RESEARCH COMMUNICATION

# Detection and Genotyping of High-Risk HPV and Evaluation of Anti-Oxidant Status in Cervical Carcinoma Patients in Tamil Nadu State, India - a Case Control Study

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## Abstract

**Cervical cancer is the second common type of cancer among women worldwide, with the human papillomavirus (HPV) recognized as the major causative agent. The HPV 16/18 prevalance in cervical cancer patients from the Trichy and Coimbatore districts of Tamil Nadu state, India, was evaluated in addition to an assessment of oxidative stress and antioxidant status. MDA, GSH, GPx, GST, SOD, vitamin C and vitamin E were estimated in the plasma and erythrocytes of the twenty patients and an equal number of age matched normal subjects as controls. 119 paraffin embedded tissue samples were collected to perform DNA extraction and genotyping of HPV 16/18 using specific primers. Plasma and erythrocyte TBARS level was significantly elevated in the cervical cancer patients compared to normal. It was observed that SOD, GPx, GSH levels in the erythrocyte and plasma was significantly lower in cervical cancer patients, as well as GST and Vitamins E and C levels in the plasma and catalase enzyme levels in the erythrocytes. Genotyping showed 57% positive for HPV16 and 18% for HPV18, indicating that vaccination against these two will effectively reduce the burden associated with the disease. These findings suggest possible use of antioxidant supplementation as prophylactic agents for prevention and treatment of cervical cancer.**

**Keywords:** Human papillomavirus - cervical cancer - HPV genotyping - free radicals - antioxidants - India

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## Introduction

Cervical cancer is one of the most common malignancies and is the major cause of cancer mortality among Indian women (Pal and Mittal, 2004). There is an annual global incidence of nearly 5,00,000 new cases, with 2,74,000 deaths per year, and these cases are expected to increase to nearly 90% by the year 2020. More than 80% of these cases occur in developing countries (Kling and Zeichner, 2010). In India, nearly 366.58 millions of women are at risk for cervical cancer according to a report in 2010 (WHO/ICO, 2010). Though a slow and steady decline in cervical cancer incidence rates is observed in some urban populations, the rates are still high, particularly in rural areas, and the absolute number of cases is on the increase due to population growth (Sankaranarayanan et al., 2003). Virtually all cervical cancers are caused by infections with oncogenic HPV types (Walboomers et al., 1999). Of more than 35 HPV types found in the genital tract, HPV16 accounts for 50 to 60% of the cervical cancer cases in most countries, followed by HPV18 (10-12%), and HPV31 and 45 (4-5% each) (Bosch and Sanjose, 2003). However, the prevalence and oncopotency of specific HPV types in cervical cancer may vary with the geographic origin of the specimen (Bosch et al., 1995). Because of subtle regional cultural differences that exist in various states in India, it is important to describe the distribution of HPV genotypes in cancer cases and community samples from multiple representative populations before these data can be generalized for application in national cancer prevention strategies (Sowjanya et al., 2005). The studies on the HPV prevalence and genotype distribution are very few in southern region.

Oxidative stress can lead to increased production of ROS either by metabolic, dietary, environmental, or other means (Valko et al., 2006). Initiation and progression of carcinogenesis may also involve reactive oxygen species (ROS) (Punnonen et al., 1993). The levels of free radical molecules are balanced by various cellular defense mechanisms consisting of enzymatic components such as catalase, glutathione peroxidase, superoxide dismutase etc. and nonenzymatic components like vitamins E, C, glutathione etc (Halliwell, 1996; Mates et al., 1999). Oxygen free radical (OFR) induced lipid peroxidation has been implicated in malignant transformation. Lipid peroxidation products such as malondialdehyde (MDA) is formed by the target of peroxidation by ROS on the polyunsaturated fatty acids (PUFA) in the membrane lipids (Samir and Kholy, 1999). The elevated lipid peroxidation and decline in enzymatic antioxidant status

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were noticed previously in cervical cancer and other types of cancer (Manju et al., 2002; Baskar et al., 2004; Gokul et al., 2010). The formation of lipid peroxidation products is normally prevented or scavenged by a host of antioxidant enzymes which are rich in normal erythrocytes and low level of these enzymes are associated with the risk of cancer (Mates et al., 1999; Diplock, 1997). Therefore, there is a growing interest in studying the lipid peroxidation and antioxidant status in cervical cancer patients and in this present study, the status of enzymic and non enzymic antioxidants by analyzing the plasma and erythrocytes of women with cervical cancer and in normal patients were assessed. The aim of this study is to analyze the prevalence of HPV16/18 high risk types among the populations of Trichy and Coimbatore, Tamilnadu, South India and to find the prevalence of other high risk types. The study was carried out from 2007 to 2009 on invasive cervical cancer patients.

