RESEARCH ARTICLE

Papanicolaou smear in pregnant women: a prospective study

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ABSTRACT

Objectives: Aim of the study is to assess the prevalence of abnormal pap smear in pregnant women and its associated risk factors. **Methods:** This is a prospective observational study, 200 pregnant women were recruited who came for regular antenatal visit. Papanicolaou smear was done as per conventional method and reported using 2014 Bethesda system. **Results:** 53.6% of pregnant women had abnormal pap smear. Among them, 29% had inflammatory smears, 9% had bacterial vaginosis, 3% showed trichomonal and 7% moniliasis. The abnormal smears were more in patients from rural background (58.5%) than urban (29%), which is statistically significant. Most of them in lower class (88.8%) had abnormal pap. The frequency of abnormal pap in illiterates was 83.3%, 64.7% in middle school, in senior secondary it was 50.7% and graduates was 37.83%. There were 86.5% abnormal smears in those < 21 years of age at first intercourse as compared to 33.06% in those ≥ 21 years of age and the difference was statistically significant. There were 4 (80%) abnormal smears among those with more than one sexual partner as compared to 1(20%) normal smear. 26 (30.58%) abnormal smears in primigravida and 74 (67.88%) abnormal smears in multigravida was noted which was statistically significant. **Conclusion:** Clinicians should make every effort to educate, counsel and screen pregnant women if they have not had a pap test in the past in order to reduce the burden of cervical cancer in our country. This opportunity for screening cervical cancer should not be missed in the antenatal period.

Keywords: Risk factors, abnormal pap, cervical cancer screening, pregnancy.

Cervical cancer is the commonest cancer and leading cause of death among women in developing countries.¹ It is the fourth most common cancer among women globally, with an estimated 570,000 new cases in 2018.² Cervical cancer is the second most common cancer in India, accounting for 22.86% of all cancer cases in women and is the third largest cause of cancer mortality accounting for nearly 10% of all cancer related deaths in the country.^{3,4} It is an important public health problem for women leading to annual mortality of 70,000 and India has the largest burden of cervical cancer patients in the world.^{5,6} The relative five year survival averages to 69% and the length of survival depends on the cancer stage at the time of detection.⁷ The survival chance of a person becomes better if the cervical cancer is detected and treated at earlier stages. Therefore, it is important to avail of cervical cancer screening.

The introduction of cytology screening by Dr. George Nicholas Papanicolaou during 1940s was a great public health success in cervical cancer prevention as it detects potentially pre-cancerous and cancerous lesions in the cervix.⁸ Since the introduction of the conventional cytology or pap smear, the incidence and mortality of cervical cancer has dramatically decreased in many developed countries.⁹ Abnormalities in cervical cytology are found in up to 5% of all pregnancies. Risk of invasive carcinoma ranges between 1-10/10,000 pregnancies.¹⁰ In western countries, cytological screening is a standard part of antenatal protocols, but no such screening program exists in India.

The peak age of incidence of pre-cancerous lesions of the cervix is between 25–35 years of age.¹¹ Antenatal period is a time when women come voluntarily to seek health care for pregnancy. This opportunity should be utilized to down stage

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cancer cervix by detection of pre-invasive lesions with cytology who otherwise might not report for screening. Pap test detects 60-70% of cancer cervix. The accuracy of pap smear in pregnancy is almost similar to that of non-pregnant women.

Most of the reproductive tract infections are asymptomatic making their detection and diagnosis difficult. During pregnancy, the symptoms of vulvo-vaginitis depend on clinical, behavioral and demographic factors.¹² Pap smear can also detect vaginal infections. The 3 most common causes of vaginal infections are bacterial vaginosis, followed by candidiasis and trichomoniasis¹³. These can cause various types of morbidity in women particularly during pregnancy resulting in abortion, preterm labour and stillbirth.^{14, 15} Vaginal infections can lead to many complications; it is of the prime importance to know the causative organisms, prevalence and its attributing factors affecting the pregnant women.

Numerous studies have shown an association between certain risk factors, dysplasia and cervical cancer which include gravidity, contraceptive use, age of first coitus, multiple partners, coexisting microbial infections, altered immune status, smoking and oral contraceptive use. In Indian setup, data on abnormal pap smear in pregnancy is lacking. The present study was designed to know the prevalence of abnormal pap smear and its associated risk factors in pregnancy.

