Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/mjafi

Short Communication

Detection of human papillomavirus in women attending Pap cervical screening camp at a peripheral hospital of North-Eastern India

Sibnarayan Datta [a,*], *Maj Manisha Agarwal* [b], *Soumya Chatterjee* [c], *Hemanta Kumar Gogoi* [c], *Vijay Veer* [c], *Lokendra Singh* [c]

[a] *Scientist 'C', Biodefence & Biotechnology Division, Defence Research Laboratory Tezpur (DRDO), Tezpur 784 001, Assam, India*

[b] *Graded Specialist (Pathology), Military Hospital Allahabad, C/o 56 APO, India*

[c] *Defence Research Laboratory Tezpur (DRDO), Tezpur 784 001, Assam, India*

ARTICLE INFO

Article history:
Received 1 April 2012
Accepted 25 April 2013
Available online 6 August 2013

Keywords:
HPV
HPV 16
Cervical cancer
High risk type
Pap test
PCR

ABSTRACT

Human papillomavirus (HPV) associated cervical cancer is the leading cause of deaths in India. However, cytological/HPV screening may result in early detection of cervical cancer, resulting in early treatment and reduced mortality. Although reports related to general population is available, data on HPV prevalence among women attending AFMS health care facilities is scarce. Cervical samples were collected for cytological staining by Pap test and molecular detection by PCR, genotyping by HPV specific primers and sequencing. Apart from finding of atypical cells of undetermined significance (ASCUS) in one subject, no evidence of malignancy was observed. A high prevalence of HPV was found in this study group, which was intermediate between previous reports from general population and cervical cancer patients. All the subjects had infection of high risk HPV type16. HPV prevalence was found similar between different age groups. Although, none of the study subjects had malignant changes, but due to high prevalence of high risk HPV infection and other associated risk factors, these subjects might be at an elevated risk of developing cervical cancer. Regular follow-up of these patients who were detected HPV positive are required to screen for cervical malignancy.

Introduction

Worldwide cervical cancer is the second leading cause of uterine cancer in developing countries and epidemiological studies have clearly established HPV as the causal agent.[1] In India it is the primary cause of female mortality.[1–3] Unlike most other cancers, cervical cancer is preventable and curable, if detected early. Developed countries have radically reduced cervical cancer incidence through population screening.[1,2] Method such as Pap test and HPV DNA PCR are used to screen large populations.[2–4] HPV is a double-stranded DNA virus (family-*Papillomaviridae*), comprising more than 100

* *Corresponding author*. Tel.: +91 94353 81750.
E-mail address: sndatta1978@gmail.com (S. Datta).

0377-1237/$ – see front matter © 2013, Armed Forces Medical Services (AFMS). All rights reserved.
http://dx.doi.org/10.1016/j.mjafi.2013.04.014

genotypes, some of which have high oncogenic potential ("high-risk genotypes" such as HPV 16 and HPV 18).[2] HPV genotyping thus enables identification of patients at risk of developing cervical cancer.

In India, inadequate HPV screening programs have contributed to enormous cervical cancer burden.[1,2] Some data on HPV screening among women attending general health care facilities is available from our country. However, there is a scarcity of data on HPV among women, who access health care facilities provided by AFMS. We thus aimed to assess the prevalence of HPV high-risk genotypes (16 & 18) within this group of women.

Material and methods

Cervical samples analyzed in this study were collected from women, attending a Pap test camp (as a part of welfare measure), organized by a peripheral hospital under AFMS (situated in North-Eastern India). Total 112 women were interviewed based on a standard questionnaire and informed consent was obtained before sampling. Besides basic demographic data, marital status, number of pregnancies, parity and other risk factors associated with HPV or cervical cancer was also collected. Subjects with ongoing pregnancy, menstrual bleeding, and unmarried girls were excluded. Cervical examination and sample collection (using Ayre's Spatula/endocervical cytobrush) was done by trained medical personnel according to the standard procedures. To avoid bias, comparison of the cytological results & molecular detection results were done independent of each other.