## Materials and Methods

*HPV sample collection*

Formaldehyde-fixed, paraffin-embedded samples of patients stored for one to two years in pathology laboratory of G.V.N Institute of Oncology, Trichy and G.K.N.M Hospital, Coimbatore were tested for HPV genotypes. The patients attending the oncology unit formed the study group. Pathologists confirmed the histological diagnosis of cancer through pathological slides. All patients had undergone surgical procedures for assessment to stage the extent of disease or for treatment. Informed consent was obtained from patients to use their samples. The ages of patients are between 22 to 67 yrs. The selection criterion for the patients was gender (female), and invasive Squamous cell carcinoma patients. Controls whenever possible were matched to cases according to age and they had a normal cervix.

*Extraction of DNA*

For each of the cases, 5$\mu$m thick slices were cut with standard microtome from every paraffin wax block and transferred into a 1.5ml microtube. The Microtome blade was washed with xylene and ethanol to prevent cross contamination between the samples after sectioning of each block, triplicate. The area to be sectioned was examined carefully for the presence of tumour tissue and that an equal amount of tissue was included in each set. The sections were then dewaxed by xylene followed by ethanol wash to remove residual xylene. Procedures for DNA extraction was performed according to a standard phenol/chloroform extraction (Chan et al., 2001). The extracted DNA was stored in 4ºC until the PCR amplification was performed.

*PCR amplification*

The samples were subjected to four sets of primers targeting a 155 bp fragment of L1 gene (GP5/GP6), general primers for HPV, HPV types 16 and 18 specific primers (TS16-a/TS16-b and TS18-a/TS-18b), and PC03/PC04 for β-globin as internal control (Khan et al., 2007). The primers were procured from Sigma Aldrich, Bangalore, India (Table 1).

**Table 1. Primers Used forGenotyping HPV**

| Primer | | Sequence | Amplimer length(bp) |
|---|---|---|---|
| β-Globin | | ACACAACTGTGTTCACTAGC | 110 |
| | | CAACTTCATCCACGTTCACC | |
| New TS 16 | a | GGTCGGTGGACCGGTCGATG | 96 |
| | b | GCAATGTAGGTGTATCTCCA | |
| New TS 18 | a | CCTTGGACGTAAATTTTTGG | 115 |
| | b | CACGCACACGCTTGGCAGGT | |
| GP5 | | TTTGTTACTGTGGTAGATAC | 155 |
| GP6 | | GAAAAATAAACTGTAAATCA | |

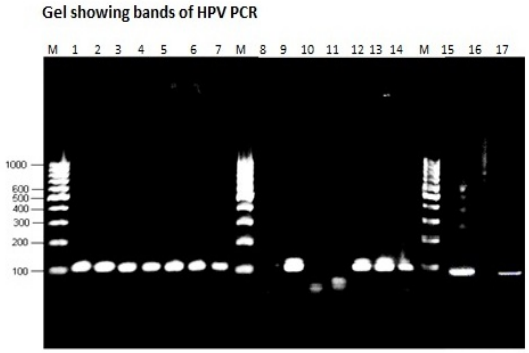


**Figure 1. Gel Showing Bands of HPV PCR** M- markers of 100 to 1000bp, Lane 1-7 – shows β-globin positive samples, Lane – 8 – Negative control, Lane 9 – Positive control for HPV, Lane 12 – HPV 18 positive control, Lane 13, 14 – HPV 18 positive samples, Lane 15 – HPV 16 positive control, Lane 17 – HPV 16 positive sample

DNA amplifications were followed by the method of Saiki et al., (1988) with a slight modification. The reaction mixture for genotyping HPV of type 16 and 18 contained 10$\mu$l of sample, 10Mm Tris-Hcl (pH-9.0), 50mM KCl, 2.5mM MgCl2, 0.1% Triton X-100, 0.01% Gelatin, 200 $\mu$M Deoxynucleoside triphosphates, 50pmol of each primer and 0.25U Super Taq. The PCR conditions were initial denaturation at 95°C, 5 min, followed by denaturation at 95°C, 1min, annealing at 55°C, 1min and 72°C, 45s, elongation at 72°C, 2 min, the process was repeated for 40 cycles and then incubated at 72°C, 3 min, final extension at 4°C ∞.

