). In the present study, we observed a positive association of periodontitis with preterm birth [Risk ratio: 1.9 (1.06-3.71), p= 0.04] and low birth weight (Risk ratio: 2.54 (1.62-4.0), p<0.01). When tested for the association between the presence of periodontal pathogens and adverse pregnancy outcomes, we noticed a statistically significant association between the presence of _Prevotella.nigerensis_ [Risk ratio: 2.02 (1.30-3.15), p= 0.004] with low birth weight. While few other putative periodontal pathogens like _P. gingivalis_, _A. actinomycetemcomitans_ and _C. rectus_ were also commonly observed among our study population with periodontitis; their presence did not have any association with adverse pregnancy outcomes. In the present study, interventions in the form of therapeutic management of women with periodontitis were not employed. Efficacy of treatment for periodontitis in reducing the rates of PTB and LBW was assessed by various authors with debatable results (Lopez NJ et al 2002, Michalowicz BS et al 2006, Offenbacher S et al 2006 and Polyzos NP 2010). In Indian pregnant women, a decrease in the incidence of preterm births was reported among women who underwent treatment for periodontitis.
Discussion

More recently from a meta-analysis, it was reported that the treatment for periodontitis did not reduce the rate of PTB and low birth weight (Rosa MI et al 2012). Nevertheless, considering the significance of periodontitis as an independent risk factor for both preterm birth (Relative risk: 2.3(1.08-5.50), p=0.04) and low birth weight (RR: 2.8 (1.40-5.76), p= 0.004) in the present study, we emphasize the need for further studies to find if early diagnosis and treatment (<20 weeks of gestation) of these subtle infections can show a reduction in rates of preterm birth and low birth weight. Similar association of periodontitis as an independent risk factor after adjusting the influence of known confounders like smoking, alcohol consumption, low BMI and bacterial vaginosis was reported with preterm birth and low birth weight among low risk Brazilian pregnant women (Vogt et al 2010).

In addition to the maternal genital and periodontal infections, we also observed few non-infectious maternal parameters associated as independent risk factors with preterm birth and low birth weight in the present study population. While women with pre-gestational hypertension were excluded from recruitment in the present study, 22 (3%) of the 726 (after excluding 64 women who were lost during follow up visits) women developed gestational hypertension (after 20 weeks of gestation). All these women were medically managed by physician as a part of their routine antenatal care. In the present study, preeclampsia was seen among 5 (0.6%) of the 726 women. Gestational hypertension was found to be an independent risk factor on multivariate logistic regression model for both PTB [Relative risk: 5.1 (1.52-17.59), p= 0.008] and low birth weight [RR: 6.0 (2.05-17.80), p= 0.001]. Similar association of gestational hypertension with PTB and LBW was reported among various study populations globally (Zhang YP 2012, Blencowe et al 2013, Hematram Yadav & Nagarajah Lee 2013, Chythra Rao et al 2014). Oligohydroamnios (amniotic fluid index <8 cm) during third trimester of the pregnancy was diagnosed among 31 (4.2%) of the women. In the present study, oligohydroamnios was found to be an independent risk factor for both preterm birth (Relative risk: 4.8 (1.8-13.08), p=0.002) and low birth weight (RR: 4.1 (1.63-10.29), p=0.003). Similar association of oligohydroamnios with adverse pregnancy outcomes was reported previously (Goldenberg RL et al 2005, Kim BJ et al 2011, Yi Chen et al 2013). Poor maternal weight gain during pregnancy (<6kg) was found to be an independent risk factor for low birth weight (Relative risk: 2.0 (1.16-3.74), p= 0.014) in the present study. Similar association of poor maternal weight gain during pregnancy and low birth weight of the infants was reported
Discussion

previously (Schieve LA et al 2000, WY Hsu et al 2013). From an interventional observation study, a reduction in the rate of low birth weight babies among women who were provided antenatal care to resolve their inadequate weight gain during pregnancy was reported (Ricketts et al 2005).

Our study has following merits:

1. Screening for genitourinary and periodontal infections was done for all the women recruited in the study using standard clinical and microbiological examination techniques during their first visit for antenatal care (within 8-24 weeks of gestation).

2. While there have been few studies which have evaluated the association of individual infections like BV (Lata et al 2010), urinary tract infections (Lata et al 2010, Jain V et al 2013) and periodontal infections (Gandhimadhi D et al 2010, Mannem S & Vijay K Chava 2011) among Indian pregnant women with adverse pregnancy outcomes, the present study was conducted to screen all the above infections in a cohort of antenatal women and follow them up until their delivery.

3. To determine the risk factors associated with adverse pregnancy outcomes, we sequentially analyzed the risk ratios of all maternal demographic, physical, infectious and non-infectious factors using Chi-square test followed by estimating the relative risks using univariate analysis. Finally, all the risk factors that had statistically significant association with adverse pregnancy outcomes on univariate analysis were used to construct a multivariate logistic regression model to identify independent risk factors for preterm birth and low birth weight among the study population.