Cervical scraping was smeared on microscopic glass slides, fixed and Papanicolaou staining was done following standard procedure followed by visualization under a compound microscope (Olympus CH20, Singapore) by a trained pathologist. Observations were recorded as per 2001 Bethesda system terminology for reporting cervical cytology results.[5] Details of the procedures are available from the corresponding author.

For molecular detection of HPV DNA by Polymerase Chain Reaction (PCR), cervical cells were processed according to modified freeze-thaw and boiling procedure for preparation of DNA extracts.[6] Extracted DNA was confirmed by performing PCR of housekeeping gene coding for human interleukin – 1b promoter. HPV PCR was performed by using HPV MY-PCR primers (MY09/MY11) and positive samples were further subjected to HPV type specific PCR for HPV 16 and HPV 18.[7,8] Details of PCR (reaction mixture composition, primer concentration and thermal profiles) are available from the corresponding author.

Amplicons generated in the type specific HPV PCR were randomly selected, purified, and sequenced (Biolinkk, New Delhi). Sequence electropherograms were checked manually and edited sequences were subjected to similarity search using NCBI BLAST algorithm [http://blast.ncbi.nlm.nih.gov/Blast.cgi].

Categorical data were analyzed and *P* value computed (two tailed) using 2 × 2 contingency table [www.graphpad.com/quickcalcs/contingency1.cfm]. *P* values less than or equal to 0.05 was considered statistically significant.

Results

Of the 112 study subjects, 94 fulfilled the study criteria and were included in the study. Age of the subjects ranged from 20 to 55 (median 30 years). Parity ranged from 0 to 6 (median 2). For analysis, the subjects were further divided into three age groups: Gr I (20–30 years), Gr II (31–40 years) and Gr III (more than 41 years). Almost 92% subjects comprised the first two groups (48% and 44% respectively), rest comprise the third age-group. Demographical details and cytological findings in these groups are summarized in Table 1.

Negative for Intraepithelial Lesion or Malignancy (NILM) was statistically similar between Gr I and Gr II (58% vs. 46%, $P = 0.119$); between Gr I and Gr III (58% vs. 63%, $P = 0.563$) while significantly lower in Gr II as compared to Gr III (46% vs. 63%, $P = 0.022$). No significant difference was observed in incidence of inflammation (INFLM) between Gr I and Gr III (11% vs. 13%, $P = 0.828$), but was significantly higher in Gr II as compared to both Gr I and Gr III (11% vs. 32%, $P = 0.000$ and 13% vs. 32%, $P = 0.002$, respectively).

Interestingly 54 (57%) of the samples had amplifiable HPV DNA (with HPV primers MY09/MY11). Moreover, all these positive samples were also amplifiable only with HPV

Table 1 – Basic demographic data and cytological findings.

Age group	*n*	Age median (range)	Parity median (range)	Cytological findings				*P* values
				Inadequate cells	Adequate cells			
					NILM (%)	INFLM (%)	ASCUS (%)	
Gr I (20–30 years)	45	25 (20–29)	2 (0–6)	14	26 (58)†,‡	05 (11)$^{@,\&}$	00 (0)	$^{\dagger}P = 0.119$,
Gr II (31–40 years)	41	32 (31–38)	2 (0–6)	08	19 (46)$^{\dagger,\#}$	13 (32)$^{\&,*}$	01 (2)	$^{\ddagger}P = 0.563$,
Gr III (41 and more)	08	45 (40–66)	2 (0–5)	02	05 (63)$^{\ddagger,\#}$	01 (13)$^{@,*}$	00 (0)	$^{\#}\underline{\mathbf{P = 0.022}}$,
Total	**94**	**30 (20–55)**	**2 (0–6)**	**24**	**50 (53)**	**19 (20)**	**01 (1)**	$^{@}P = 0.828$,
								$^{\&}\underline{\mathbf{P = 0.000}}$,
								$^{*}\underline{\mathbf{P = 0.002}}$

NILM – Negative for intraepithelial lesion or malignancy; **INFLM** – Inflammatory cells; **ASCUS** – Atypical squamous cells of undetermined significance.
Significant differences are shown in bold underlined *P* values.