The PCR conditions for New TS-16 and TS-18 primers were the same as for the GP5/GP6 primers, except that for TS-16 and TS-18 the annealing temperatures were 61 °C and 63 °C, respectively.

The quality of the DNA preparation was assessed by PCR using β globin primers as an internal control (Khan et al., 2007). The reaction mixture for β globin contained 0.1% Triton X-100, 5µl of sample, 10mM Tris-HCl, pH 9.0, 50mM KCl, 1.0mM $MgCl_2$, 0.2mM Deoxynucleoside triphosphate (dNTPs), 0.2pmol of each primer ($PCO_3$,$PC0_4$) and 0.2U Taq-polymerase. The PCR conditions were initial denaturation at 94°C, 5 min, followed by denaturation at 94°C, 1min, annealing at 51°C, 1min and 72°C, 30s, elongation at 72°C, 5 min, the process was repeated for 40 cycles and then incubated at 72°C, 3 min, final extension at 4°C.

Positive β globin amplification proved that the sample contained a sufficient amount of DNA and that no PCR inhibitors were present. After PCR, 25 to 30 $\mu$l of all

samples was run on an agarose gel stained with ethidium bromide. It was visualized using UV- transilluminator and the final results were read according to the size of the fragment.

Positive control was validated using cervical carcinoma samples known to be positive for HPV type 16 or 18. Negative PCR control was included without DNA template (Figure 1)

*Lipid peroxidation and antioxidant subjects*

Twenty newly diagnosed cervical cancer patients (stage III) attending the oncology unit of GVN Hospitals, Trichy were taken as subjects. Patients who were treated for other chronic diseases or cancer tumor were not included in the study. Twenty control subjects were selected according to their age. Oral consent was obtained from all the patients. Papanicolaou smear was done on the subjects to identify the cervical cancer patients and control subjects. Control subjects had normal cytological cervical cells in the Papanicolaou smear and normal pelvic examination. The age of the patients ranged from 26 – 57yrs. The mean age of the patients was 41.35 ± 7.35 yrs.

*Sample collection*

5 mL of peripheral venous blood samples was collected in heparinised screw cap vials and centrifuged at 1000 rpm for 15 mins, to separate plasma and erythrocytes for biochemical assays and stored at -70 ºC for processing. After centrifugation, the buffy coat was removed and the plasma and erythrocytes were washed with physiological saline. The erythrocyte suspension was prepared by the method of Dodge et al., (1963) modified by Quist, (1980). The erythrocytes were haemolysed by rigourous vortexing. The packed cells were used for the analysis of TBARS, SOD, GPx, GSH and Catalase.

*Estimation of lipid peroxidation*

Thiobarbituric acid reactive substances (TBARS), Malonyldialdehyde (MDA), an end product of fatty acid peroxidation, reacts with TBA to form a colored complex that has maximam absorbance at 532nm. Lipid peroxidation in the erythrocytes was measured by the formation of Thiobarbituric acid reactive substances (TBARS) described by Donnan et al. (1950). Lipid peroxidation in the plasma was measured by the method of Ohkawa et al. (1979).

*Estimation of superoxide dismutase*

The activity of superoxide dismutase (SOD) in the plasma and erythrocytes was derived from inhibition of the reduction of nitroblue tetrazolium (NBT) by superoxide radicals generated in the xanthine oxidase-catalyzed oxidation of xanthine. The unit of activity is the amount of the enzyme that inhibits the rate of formazan dye formation by 50% per mg of hemoglobin content in the sample (Sun et al., 1988).

*Estimation of glutathione peroxidase*

The activity of glutathione peroxidase (GPx) in the plasma and erythrocytes was measured as described by (Paglia and Valentine, 1967) where GPx catalyses the oxidation of glutathione by cumene hydroperoxide.

*Estimation of reduced glutathione*

Reduced glutathione (GSH) in the plasma and erythrocytes was determined by the method of Ellman, (1959). It is measured by following the decrease in absorbance due to the oxidation of NADPH.

*Estimation of glutathione-S-transferase*

The activity of glutathione-S-transferase in the plasma was measured by the method of Habig et al. (1974) by following the increase in absorbance at 340 nm.

