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Full Length Research Paper

Diagnosis of invasive squamous cell carcinoma: Impact of opportunistic screening in >70 years old women in Trentino (northern region of Italy)

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To assess the value of opportunistic screening in diagnosis of invasive squamous carcinoma found in >70 years old women in Trentino (northern region of Italy) in the period 2007 to 2010, the cytopathology Section of Rovereto Hospital examined 28589 opportunistic Pap smears. papillomavirus (HPV) genotyping by polymerase chain reaction (PCR) was performed in all histological specimens with diagnosis of low grade and high grade intraepithelial lesions and invasive cervical carcinoma. 111 cases (0.38%) of cervical intraepithelial neoplasia-3-squamous cell carcinoma were identified in 28589 opportunistic Pap smears. The cytological diagnosis of cervical intraepithelial neoplasia-3 was performed in three cases, confirmed by cone biopsy in two patients with the presence of HPV-16 and HPV-58 with PCR. The diagnosis of keratinizing squamous cell carcinoma was performed with Pap smear in three patients, histologically confirmed by the biopsy with the presence of HPV-58 in one case. Non-keratinizing squamous cell carcinoma was diagnosed with Pap smear in two cases, histologically confirmed by hysterectomy with bilateral salpingo-oophorectomy in absence of HPV. It is necessary to note that early diagnosis decrease to mortality, morbidity and management costs of new cases of cervical cancer diagnosed in > 65 years old women. The present study supports the screening policy to perform Pap test every 3 years until aged 69 years, independently to sexual activity.

Key words: Invasive cervical carcinoma, opportunistic screening, cancer in elderly women.

INTRODUCTION

Since 1996, Italian national guidelines have recommended to different regions, the implementation of organised screening programmes for cervical cancer. These recommendations, largely based on European guidelines, include personal invitations to women aged 25 to 64 years for a Pap smear every three years, a monitoring system and quality assurance for each phase of the programme. Surveys designed to assess the level of implementation of organised programmes in Italy and to collect process indicators have been conducted by Italian Group for Cervical Screening since 1997 (Ronco

et al., 2007). Their results have been published by the Osservatorio Nazionale Screening (ONS; National Centre for Screening Monitoring) since 2002. Since 1993 in Trentino region (North Italy), an Organized Screening (OrS) exists for women 25 to 65 aged. The target population comprises 146737 women. In the period 1993 to 2006, the Pap-smears of OrS were examined in the Institutes of Anatomic Pathology and Cytopathology of S. Chiara Hospital Trento and Rovereto Hospital. Since 2007, the Cytopathology Section of Institute of Anatomic Pathology of Rovereto Hospital has examined only Pap-tests of Opportunistic Screening (OpS); left to the woman's initiative. OpS may be considered as all Pap-test performed outside an OrS program. For example, some women have Pap-test at their doctor's office during their physical examination independently of personal

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letter invitation of OrS. An estimated 2,927 new cases of cervical cancer occurred in Italy in 2005 (crude incidence 9.7/100,000; world age-standardized incidence 6.0/100,000). 1014 (34.64%) has been diagnosed in >65 years old women (AIRTum, 2006; ISTAT, 2005). Early diagnosis is necessary to decrease the direct management costs of disease. In the present study we have examined the screening histories, treatment, human papillomavirus (HPV) detection of cervical intraepithelial neoplasia (CIN)3- invasive squamous cell carcinoma (SCC) in >65 years-old women, diagnosed in the period 2007 to 2010 with opportunistic Pap-tests in the Cytopathology Section of Institute of Anatomic Pathology in Rovereto Hospital. The aim of the paper is to study the incidence of invasive SCC diagnosed with opportunistic screening in order to decrease the direct management cost of disease in Trentino region in >65 years-old women.

MATERIALS AND METHODS

The Pap smear was performed by gynaecologist to the woman's initiative. An experienced cytopathologist (TP) whose diagnostic experience exceeds 20 years examined all abnormal smears and 10% of the normal smears previously observed by a senior cytotechnologist. Colposcopic and cervical biopsies were taken by an experienced colposcopist (in practice for more than 10 years) and review by a senior colposcopist as part of the routine. Cervical biopsy was performed in two cases, cone biopsy in two patients and hysterectomy with bilateral salpingo-oophorectomy in three cases. Consensus polymerase chain reaction (PCR) and direct sequencing of PCR products (DNA HPV typing) were used to determinate the type or types of HPV in histological specimens. PCR consensus primer sets which hybridize to highly conserved regions of the HPV genome has been designed to detect many known HPV types in a single amplification procedure (Gravitt et al., 1991; Lungu et al., 1992; Rady et al., 1995; Zheng et al., 1995). In the present study, we used the consensus primer sets to detect HPV in histological specimens. These primers promote the amplification of a 450 bp fragment spanning the L1 open reading frame (ORF) from at least 25 distinct genital HPV types (Manos et al., 1989). Additionally, samples positive by L1-PCR were digested with three restriction enzymes (Hae III, BstN I and Dde I) for accurate HPV typing. Cervical tissue samples were obtained from 8 patients (Table 2). Specimens were stored in liquid nitrogen until use. DNA extraction was carried out according to standard procedures (Strauss, 1987). DNAs of several cloned HPV types were tested by the PCR using general HPV primers. The PCR amplifications, which target a portion of the HPV L1 region (approximately 450 bp), were performed on 5 to 10 µl of each sample preparation as previously described (Manos et al., 1989). Reactions contained aliquots of clinical samples or of control DNAs in 100 µl of solutions containing 50 mM KCl, 1.5 mM MgCl₂, 10 mM Tris (pH 8.3), 200 µM of each dNTP (dATP, dCTP, dGTP, dTTP), 100 mg/ml gelatin, 2.5 units Taq polymerase (Perkin Elmer Cetus Instruments, Norwalk, CT) and 100 pmoles of each consensus primer (MY 11 and MY 09). In addition to HPV-negative and HPV-positive control samples, no-DNA controls (that is, reaction mixtures to which no DNA were added) were included during each amplification series for the detection of contamination during reaction set-up. Furthermore, to minimize contamination, we used only positive displacement pipettes and disposable pipettes in the assembly of amplification reactions. Pre- and post- amplification