Methods

It is a prospective observational study conducted from 2018 to 2020 at tertiary care centre, Dakshina Kannada Karnataka, India. The study was approved by the institutional ethics committee. Considering the prevalence of 8% of abnormal smear in pregnancy and 4% allowable error at 95% confidence level and 5% of level of significance, the sample size was calculated to be 200.¹⁶ Hence, 200 pregnant women were enrolled in the study who fulfilled the criteria mentioned below.

Inclusion criteria: All pregnant women who will come for regular antenatal check up.

Exclusion criteria:

- Women with history of threatened abortion in present pregnancy.
- Pregnant women not willing for pap smear.
- History of intercourse, vaginal examination, vaginal medication in the past 48 hours.
- Bleeding per vagina.
- Preterm premature rupture of membranes, preterm labor or obvious cervical dilatation on speculum examination.

Informed written consent was obtained from all the study subjects. A proforma containing general information on maternal age, parity, age at marriage, education, occupation, residence, socioeconomic status, contraception, sexual partners, smoking history and family history regarding cervical cancer was taken. Those with abnormal pap tests were managed as per protocol.

After emptying the bladder, patient was put in dorsal position. After exposing the cervix using Cusco's self retaining speculum or Sim's bivalve speculum with an anterior vaginal wall retractor, speculum findings were noted, ectocervix was sampled using an Ayer's spatula by rotating 360 degrees and then smearing on glass slides. It was immediately fixed with 95% ethyl alcohol, stained with papanicolaou stain and results were reported according to modified Bethesda classification 2014.

Statistical analysis: Descriptive statistics, the data was entered in Microsoft excel 2019 and all statistical data was analysed using statistical package for social sciences 11.0. Frequency, percentages and ratios were analysed from the data and the results were displayed in tables. Chi square test and t test was used to assess the statistical significance of the findings. Statistical significance was determined at $p < 0.05$.

Results

A total of 200 pregnant women were included in the study and results were analysed. Out of the 200 smears that were taken in our study, 6/200 (3%) of them were inadequate and the remaining 194 smears were adequate with a percentage of 97% smear adequacy as shown in figure 1.

Figure 1: Distribution of smears based on pap smear adequacy (n=200)

The Pap smear results revealed that out of 194, 100 (51.55%) of them had abnormal findings and the remaining 94 (48.45%) were negative for intra-epithelial malignancy. Among the abnormal smears, 58 (29%) women had inflammatory smears, 18 (9%) had bacterial vaginosis while 14 (7%) and 6 (3.34%) had monilial and trichomonal

pathology, only 1.03% were low grade squamous intra epithelial lesion (LSIL) and atypical squamous cells of undetermined significance (ASCUS) each respectively (figure 2).

Figure 2: Distribution of smears based on cytology (n=200)

In our study it was seen that more than half of the participants belonged to the age group between 20 to 30 years in both the groups, there was no much difference in age distribution. The mean age of the participants with normal Pap smear was 27.24 ± 4.49 years and that of abnormal pap smear was 26.81 ± 4.48 years, hence the difference between the ages of women in the normal and abnormal pap smear groups is not statistically significant (table 1).

According to residence, there were 147 subjects from rural background and 47 subjects from urban background. There were 86 (58.50%) from rural and 14 (29.78%) from

As shown in table 1, all the subjects were classified according to the modified Kuppuswamy socioeconomic status scale (2016). In our study, majority of the participants were from lower middle class i.e., 78 (51.65%), followed by upper middle class (9.80%), upper lower (7.7%) and lastly lower class (4.63%). Most of the participants in the lower class (88.8%) had abnormal pap smears. The odds of having an abnormal pap smear was more in lower middle, upper lower and lower class and the difference was statistically significant with p value less than 0.05.

The frequency of abnormal pap smear in illiterate population was 10 (83.33%), 11 (64.70%) in middle school educated women, in senior secondary it was 65 (50.7%) and in graduates was 14 (37.83%). The odds of having an abnormal pap smear was noted with lower educational status as it showed raise in occurrence of abnormal pap smear findings and in our study low odds (0.123) of abnormal Pap smear is seen with graduate education (table 1).