*Estimation of catalase*

The activity of catalase was assayed by the method of Sinha, (1972) based on the utilization of hydrogen peroxide by the enzyme in the erythrocytes. The color developed was read at 620 nm. Values were expressed as μmol/l of $H_2O_2$ consumed per minute per mg of hemoglobin.

*Estimation of vitamins C and E*

Plasma ascorbic acid was estimated by the method of Tietz, (1986). Plasma vitamin E was measured by the method of Baker et al. (1980).

*Statistical analysis*

Statistical analysis was performed by SPSS statistical software package version 11, using Student's t test for calculating means and standard deviations. Fisher's exact test or Chi square test was used for comparing age and finding any relations between HPV positivity and other variations. Statistical significance was assumed at the $P< 0.05$ level.

## Results

In this study, formaldehyde- fixed paraffin embedded tissue sample of 119 patients was tested for detection and typing of HPV. In all, cervical biopsies from patients with histology confirmed invasive cervical cancers were received for HPV analysis. The prevalence of HPV in cervical carcinoma tissue samples was estimated. β globin gene was used as an internal control. HPV types were found by using specific primers for each type. The results for the presence of HPV and the HPV positive type distribution for cervical cancer positive patients are listed below (Table 2).

The mean age of patients was 44.2 years (ranging from 22 to 67 years). The patient demographic characteristics with their odds ratio and 95% CI were shown in Table 4.

Figure 2 showing the lipid peroxidation and antioxidant status in plasma of healthy and cervical cancer patients.

**Table 2. HPV Positive Type Distribution for Cervical Cancer Positive Patients**

| HPV type | Patients (N) | % |
|---|---|---|
| HPV 16 | 36 | 56.2 |
| HPV 18 | 12 | 18.7 |
| Other types | 16 | 25 |
| Total | 64 | 100 |

**Table 3. PCR Positive Patients According to Their Age**

| Age | Positive (N) | % |
|---|---|---|
| 20 – 30 | 9 | 14 |
| 30 – 40 | 24 | 37.5 |
| 40 – 50 | 15 | 23.4 |
| 50 – 60 | 10 | 15.6 |
| 60 – 70 | 6 | 9.37 |
| Total | 64 | 100 |

**Table 4. Patient Demographic Characteristics and Associated Odds Ratio for Invasive Cervical Cancer**

| Characteristic | Cases N (%) | Controls N (%) | OR | 95% CI |
|---|---|---|---|---|
| HPV – DNA | | | | |
| Positive | 49 (83) | 15 (25) | 1.00 | |
| Negative | 10 (17) | 45 (75) | 14.70 | 5.99-36.04 |
| | | | $\chi2$ =38.02 | P <0.0001 |
| No of sex partners | | | | |
| 1 | 24 (40.6) | 27 (45) | 1.00 | |
| 2 | 26 (44) | 24 (40) | 0.82 | 0.37-1.97 |
| 3+ | 9 (15.2) | 9 (15) | 1.08 | 0.36-3.18 |
| | | | $\chi2$ =0.08 | P =0.3 |
| Age at first intercourse | | | | |
| 10-15 | 4 (6.7) | 3 (5) | 1.00 | |
| 16-20 | 33 (55.9) | 33 (55) | 1.33 | 0.27-6.47 |
| 20-25 | 17 (28.8) | 31 (35) | 1.23 | 0.50-2.75 |
| 25+ | 5 (8.47) | 3 (5) | 0.48 | 0.10-2.33 |
| | | | $\chi2$ =0.20 | P =0.3 |
| Parity | | | | |
| 1-3 | 15 (25.4) | 29 (48.3) | 1.00 | |
| 4-6 | 33 (55.9) | 24 (40) | 0.37 | 0.16-0.85 |
| 7+ | 11 (18.6) | 7 (11.6) | 0.87 | 0.29-2.58 |
| | | | $\chi2$ =4.7 | P =0.01 |
| Age at first pregnancy | | | | |
| 15-20 | 37 (62.7) | 29 (48.3) | 1.00 | |
| 20-25 | 16 (27.1) | 23 (38.3) | 1.83 | 0.80-4.82 |
| 25-30 | 6 (10.1) | 8 (13.3) | 0.92 | 0.26-3.19 |
| | | | $\chi2$ =1.6 | P =0.09 |
| Menopause | | | | |
| Premen-opausal | 43 (72.8) | 40 (66.6) | 1.00 | |
| Postmen-opausal | 16 (27.1) | 20 (33.3) | 1.34 | 0.61-2.94 |
| | | | $\chi2$ =0.28 | P =0.29 |
| Smoking | | | | |
| Smoker | 33 (55.9) | 29 (48.3) | 1.00 | |
| Non smoker | 26 (44.1) | 31 (51.6) | 1.35 | 0.65-2.97 |
| | | | $\chi2$ =0.41 | P =0.25 |
| Marriage | | | | |
| Single | 8 (13.5) | 5 (8.3) | 1.00 | |
| Married | 51 (86.4) | 55 (91.7) | 1.72 | 0.52-5.62 |
| | | | $\chi2$ =0.38 | P =0.26 |
| Education | | | | |
| None | 21 (35.5) | 27 (45) | 1.00 | |
| Primary | 35 (59.3) | 28 (46.6) | 0.62 | 0.29-1.32 |
| Secondary | 3 (5.2) | 5 (8.3) | 2.08 | 0.45-9.48 |
| | | | $\chi2$ =0.34 | P =0.27 |