reagents were kept physically separated throughout the experiments to avoid contamination (Kwok and Higuchi, 1989) Each L1 amplification reaction contained L1 degenerate primers MY11 (GCMCAGGGWCATAAYAATGG) and MYO9 (CGTCCMARRGGAWACTGATC) (Manos et al., 1989), with the following modification: 5 pmol each of the β-globin primers GH20 (5' GAAGAGCCAAGGACAGGTAC 3') and PCO4 (5' CAACTTCATCCACGTTACC 3') were included for the simultaneous amplification of a β-globin product of 268 bp that served as an internal control. Each reaction was subjected to 30 amplification cycles in a DNA Thermal Cycler, using thermocycle-step parameters of 95°C for 30 s, 55°C for 30 s and 72°C for 1 min. An additional 5 min was included at the final 72°C elongation cycle. If the initial result was HPV-negative, a portion (usually, 1/10th) of the first amplified reaction mixture was subjected to another 30 cycles of amplification under the same conditions with freshly supplemented primers, deoxyribonucleotide triphosphates, and Taq polymerase. 10 ml of PCR product was visualized with UV-light after agarose gel electrophoresis (2%) containing ethidium bromide (5 µg/25 ml). Three restriction enzymes (Hae III, BstN I, Dde I) were chosen based on their restriction patterns of the amplified product produced from L1-PCR. Samples that were L1-PCR-positive for any HPV type assayed and produced a visible band on agarose gel, it was digested with the 3 chosen restriction enzymes for confirmation of the results. In L1-PCR, amplification products were almost free from nonspecific bands and therefore aliquots of the amplification reaction were subjected to restriction digestion without further purification. Volumes of 12 µl of each sample were added to a restriction enzyme cocktail under conditions specified by the manufacturer. Reactions were stopped with 12 µl of gel loading buffer and separated on a 12% polyacrylamide gel. DNA was visualized with ethidium bromide staining.

RESULTS

The distribution of women for decades is reported in Table 1. Between the women (> 64 years old) with CIN3-SCC cytological diagnosis all were > 70 years old and were not invited to OrS because of age > 64 years. We have reported in Table 2 the age, histological diagnosis, treatment and HPV detection of 8 patients over 70 years with CIN 3 SCC cytological diagnosis. Each specimen was tested for amplification of the L1-ORF of HPV 6, 11, 16, 18, 31, 33, 35, 42, 51 and 58 using consensus primers. PCR product of each sample was revealed by 2% agarose gel electrophoresis. About 100 pg of HPV types 6b, 11, 16, 18 and 33 in viral plasmids as positive controls were routinely detected by PCR. Negative controls were satisfactory. Tissues tested for the presence of HPV DNA by PCR were also tested for the presence of the β-globin DNA to determine if the cellular DNA in the specimens was accessible for PCR. The β-globin sequences were successfully amplified in all 8 cases. The amplified β-globin fragment (268 bp) and HPV amplification products (450 bp) of the expected size was visible in the ethidium bromide-stained gels. In cervical biopsies obtained from 8 women, an HPV rate of 37.5% (3 of 8) was found. One of these HPVs was HPV 16 and two was HPV 58. Detection rates for HPV infection were not increased after second sampling. HPV detection by PCR correlated with that by restriction enzyme analysis.

Table 1. Opportunistic screening: Decades of age of 28.589 women in the period 2007 to 2010.

Total number opportunistic Pap-tests	≤ 20 Years (%)	21 – 40 Years (%)	41 – 70 Years (%)	> 70 Years (%)
28.589	892 (3.2%)	11240 (39.3%)	14848 (51.9%)	1620 (5.6%)

Table 2. Age, treatment, histological diagnosis, HPV detection in women over 70 years-old with CIN3-squamous cell carcinoma.

No. of patients	Age	Treatment	Histological diagnosis (pT)	HPV finding
1	81	Hysterectomy with bilateral salpingo-oophorectomy	CIN3	HPV16
2	82	Cone biopsy	CIN3	Negative
3	71	Cone biopsy	CIN3	HPV58
4	79	Cone biopsy + radiotherapy	Keratinizing squamous cell carcinoma NOS	Negative
5	75	Hysterectomy with bilateral salpingo-oophorectomy	Non keratinizing squamous cell carcinoma (pT1b1)	Negative
6	82	Biopsy	Keratinizing squamous cell carcinoma NOS	Negative
7	72	Biopsy + radiotherapy	Keratinizing squamous cell carcinoma NOS	HPV58
8	75	Hysterectomy with bilateral salpingo-oophorectomy + radiotherapy	Non keratinizing squamous cell carcinoma (pT1b1)	Negative

Samples that were L1-PCR positive for 2 HPVs were digested with 3 restriction enzymes, Hae III, BstN I and Dde I. The restriction fragment lengths are useful for typing the HPV DNA fragments amplified by L1-PCR.