The number of subjects with age of coitarche (first intercourse) less than 21 years of age was 67 (34.54%) and those more than or equal to 21 years were 127 (65.46%). There were 58 (86.56%) abnormal smears in those under 21 years of age at first intercourse as compared to 42 (33.06%) in those ≥ 21 years of age at time of coitarche. This difference was proved to be statistically significant with p value less than 0.05 and the odds ratio was 0.0767 (table 2).

Demographic characteristics	Normal pap (n=94)		Abnormal pap (n=100)		P value/ Odds ratio
	Number	Percentage	Number	Percentage	
Age					P value
< 20 yrs (n = 18) 9.28%	7	38.88%	11	61.11%	0.692196
20 - 30 yrs (n = 126) 64.95%	62	49.20%	64	50.79%	(Not significant)
> 30 yrs (n = 50) 25.77%	25	50.0%	25	50.0%	
Residence					P value
Urban (n = 47) 24.23%	33	70.21%	14	29.78%	0.000606
Rural (n = 147) 75.77%	61	41.49%	86	58.50%	(significant)
Socio-economic status					Odds ratio
Upper Middle Class (n = 19) 9.80%	17	89.47%	2	10.52%	0.176
Lower Middle Class (n = 151) 77.84%	73	48.34%	78	51.65%	15.60
Upper Lower Class (n = 15) 7.73%	3	20.0%	12	80.0%	1.33
Lower Class (n = 9) 4.63%	1	11.1%	8	88.8%	1.143
Education					Odds ratio
Graduate (n = 37) 19.07%	23	62.16%	14	37.83%	0.123
Senior secondary (n = 128) 65.98%	63	49.21%	65	50.7%	32.50
Middle school (n = 17) 8.76%	6	35.29%	11	64.70%	2.20
Illiterate (n = 12) 6.19%	2	16.66%	10	83.33%	1.250

urban background with abnormal Pap smears and 61 (41.49%) and 33 (70.21%) of normal Pap smears belonged to subjects from rural and urban background. The abnormal smears were more in the patients from rural background than urban, which was found to be statistically significant (table 1).

There were 189 (97.42%) subjects with one sexual partner and 5 (2.58%) subjects with >1 sexual partner. There were 4 (80%) abnormal smears and 1(20%) normal smear in subjects with >1 sexual partner. The frequency of abnormal smear was more in subjects with >1 partner in our study, hence it is one of the risk factors (table 2).

As shown in table 2, abnormal smears were 5 (83.33.91%) in those with history of copper T use, 8 (50.0%) with history of use of oral contraceptives (OCP) and 15 (38.46%) in those using barrier methods. The frequency of abnormal smears among Cu-T users was more than other contraceptive methods.

Table 2: Distribution of smears based on sexual history and contraception

Sexual history	Normal pap (n=94)		Abnormal pap (n=100)		P value/ Odds ratio
	Number	Percentage	Number	Percentage	
Age at first intercourse					p value
< 21 years (n = 67) 34.54%	9	13.43%	58	86.56%	0.00001
> 21 years (n = 127) 65.46%	85	66.92%	42	33.07%	(significant)
Number of partners					p value
1 (n = 189) 97.42%	93	49.20%	96	50.79%	0.197112
> 1 (n = 5) 2.58%	1	20.0%	4	80.0%	(not significant)
Contraception					Odds ratio
Barrier methods (n = 40) 20.10%	25	62.5%	15	38.46%	1.7
OCPs (n = 16) 8.25%	8	50.0%	8	50.0%	0.93
Copper T (n = 6) 3.09%	1	16.66%	5	83.33%	4.9
Nil (n = 132) 68.04%	60	45.45%	72	54.54%	1.5

It was observed that 26/85 (30.58%) were abnormal smears in primigravida and 74/104 (67.88%) abnormal smears in multigravida, hence a greater number of abnormal smears were noted in multigravida which was statistically significant (table 3).

In subjects who had previous abortions, out of 20, 15 (71.42%) had abnormal pap smear and 6 (28.57%) subjects had normal pap smear. The abnormal Pap smear was more in those with previous abortion to those without (table 3).