Lipid peroxidation levels indicated by TBARS were significantly higher in the plasma of cervical cancer patients as compared with normal subjects. The enzymic antioxidants SOD is significantly lower in the cervical cancer patients.

Figure 3 shows Vitamin C and E (non enzymatic antioxidant status) levels in the plasma lowered in cervical cancer patients than the healthy patients.

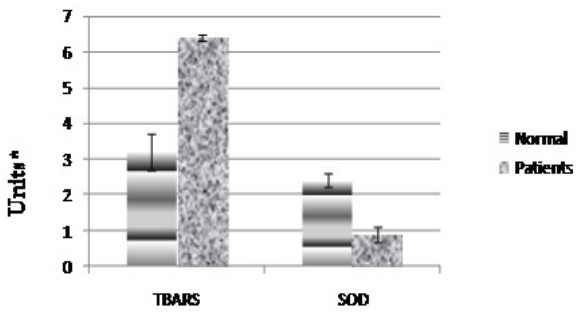


**Figure 2. Lipid Peroxidation and Antioxidant Status in Plasma of Healthy and Cervical Cancer Patients** (Mean ± S.D; n=20). SOD U/min/mg Hb; TBARS n moles of MDA formed/mg of hemoglobin. P value was <0.0001 and it was significantly different when compared with normal.

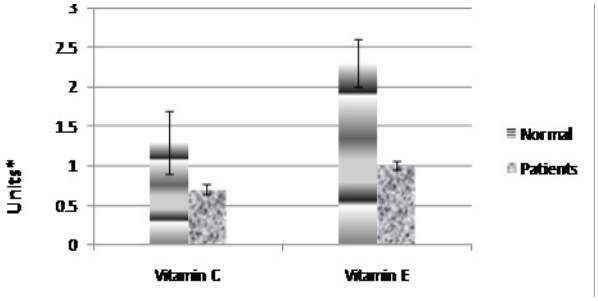


**Figure 3. Non Enzymatic Antioxidant Status in Plasma of Healthy and Cervical Cancer Patients (Mean ± S.D; n=20).** Vitamin C and E mg/dl plasma. P value was <0.0001 and it was significantly different when compared with normal

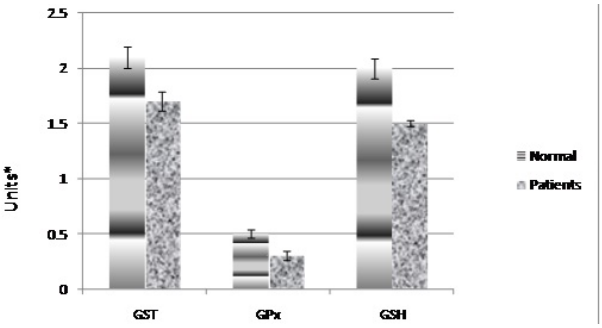


**Figure 4. Enzymatic Antioxidant Status in Plasma of Healthy and Cervical Cancer Patients** (Mean ± S.D; n=20). GST Units/min/mg Hb; GPx Units/min/mg Hb; GSH mg/dl plasma; P value was <0.0001 and it was significantly different when compared with normal

Figure 4 shows the enzymatic antioxidant enzymes such as GST, GPx, GSH status to be low in the plasma of cervical cancer patients when compared to the normal patients.