DISCUSSION

The annual incidence of invasive cervical cancer in women between 30 and 50 years of age in high-risk areas is 1/1,000. From an epidemiological point of view, an HPV infection meets the criteria as a causal agent for cervical cancer (Schiffman et al., 1993; Bosch et al., 2002). Having sexual contact is the main source of HPV infection. HPVs are a group of host specific DNA virus with remarkable epithelial cell specificity. More than 120 different HPV genotypes have been identified and almost 45 subtypes, isolated from the low genital tract have been grouped into high- and low- risk HPV types, considering their risk potential to induce an invasive cervical cancer. In a recent study Muñoz et al. (2003) classify HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82 as high risk viruses, detectable in high grade squamous intraepithelial lesions or in invasive cancer; HPV 26, 53 and 66 as potential high risk with a not well known oncogenic potential; while types 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81 and 89 can be considered as viruses with low oncogenic risk and they can be isolated from low grade epithelial lesions. Functionally high risk HPV types infection contributes to carcinogenesis and tumour progression predominantly through the action of two viral oncogenes, E6 and E7. The E6 protein exerts rapid

degradation of p53, in corporation with E6-associated protein (E6-AP), via ubiquitin-mediated proteolysis pathway (Scheffner et al., 1993; Huijbregtse et al., 1993). The E7 protein mediates the release of the E2F transcription factor from pRb-E2F complex (Nevins, 1992). Mutational analysis of HPV 16 E6 protein revealed that a certain level of the activity to degrade p53 is required for E6 to manifest its transforming function (Nakagawa et al., 1992). The p53 mutations are the most frequent genetic abnormalities found in a wide variety of human malignant tumours (Harris, 1993). Once DNA damage occurs, p53 protein is induced and arrests cells in the G1 phase to enhance DNA repair (Kuerbitz et al., 1992), or triggers apoptosis following DNA damage (Lowe et al., 1993). These functions of p53 protein are important to maintain the genomic integrity. Mutant p53 proteins are devoid of these functions, because they lose the ability of DNA contact or destabilize the structure of the core domain (Cho et al., 1994). In this way, once p53 is mutated, DNA damage is fixed and subsequent genetic rearrangement progress which may be putative mechanisms to initiate cancer. Thus far, exceptionally low prevalence (0 to 6%) of the p53 mutations had been documented in cervical carcinomas (Fujita et al., 1992; Choo et al., 1993; Helland et al., 1993; Paquette et al., 1993; Miwa et al., 1995). The p53 protein in cervical carcinoma is thought to be inactivated presumably due to complex formation with HPV E6 oncoprotein. The most common member of the high-risk group is HPV 16, which accounts for more than 60% of all cervical cancers. The high-risk types account for more than 95% of all cases of cervical cancer. One of the main differences between

high- and low-risk types is the possibility of integration in the genome. Approximately 1% of the high-risk HPV types and only 0.1% of the low-risk HPV types will lead to the development of cervical cancer (Ferlay et al., 2000). In our case HPV 16 and HPV 58 were detected in two cases of CIN3 and HPV 58 in one case of SCC. In one case of CIN3 and in 4 cases of squamous cell carcinoma the HPV type has been not detected. Two hypotheses may be made. There is a subset of SCCs that is unrelated to HPV. Other hypothesis takes into account that HPV 16 is only integrated in 72 percent of all invasive cervical cancers (Walboomers et al., 1999). The finding of the absence of HPV 16 DNA integration in some carcinomas implies that integration is not always required for malignant progression, but does not exclude the importance of HPV integration in the initiation of cervical cancer. Hypothetically, after the development of a carcinoma, the abnormal clone could lose the viral DNA. HPV 18, on the other hand, shows 100% integration. In light of recent studies demonstrating that mutation of p53 gene was found in over 20% of the patients with vulvar carcinoma (Lee et al., 1994; Milde-Langosch et al., 1995), a disease of elderly women and a known HPV- related malignancy, Nakagawa et al. (1999) analysed mutation of the p53 gene in 46 women with cervical carcinomas at the age of 60 or more (mean; 71 years, range; 60 to 96 years). Of the 46 patients, 41 had squamous cell carcinoma and 5 had adenocarcinoma. Point mutation of the p53 gene was detected in 5 out of 46 (11%) cervical carcinomas: 1 of 17 (6%) samples associated with high-risk HPVs (HPV 16 and HPV 18) and 4 of 27 samples (15%) with intermediate-risk HPVs, whereas no mutation was found in 2 HPV negative cases. Although falling short of statistical significance reduces the strength of the conclusion. Data presented by Nakagawa et al. (1999) imply that p53 gene mutation may constitute one pathogenetic factor in cervical carcinoma affecting elderly women. To clarify the age-related genetic events in cervical cancer in elderly (>65 years) women, a large variety of nucleic acid hybridization assays has been employed for the detection and specific identification of HPV DNAs. These include Southern, dot blot, filter *in situ* (FISH) and tissue *in situ* hybridizations. Recently, much attention has focused on the potential utility of PCR methods to amplify HPV-specific DNA sequences (Manos et al., 1989). PCR promises to be much more sensitive than previously used methods of HPV DNA detection. Moreover, PCR consensus primer sets, which hybridize to highly conserved regions of the HPV genome, have been designed to detect many known HPV types in a single amplification procedure. Saito et al. (2000) have analyzed for HPV typing via PCR, the expression of p53, 66 tissue specimens obtained from patients with stage Ib-IIb cervical carcinoma. Of this group, 50 women aged 64 years and younger were designated as the younger group (mean age 46.7) and 16 women aged 65 years