In subjects who had previous preterm delivery (n = 13), abnormal pap smear was seen in 10 (76.92%) while normal pap smear was seen in 3 (23.07%) subjects. The abnormal pap smear was more in those with previous abortion (table 3).

There was 1 participant with history of smoking in our study. We observed, 99 (51.29%) abnormal smears in non-smokers and 1(100%) in smokers but it is a very small sample to generalize the result finding. Here, the p value is

Table 3: Distribution of smears based on previous obstetric history

Obstetric history	Normal pap (n=94)		Abnormal pap (n=100)		P value
	Number	Percentage	Number	Percentage	
Gravidity					
Primigravida (n = 85) 43.81%	59	69.41%	26	30.58%	0.00001
Multigravida (n = 109) 56.19%	35	32.11%	74	67.88%	(significant)
History of abortion					
Yes (n = 21) 10.82%	6	28.57%	15	71.42%	0.053534
No (n = 173) 89.18%	88	50.86%	85	49.13%	(significant)
History of pre-term delivery					
Yes (n = 13) 6.70%	3	23.07%	10	76.92%	0.05836
No (n = 181) 93.30%	91	50.27%	90	49.72%	(significant)

0.3 which is not statistically significant.

Out of 70 women with gestational age <20weeks, 28 (40%) had normal pap smear and 42 (60.0%) had abnormal pap. Among 124 women with gestational age >20 weeks, 66 (53.22%) had normal pap smear and 58 (46.77%) had abnormal pap smear. We observed that the gestational age had no role to play in occurrence of abnormal pap smear.

Among those with a healthy cervix (n = 165), 90 (54.54%) had normal pap smear and 75 (45.45%) had abnormal pap smears. And in those with an unhealthy cervix,

4 (13.79%) had normal pap smear and 25 (86.20%) had abnormal pap smears. This difference was statistically significant with p value of less than 0.05 and OR of 7.5 which indicates that the odds of finding unhealthy cervix in an abnormal pap smear was 7.5 times more than that in normal pap smear.

Discussion

The pap smear test used as a screening method to detect cervical cancer is an effective way to prevent the development of cervical cancer, but awareness within the community about the pap smear test is very low. Pregnancy provides an opportunity for women of a reproductive age to meet an obstetrician and discuss their health concerns and prenatal care offers an excellent opportunity to implement cervical screening in patients of young age.

In our study, the percentage of adequate smears was 97% which was similar to studies performed by Manikkam et al¹⁷, Prabhu TRB et al¹⁸. The percentage of smear inadequacy was 3% which may be due to dryness of the smear or a technical error (improper training of personnel and the use of the improper technique).

The prevalence of abnormal smear in our study is 51.55%. The abnormal smears indicative of inflammatory smears, bacterial vaginosis, monolial infection, trichomonas vaginalis, ASCUS and LSIL were 29%, 9%, 7%, 3%, 1% and 1% respectively, whose significance has been a matter of much interest in relation to pregnancy complications. These results were in accordance with Ngaojaruwong N et al¹⁹ who reported the prevalence

of abnormal smears at 38% in their study, which included smears indicative of candida infections (23.4%), bacterial vaginosis (14%), trichomonas infection (0.2%) and LSIL (0.4%). Khaengkhor et al²⁰ (2011), also reported similar prevalence of abnormal smears at 40.6% in their study, which included smears indicative of candida infections (11%), bacterial vaginosis (21%), trichomonas infection (0.6%) and LSIL (3.5%) and prevalence of these infections was further correlated with premature rupture of membranes (PROM), highlighting their diagnostic importance.

In contrast, studies like Sueblinvong et al²¹ (2005) had only 9.6% smears with infections, 0.4% LSIL and 0.4% HSIL compared to our study with 1% LSIL. While in the study conducted by Ghuge et al²² the prevalence of bacterial vaginosis was 65.6% compared to our study of 9%. This emphasizes the importance of regular screening programme during antenatal period, which is in accordance with the American College of Obstetricians (ACOG) guidelines for perinatal care. According to the guidelines, pap smear at first antenatal visit must be taken, if not done prior to pregnancy.