Figure 5 shows the lipid peroxidation and antioxidant status in erythrocytes of healthy and cervical cancer patients. Lipid peroxidation levels indicated by TBARS were significantly higher in the erythrocytes of cervical cancer patients as compared with normal subjects. The enzymic antioxidants SOD is significantly lower in the cervical cancer patients. The catalase activity was also lower in cervical cancer patients.

Figure 6 shows the erythrocytes exhibiting low

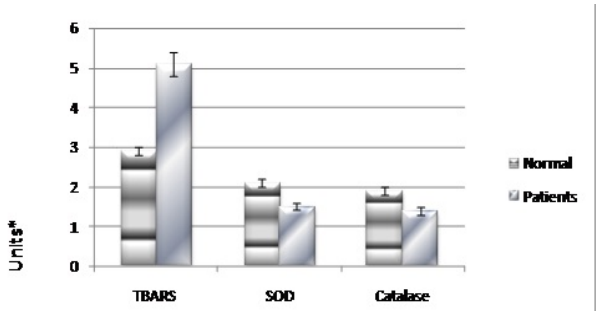


**Figure 5. Status of Lipid Peroxidation and SOD in the Erythrocytes of Healthy and Cervical Cancer Patients** (Mean ± S.D; n=20). SOD U/min/mg Hb; TBARS n moles of MDA formed/mg of hemoglobin; Catalase µmoles of $H_2O_2$ utilized/ min; P value was <0.0001 and it was significantly different when compared with normal.

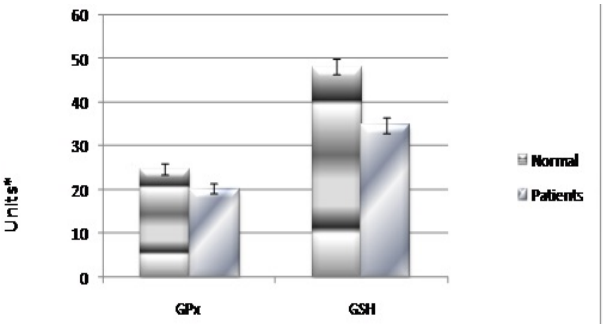


**Figure 6. Status of Enzymatic Antioxidants in Erythrocytes of Cervical Cancer and Healthy Patients** (Mean ± S.D; n=20). GPx Units/min/mg Hb; GSH mg/dl plasma; P value was <0.0001 and it was significantly different when compared with normal

levels of enzymatic antioxidants such as GSH, GPx in the cervical cancer patients when compared with normal patients.

## Discussion

The results from this present study have confirmed that HPV infection is also present in the vast majority of cervical cancers in an Indian population. Paraffin embedded tissue samples were collected from 119 patients and out of which 64 samples showed positive for Human papillomavirus. In this study we observed that HPV16 was the most prevalent type in cervical cancer patients which supported the other reports referring to this study (Singh, 2005; Das et al., 2008; Pillai et al., 2009). HPV 16 was found to be more in patients than HPV 18. In India, a high prevalence of HPV16 has been reported among women (Walboomers et al., 1999). This is in consistent with the results of other reports who have mentioned HPV16 as the main oncogenic type of HPV associated with cervical cancer (Eslami et al., 2008). Similar HPV type distribution has been recently reported from other countries, where HPV16 was shown to be the most common type (Menegazzi et al., 2009; Fernandes et al., 2010; Wu et al., 2010). The risk of cervical cancer increased with sexual intercourse and pregnancy at young age. Health concerns should be made known to people to improvise their personal hygiene. Moreover due to low socioeconomic status, women are malnourished and thus are more prone to malignancy. High parity continued to be at risk.