and older were designated as the older group (mean age 67.6). The prevalence of HPV DNA was higher in the younger group than in the older group (84.0 vs. 50.0%) as was the detection rate of HPV 16 (44.0 vs. 6.3%). In contrast, HPV 18, 33, 52, 58, were frequently detected in older patients. The positive rate of p53 overexpression in the older group was similar to that in the younger group (46.7 vs. 48.8%). There was no significant difference in the incidence of lymph node metastasis, histology, and the distribution of clinical stage between the two groups. EGFR and Cox-2 overexpression has been reported in many neoplasms (Tsuji and DuBois, 1995; Tsujii et al., 1997). To find information on invasive SCC in the elderly, Giordano et al. (2011) have analyzed 110 invasive SCCs obtained from 2 groups of patients for HPV status by PCR study, for immuno-histochemical EGFR, Cox-2 expression and clinicopathologic features. In this study, 64 women aged 60 years or younger were designated as the younger group and 46 who were 61 years or older were designated as the older group. The HPV status and the expression of Cox-2 and EGFR in the younger and older women were compared and correlated with the grading, staging neoplasm, lymph nodal status and overall survival. The number of neoplasms with higher staging was significantly greater than those in the younger women. The mortality was higher in the older group than in the younger patients. In the elderly, the presence of HPV DNA in 65% of cases and in the absence of sexual activity could be due to reactivation of latent HPV infection. In accordance with data provided by the literature, this finding demonstrated that HPV DNA can be detected in elderly women and can be associated with cervical carcinoma (Baay et al., 2001; García-Piñeres et al., 2006; Subbaramaiah and Dannenberg, 2007). Thus, it is possible that, in elderly women, HPV presence, in the absence of sexual activity, could be due to reactivation of latent HPV infection because of impairment of host immunologic response (Mubiayi et al., 2002). Inadequate immunologic control of HPV infection resulting in viral persistence is likely an important determinant of risk of progression to cervical neoplastic disease. Immunologic competence has been reported to decrease with aging. Garcia-Piñeres et al. (2006) examined the association between lymphoproliferative responses to antigens/mitogens and persistent HPV infection in women older than 45 years. Women included in this study were participants in a 10,000 woman population-based cohort study of cervical neoplasia in Costa Rica. Women older than 45 years and HPV DNA positive at a screening visit were selected as cases (n = 283). Garcia Piñeres et al. (2006) selected a comparably sized control group of HPV DNA-negative women, matched to cases on age and time since enrollment (n = 261). At an additional clinical visit, women were cytologically and virologically re-screened and cervical and blood specimens were collected. Proliferative responses to phytohemagglutinin (PHA), influenza virus

Table 3. Organised cervical cancer screening programmes in Italy: Value of some process indicators in Trentino region between 2005-2008 (National Centre for Screening Monitoring).

	TRENTINO			
	2005	2006	2007	2008
Nominal extension (%)	37	30	30,1	29,8
Number of invited woman	52.305	43.455	45.104	44.852
Compliance with recommendation to repeat cytology (%)	35,7	36,2	37	53,2
Inadequate cytology (%)	4,8	5,7	5,5	5
Recommendation to repeat cytology(%)	1,5	1,3	1,3	1,2
Compliance colposcopy with referral for ASCUS+ DR° for cytologyc lesions CIN2+ unadjusted	75,5	78,5	79	76
PPV for CIN2+ of ASCUS+ referred to colposcopy	29,6	23,9	31,5	28,3

(Flu), and HPV16 virus-like particle (VLP) were lower among women with persistent HPV infection than for the control. The decreases were most profound in women with long-term persistence and were only observed for the oldest age group (≥ 65 years). The results of this study indicate that impairment in host immunologic responses is associated to persistent HPV infection. Since 1993, at least 7 studies have described the screening histories of women with invasive cervical cancer (Ciatto et al., 1993; Kenter et al., 1996; Stuart et al., 1997). In 2007 the almost 30% of the Italian population not included in organised programmes is partly the result of an implementation process still in progress in some Regions in Southern Italy, but mainly of a very limited or completely absent implementation in a few Regions in Northern Italy. In 2007, 121 active programmes had a target population 11,872,810 women, corresponding to 71.8% of Italian women aged 25 to 64 years vs. 69% in 2006. During 2007, 39.8% of invited women were screened vs. 38.5% in the previous year. The last report of National Centre for Screening Monitoring as been published in 2008 and various process indicators of all regions have been described with exclusion of Liguria. Only 39.7% of invited women were screened vs. 39.8% in the previous year. The data of Trentino Region has been reported in Table 3. The nominal extension varied from 9,9% (Puglia) to 54,4% (Basilicata), the compliance with recommendation to repeat cytology from 17.2% (Puglia) to 73.3% (Valle D'Aosta), the inadequate cytology from 0.8% (Valle D'Aosta) to 12.1% (Molise), the recommendation to repeat cytology varied from 1.2% (Trentino and Puglia) to 4.5% (Abruzzo). The main examined process indicator has not been reported in all the regions.