Abnormal pap smears reported in age group of <20 years is 61.11% which was similar to a study done by Himabindu et al²³. Abnormal smears in age group of 20-30 years accounted for 50.79% in our study and Bijeta et al²⁴ also reported a similar prevalence of abnormal smears in the 20-30 years age group.

Rural population showed more abnormal pap smears in our study. This was in accordance with the study done by Shrestha et al²⁵ as well as Arora et al²⁶. We note that the correlation of abnormal smears and socioeconomic status was statistically significant ($p < 0.05$). Thus, socioeconomic status is a risk factor as per our study which is also mentioned in a study done by Seppo Virtanen et al²⁷, which shows the impact of socio-economic status on pap smear finding.

83.33% among illiterate had abnormal pap smear, among middle school it was 64.70%, 50.7% among senior secondary, 37.83% among graduates. As the literacy level in Karnataka (rural) is 69.2%²⁸ and in our study 65.9% among 194 subjects had qualification till senior secondary, abnormal pap smears were comparable with normal pap smears among them. This was in accordance with the study conducted by Shrestha et al²⁵ except for the illiterate population.

86.56% had abnormal smears among 67 pregnant women who had first intercourse before 21 years. Hence age at first intercourse is one of the risk factors in our study. As in Karnataka mean age for marriage is 20.5 years in total (District Level Household and Facility Survey-4, 2012-2013)²⁸ and there is early child bearing, hence age of coitarche is less in our study. Mannikam et al (2016)¹⁷, Sueblinvong et al (2005)²¹, Mohindroo N et al. (2019)³⁰ found a similar relation between abnormal smears in pregnant women and early age at first intercourse. Among women with multiple sexual partners, occurrence of abnormal smears was more that is 80% had abnormal smears which is in accordance with the study done by

Ngaojaruwong N et al¹⁹ which shows a link between multiple sexual partners and cervical lesions.

Multigravida account for 30.58% of abnormal smears, it is one of the risk factor in our study. Manikkam et al (2016)¹⁷, Prabhu TRB (2016)¹⁸, Himabindu et al (2015)²³ and Bijeta et al (2015)²⁴, also reported higher number of abnormal smears in multipara. Hence, screening should take place regardless of gravidity and the first pregnancy is ideal for a first pap smear in an area with no comprehensive screening program.

Our results pertaining to use of contraception showed abnormal smears more in IUCD users, which was similar to study conducted by Mohindroo N et al.³⁰ (2019). Only long-term use of OCPs increases the risk of cervical cancer and the changes caused by OCPs revert to never users after 10 years or more. This suggests that hormonal contraceptives may not increase the risk of cervical neoplasia. There is a need to conduct a cohort study on this topic, to further strengthen or refute the safety of hormonal contraceptives.^{31, 32}

The percentage of smokers in our study was 0.52% which is similar to study done by Mohindroo N et al. (2019)³⁰ which shows 0.9%, but in the study conducted by Ngaojaruwong et al (2008) it was 6.8%, lower incidence could be because women in the studied population smoke less or were not forthcoming in history. We did not find significant relation between history of smoking and abnormal smears. This was not in accordance with the study done by Ngaojaruwong et al¹⁹, which shows a link between smoking and abnormal cervical smears.

Abnormal smears were reported more in women with unhealthy cervix ($p = 0.000051$) which is similar to a study conducted by Himabindhu et al²³ and Mohindroo N et al. (2019)³⁰. On per speculum examination, if cervix is found to be unhealthy, it is considered as an independent risk factor per se for abnormal smear and cervical pathology.

Conclusion

This study highlights the risk factors associated with abnormal smears and particularly those patients must be counseled, educated and followed up to prevent the progression to invasive cancer. In order to reduce the burden of cervical cancer in developing country where the medical services are sparse in rural areas, pregnant women seeking obstetricians for regular antenatal visit must be screened for possible pre invasive lesion of cervix, this opportunity must not be missed (opportunistic screening). Pap smear test is easy to perform, cost effective and doesn't affect the course

of pregnancy and hence, plays a major role in decreasing this preventable disease.

Conflict of interest: None. **Disclaimer:** Nil.

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