It is important to identify those HPV positive women with increased risk of developing cervical carcinoma by methods that detect viral oncogene expression. The association of high-risk types HPV 16/18 might be due to their DNA integration into the host DNA through the disruption of E2, which led to an uncontrolled production of oncogenic proteins E6 and E7, which could cause malfunction in the cell cycle regulators p53 and Rb and a highly significant association of high-risk HPV 16/18 with p53 and bcl-2 expressions in the cervical lesions (Grace et al., 2003). There is a need for genotyping of HPV in rural areas particularly in southern regions of India. Prevention of cervical carcinogenesis is possible using vaccines as it is associated with HPV infection (Hendrix, 2008). HPV is less prevalent in older age which was proved in many studies (Higgins et al., 1991) and the same was found in our study also. However, we found a negative association between HPV DNA detection and postmenopausal status and the negative association between detection of HPV 16 and 18 with postmenopausal status was highly significant (P<0.001). Some studies have stated that hormones such as estrogen can stimulate differential transcription of HPV in cervical carcinoma cells (Mitrani-Rosenbaum et al., 1989). May be alteration in the female hormone profile associated with the menopause might have influenced the HPV DNA replication and therefore, contributed to the clearance of HPV infection, especially high risk types 16 and 18. There is a necessity to do screening program for women at the age of 30, which may help to identify the women at high risk of developing invasive cancer and treat them. Several prophylactic vaccines for HPV 16, 18, 6, and 11 are under clinical trial (Paavonen et al., 2007). However, 75% (37/49) of cases showed infection with HPV16 or HPV18. The approved quadrivalent HPV prophylactic vaccine might be able to protect only about two thirds of cervical cancer in southern India.

Antioxidant enzymes were analysed in the erythrocytes and plasma of human peripheral blood. In this study, the influence of oxidative stress and antioxidants in the healthy and cervical cancer patients was estimated. HPV-DNA integration involves a break which is created in both the host DNA and in the circular viral episome for integration to occur, and studies have shown that viral integration is in fact increased by the induction of DNA double strand breaks. Inflammation generates reactive oxygen species, which in turn have the possibility to create such DNA strand breaks. It is likely that these breaks enable a greater frequency of HPV-DNA integration, and in this way contribute to carcinogenesis (Williams et al., 2011), thus it is possible that HPV infection induces an oxidative stress in cervical cells (Keegan and Keegan, 2004). An extensive search over the past several decades has suggested the role of free radicals in a number of diseases including carcinogenesis (Maeda and Akaike, 1998). Lipid peroxidation plays an important role in the control of cell division. The end product of lipid peroxidation, malondialdehyde (MDA) has its high cytotoxic and inhibitory action on protective enzymes. Since MDA is an index of lipid peroxidation, it was

estimated in patients with cervical cancer to estimate the extent of lipid peroxidation. In present study, increased levels of MDA in the circulation of cervical cancer patients can be attributed to increase in oxidative stress due to the deficiency of antioxidant mechanism (Otamiri and Sjodahl, 1989; Diplock et al., 1994). SOD protects cells against superoxide radical by dismutation of the highly reactive superoxide anion to oxygen and to a less reactive oxygen species, hydrogen peroxide. In the present study, the decline in SOD activity is observed with the increase in circulating lipid peroxides of cervical cancer patients. Vitamin E safeguards intracellular molecules against damage produced by free radicals. In present study, the decreased levels of plasma vitamin E may be due to their increased utilization in scavenging lipid peroxides (McCord, 2000; Manju et al., 2002). Blood glutathione levels are believed to be predictors of morbidity and mortality. The lower GSH levels, glutathione related enzymes, glutathione peroxidase enzyme present in cervical cancer patients support the hypothesis that this status is inversely related to malignant transformation. The depletion of vitamin E (alpha-tocopherol) and vitamin C (ascorbic acid) in the diet can result in the enhanced lipid peroxidation observed in cervical cancer patients. Women from the low socioeconomic status are malnourished and thus are more prone to malignancy. Other studies further indicate that women who consume fewer amounts of fruits and vegetables are associated with cervical cancer (Eastwood, 1999).

Thus low levels of GSH, GPx, GST, SOD, vitamin E and vitamin C in the circulation of cervical cancer patients may be due to their increased utilization to scavenge lipid peroxides as well as their sequestration by tumor cells. Malnutrition and smoking may also be a significant cause for the increased prevalence of cervical cancer in women with a low socioeconomic status. The enhanced erythrocyte lipid peroxidation and impaired antioxidant enzyme activities observed in cervical cancer patients indicate the potential for oxidative injury to erythrocytes and in cervical cancer patients. Antioxidants neutralize oxidative stress by scavenging reactive oxygen species before they cause damage to the various biological molecules. So recommending nutritious diet to low socioeconomic status populations may help them to lead a better cervical cancer free life. Effective therapeutic agents for HPV 16 may be formulated as majority of the populations are affected. In conclusion, HPV testing of women with abnormal cervical cytology, educating women about HPV genital infection and encouraging the women of 30 years of age and older to perform routine Pap smear seems to be necessary.

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