In conclusion, the data of National Centre for Screening Monitoring provides information regarding the deludent performance of the organized screening programmes for cervical cancer. The distinction between OpS and OS screening has not been done. Ricciardi et al. (2009) have examined the direct cost of managing invasive cervical cancer in Italy. An estimated 2,927 new cases of cervical cancer occurred in Italy in 2005. The estimated numbers

of new cases by FIGO stage were: FIGO I, 1,927; FIGO II, 556; FIGO III, 259; and FIGO IV, 185. Costs for the most frequent procedures were estimated as: € 6,041 for radical hysterectomy or other surgery; € 4,901 for radio-chemotherapy; € 1,588 for brachytherapy; and € 3,795 for palliative chemotherapy. Mean management costs for incident cases (including 10 years follow-up) were estimated at: FIGO I, € 6,024; FIGO II, € 10,572; FIGO III, € 11,367; FIGO IV, € 8707; and € 5,854 for the terminal phase (1 month). The total direct management cost was estimated at € 28.3 million per year. Because the 34.64% of invasive cervical carcinoma has been diagnosed in > 65years old women, it is necessary to consider the extension of screening programs after the 65 years. With regard to screening histories of invasive cervical carcinoma in Italy, no studies have been published except OrS programme of Friuli Venezia Giulia.

In Italy the complete screening history of women diagnosed with invasive cervical cancer has been performed only in Friuli Venezia Giulia – North eastern Italy. In these regions an OrS was initiated in 1999 targeting women aged 25 to 64 years, who are invited to have a Pap-test every 3 years. The screening histories of CIN3 – SCC in > 65 years old women may be made with study of OpS because the OrS offers a free-of-charge Pap-test every 3 years to all women aged 25 to 64 years. Zucchetto et al. (2010) have examined the screening histories of 438 women with invasive cervical cancer diagnosed in Friuli Venezia-Giulia between 1999 and 2005. 82 cases (49.7%) were found in > 65 years old women. 165 (37.7%) women were not screening. 69 (15.8%) women were not invited to OrS because of age >65 years old. Histological type and HPV detection of invasive cervical cancers has been reported. The study of Zucchetto et al. (2010) shows that the lack of screening among older women and of compliance with organized programs among women in the target population are the main limitation in cervical cancer secondary prevention. The results of Zucchetto et al. (2010) are in agreement with research conducted in northern Europe. Bos et al. (2006) have analyzed the screening history of 3.175 women with invasive cervical cancer diagnosed in the

years 1994 to 1997 in the Netherland. 57% of 3175 women with invasive cervical cancer had no previous smears. Given the high proportion of women with invasive cervical cancer older than 64 years at diagnosis, the possibilities of inviting them to have at least one Pap smear test after 64 years, should be taken into consideration. According to American Cancer Society Guidelines for the early detection of cancer and the guidelines of other national regional screening programme, women 70 years of age or older who have had 3 or more normal Pap-test in a known and no abnormal Pap-test results in the last 10 years may choose to stop having Pap-test. According to National Cervical Screening Program, the current policy of screening women of New Zealand is to continue organized regular screening until aged 69 years with Pap test every three years if the women have ever been sexually active. The National Cervical Screening Program of Australian Government believes that at age 70, women should consult with your doctor about whether they need to continue to have a regular Pap smear.

The present study support the screening policy to perform Pap test every 3 years until aged 69 years, independently to sexual activity because 34.64% of invasive cervical carcinoma has been diagnosed in > 65 years old women. Consequently, it is necessarily early diagnosis that decreases mortality, morbidity and direct management costs of disease.

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Full Length Research Paper

Sero-prevalence of *peste des petits ruminants virus* (PPRV) in small ruminants in Blue Nile, Gadaref and North Kordofan States of Sudan

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The present cross-sectional survey was conducted in Blue Nile, Gadarif and North Kordofan states of Sudan. The study was conducted during the period May 2005 to September 2006 to provide an overview of the most important diseases affecting small ruminants' productivity. Results of interviews with small ruminants' keepers indicated that *peste des petits ruminants* (PPR) was found to be one of the most important diseases in all the study areas, with minor variations in importance at individual state level. Analyses of the sera collected from small ruminants gave an overall sero-prevalence of PPR as 61.8%. However, PPR had a slightly higher ranking in importance in Gadarif and Blue Nile states, while respondents in North Kordofan did not report PPR as the most important disease, but reported a syndrome with diarrhea, pneumonia and sudden death. This syndrome was considered by the pastoralists as the most important condition which reduced their small ruminants' numbers and denied them market access.

Key words: *Peste des petits ruminants* (PPR), seroprevalence, small ruminants, Sudan.

INTRODUCTION

Peste des petits ruminants (PPR) is an acute or sub-acute viral disease of small ruminants. Other names commonly used includes, pseudo rinderpest of small ruminants; pest of small ruminants; goat plague; pest of sheep and goat; stomatitis pneumoenteritis syndrome; contagious pustular stomatitis and pneumoenteritis complex (Alillo et al., 1998). It is a highly contagious, infectious and fatal viral disease of domestic and small ruminants (Roeder et al., 1999; Ozmen et al., 2009). The disease is characterized by fever, necrotic stomatitis, gastroenteritis and pneumonia. Defra (2005) described it as a rinderpest-like contagion of goats and sheep characterized by erosive stomatitis, enteritis, pneumonia.

The first report of an outbreak of a rinderpest-like disease in sheep and goats in Sudan was in 1971 in the southern part of Gadarif State near Dindir River (Elhag, 1973). The disease was diagnosed as Rinderpest (RP) on clinical signs. However, RP precipitinogens were demonstrated by agar gel precipitation test (AGPT) (Elhag, 1973). Subsequent isolates from an outbreak of a 1972 RP-like disease in Sennar and Meilig in Sudan were found to be closely related antigenically to the Nigerian *peste des petits ruminants* (PPRV). The isolates were considered as PPR and termed as SUD 72/ 1(Sinnar) and SUD 72/ 2 (Meilig) (Elhag and Talor, 1984). Other outbreaks of PPR in the Sudan include the ones from Elhilalia in Gezira state (Awad Elkarim et al., 1994) and Elfashir in North Darfur (Elsheikh, 1992). Sero-surveillance results demonstrated the prevalence of the disease in Khartoum (Zeidan, 1994), Southern Sudan states (Osman, 2005), and Khartoum, Gezira, River Nile, Kordofan, Eastern

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states (Intisar et al., 2010).

Various laboratory techniques and serological tests were employed to diagnose the PPRV antigens or antibodies in sera of infected animals including AGPT and hemagglutination test (HA) (Nusseiba et al., 2008) and experimental infection (Nusseiba et al., 2009a).

The purpose of this study was to determine the prevalence of PPR by serology in small ruminants in Blue Nile, Gadarif and North Kordofan States of Sudan.

MATERIALS AND METHODS

Study area

The study area include Blue Nile State, Gadarif State and Elkhowie area in North Kordofan State which were selected according to predetermined criteria, namely, diversity in terms of production systems, market outlets, prevalence of poverty, dominance of sheep and goats and prevalence of major trans-boundary diseases. Information on small ruminants' health and market constraints was collected through a structured questionnaire

Questionnaire survey

The questionnaires were designed by the Small Ruminant Project Scientists (ILRI/ICARDA), discussed with the national research team and agreed upon to be used for data collection from households/flocks. The structured questionnaire comprised of 262 for households [116 from Blue Nile, 101 from North Kordofan (Elkhowie administrative unit) and 45 from Gadarif States]. Occupation and main sources of family income were included in the questionnaire.

Sample size and serum samples collection

The following criteria were adopted for sampling as below:

- 1) The target population was defined as including all small ruminants in the study sites.
- 2) The study population was identified to include the small ruminants that had not been vaccinated against PPR.

The three study states were taken as clusters with known population of small ruminants. Sampling with probabilities proportional to number of small ruminants in each state (that is, probability sampling) was used to determine the number of small ruminants (sample size, n) to be included in the study in each state. The sample size determined, thus for each state was, Blue Nile, 280, Gadarif 105 and W. Kordofan, 215 samples, giving a total of 600 animals (sheep and goats).

Within each state number of small ruminants in each locality was selected conveniently (convenient sampling). Thus, the distribution within each state was (280) in Blue Nile State, (105) in Gadarif state and (215) in North Kordofan State (Elkhowie Administrative Unit). The total number sampled and bled for sera for all the three states was 600.

Competitive enzyme immunosorbent assay for detection of antibodies to PPRV

Competitive enzyme-linked immunosorbent assay (c- ELISA) was used to test 600 serum samples collected from small ruminants from all study sites to determine PPR sero-prevalence. The test

was carried out firmly as described by Nusseiba et al. (2009b). The Assay procedure followed the restricted steps mentioned in the PDSL protocol and was applied in the Central Veterinary Research Laboratory (CVRL), Khartoum.

Data analysis

SPSS software version 11.5 was used to analyze the data.

RESULTS

The survey revealed that the most dominant livestock (small ruminants) production system was seasonal movement in Gadarif and Blue Nile states; while sedentary system was the dominant in North Kordofan (Elkhowie area) (Table 1).

Information about small ruminants' diseases obtained from the respondents through interviews differed from year to year and by study areas. Thus, for 2005 study year 17.1, 64.2 and 0% of the respondents reported that PPR was the most important disease in Gadarif, Blue Nile and North Kordofan (Elkhowie area) states respectively; while 22.7% of the respondents reported that diarrhea and pneumonia conditions were the most important conditions in 2005. PPR, diarrhea and pneumonia had 33.98, 57.41 and 41.44% case fatality rate respectively during this year.

For the study year 2004, 43.6, 46.4 and 0% of the respondents reported that PPR was the most important disease in Gadarif, Blue Nile and North Kordofan States, respectively; while 20.6% of the respondents reported diarrhea and pneumonia to be the most important conditions in 2004. PPR, diarrhea and pneumonia had 48.23, 95.74 and 11.86% case fatality rate respectively during that year.

In the study year 2003, however, 21.4, 22 and 0% of the respondents reported PPR to be the most important disease in all the study states (Gadarif, Blue Nile and North Kordofan), respectively. However, 3.6% of the respondents reported diarrhea and pneumonia as the most important conditions in that year.

A Considerable number of respondents (58.9%) stated that both sheep and goats were affected equally with PPR, although goats appeared to be the most affected with PPR (57.1%) when compared to other diseases.

Further analysis revealed significant association between prevalence of PPR and winter season ($p < 0.05$). There was a strong correlation between the number of animals affected with PPR and the number of sick animals that could not be sold in Blue Nile state (Pearson correlation coefficient (r) = 0.819, r^2 = 0.671). Similarly, there were positive correlations (Pearson correlation coefficients, 0.327 and 0.622 for Blue Nile and Gadarif states respectively) between the number of goats born during the year and the number that died due to PPR during the same year.

PPR gave an overall sero-prevalence of 61.8%;

Table 1. Production systems of small ruminants in each study site as a percentage of the total population engaged in each system in each State.

State	Production system			Total (%)
	Sedentary (%)	Seasonal movement (%)	Permanent movement (%)	
Gadarif	13 (28.9)	29 (64.4)	3 (6.7)	45 (100)
Blue Nile	33 (28.5)	78 (67.2)	5 (4.3)	116 (100)
N. Kordofan	65 (64.3)	33 (32.7)	3 (3.0)	101 (100)
Total	111 (42.4)	140 (53.4)	11 (4.2)	262 (100)

Table 2. Sero-prevalence of PPR in sheep and goats tested with an indirect c-ELISA in the study areas.

State	Sheep		Goats		Total	
	No. tested	+ve (%)	No. tested	+ve (%)	No. tested	+ve (%)
Gadarif	58	15 (25.9)	47	15 (31.9)	105	30 (28.58)
Blue Nile	152	99 (65.1)	128	95 (74.2)	280	194 (69.3)
N. Kordofan	189	137 (72.5)	26	10 (38.5)	215	147 (68.4)
Total	399	251 (62.9)	201	120 (59.7)	600	371 (61.8)

Table 3. Prevalence of PPR antibodies in small ruminants by sex in the study areas.

State	Males		Females	
	No. tested	+ve (%)	No. tested	+ve (%)
Gadarif	20	2 (10.0)	85	28 (32.9)
Blue Nile	73	43 (58.9)	207	151 (72.9)
N. Kordofan	51	33 (64.7)	164	114 (69.5)
Total	144	78 (54.2)	456	293 (64.2)

(371/600); while individual study states had (69.3 %) in Blue Nile, (68.4%) in North Kordofan and 28.6% in Gadarif states. On species basis, an overall sero-prevalence in all study sites was 62.9% for sheep and 59.7% for goats with major fluctuation at state level (Table 2).

On sex basis, an overall sero-prevalence in all study sites was 54.2% for males and 64.2% for females with major fluctuation at state level (Table 3). The difference in overall sero-prevalence between sex among study states was significant when chi square was used ($p < 0.05$) and the odds ratio was found to be 1.52 which means that the female had a risk factor 1.5 times that of males, while within State significant difference was observed in Blue Nile state and Gadarif only.

Using chi-square statistics, there was significant association in the overall sero-positivity among different age groups for both species (sheep and goats) ($p < .005$). Likewise, there was significant positive correlation between percent inhibition (PI) which was used to measure the cut-off points for c-ELISA and the age of samples at the 0.01 level (2-tailed).

Economic importance of PPR

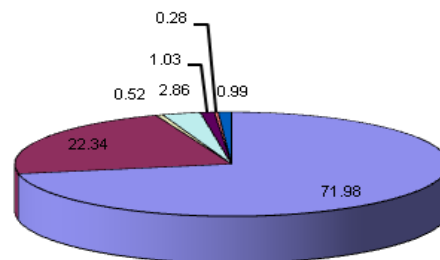
The total cost of small ruminants that died of PPR disease reported by respondents during the study years (2005, 2004 and 2003) was valued at Sudanese Dinnar (SD) 13,674,500, 10,811,950 and 1,370,000, with the equivalent values, in US Dollars being, estimated at \$ 56,977.1, 43,247.8 and 5,269.2 respectively, giving a total value of total death of SD 25,856,450, equivalent to a total value of \$ 105,494.10 for the study period (2003 to May, 2005). The approximate value of total losses other than deaths (Abortion, milk loss and emaciation) due to PPR for the same study period (2003 to May, 2005) was SD 6,321,600 (equivalent to \$ 25,838.2).

Cost of drugs for treatment against PPR over the same period (2003 to 2005) was SD 5,447,050 (or 22,146.9 US Dollars). Fees levied for services for PPR over the same period of three years was SD 6,500 (or, \$ 26.8). The combined cost due to deaths, losses other than deaths, drugs and services attributable to the PPR disease was SD 37,631,600 (\$ 153,479.2). Losses due to PPR accounted for 29.1% of the losses from all diseases

Table 4. Value of losses resulting from deaths and other costs due to PPR as reported by respondents.

Value of losses and costs	The total losses and costs of the 1 st and 2 nd most important diseases reported (SD)	PPR (SD)
Value of dead small ruminants	101,381,750	25,856,450
Value of production losses (milk, abortions lost of weight, etc.)	11,350,100	6,321,600
Cost of drugs and vaccines	16,669,510	5,447,050
Fees for services	19,500	6,500
Total (SD)	129,420,860 (100%)	37,631,600 (29.1%)

- Livestock rearing
- Crop production
- Service
- Livestock trade
- Other trade/business
- Remittance from family members working away from household
- Other (specify)

**Figure 1.** Occupation and main sources of family income reported.

studied during the same study period (2003 to 2005) Table 4.

Occupation and main sources of family income reported

71.98% of the respondents depend on livestock rearing, 22.34% on crop production, 2.86% on livestock trade, 1.03% on other/trade and business, 0.52% on services and 0.28% on remittance from family members working away from household Figure 1.

DISCUSSION

Diarrhea and pneumonia, together with unknown conditions, leading to sudden deaths, dullness and abortion in animals were observed. Such symptoms and laboratory results led to suspicion that PPR might have been the cause of deaths, but misdiagnosed by small ruminants' producers. Mariner and Paskin (2001) had reported that it was usual for communities to recognize one or two major diseases for which they had no name or names in the local languages. These researchers suggested that such unknown ill-health conditions

become major problems and may often be considered as new diseases.

Generally, ranking of diseases' importance by the respondents tends to agree with our laboratory results, confirming observation of Mariner et al. (2009) on farmers' rich practical agricultural knowledge and its relationship to the degree of economic dependence a society has on that activity. Moreover, the study revealed that 72% of the respondents reported livestock rearing as their main occupation and the main source of income.

The observation that case fatality rate of PPR in goats was (53.3%) compared to only (39.7%) in sheep indicates that goats are more susceptible to PPR than Sheep. This finding is in agreement with Lefèvre and Diallo (1990) and Roeder et al. (1994) observation that PPRV exhibits different levels of virulence between sheep and goats. It is also in agreement with Radostits' (2000) findings, which indicated that case fatality rates are much higher in goats than in sheep.

On the economic impact of PPR, this study has determined the total value of losses from deaths, losses other than deaths (abortion, emaciation and milk loss), and cost of drugs and fees for services during the three successive study years (2003, 2004, 2005) to be \$ 525,774. PPR accounted for 29.10% of these losses. Since 72% of the respondents depend entirely on livestock rearing, these losses could be taken to be of considerable economic impact on the livelihoods of small ruminants' keepers in the project areas. Additionally, small ruminants' producers are not able to access markets for their animals because of these identified diseases. Therefore, although the total losses and economic impact may have been underestimated, PPR is an economically significant disease of small ruminants as observed by Dhar et al. (2002).

Major ecological changes, which certainly affected the distribution of diseases, have occurred due to overgrazing, insecurity, tribal conflict and raiding, leading to extensive animal movement. Moreover, the newly established quarantine in Elkhwei (North Kordofan) where more animals from far areas (e.g. Darfur region), are held together without vaccination against PPR (RM Hassan, Community Animal Health Delivery services, Unpublished) could be one of the possible reasons for higher prevalence of PPRV in North Kordofan. Although the study showed higher prevalence of PPR in North Kordofan, the pastoralists are not familiar with clinical signs of PPR and have no local name for it. Pastoralist misdiagnosis is, therefore, a possibility and the higher proportion of small ruminants positive for antibodies to the disease in this site would therefore imply that PPR may have been newly introduced into the area.

This study revealed that PPR sero-prevalence in small ruminants was the highest in Blue Nile state (69.3%), which agrees with the findings of Osman (2005). This observation may be attributed to the characteristics of PPR as a trans-boundary disease and the frequent

movements of animals (small ruminants) within the state and to other parts of the country. Blue Nile and Gadarif States border Ethiopia at areas where insecurity makes veterinary services inaccessible to small ruminants' producers. Gadarif state had lower sero-prevalence (28.6%) compared to Blue Nile, which may be an indication of good PPR vaccination coverage in this state.

The study revealed that the overall (all three study states) sero-prevalence for female was 64.3% while that for males was 54.2%. There was significant difference in sero-positivity between females and males of small ruminants tested ($p < 0.05$). This significance has no biological plausibility and does disagree with Osman (2005) findings that the sex of animals had no effect on the development of PPRV antibodies. The fact that small ruminants' producers keep more females for breeding purposes may explain this observation. Hence, the probability for females getting exposed to PPRV throughout their life time is more, than for males. However, association between PPR sero-positivity and sex of tested animals in Elkhwei area was not significant ($p > 0.05$), putting doubt on this assumption.

Likewise, there was significant difference in the prevalence of antibodies to PPRV in Blue Nile and Gadarif states among different age groups. This finding disagrees with observation of Osman (2005). This can be justified by the fact that the older animals have greater probability of exposure to the PPRV throughout their life time than younger ones. Interviewees stated that they usually keep older animals for breeding purposes.

The fact that there was significant correlation between Percent Inhibition (PI) average (used to measure the cut-off point) and different age groups confirms the findings of Radostits et al. (2000) that the percentage of antibodies to PPRV in small ruminants raises with age.

The conclusion from this study is that a PPR is probably more prevalent in the Sudan than is known so far. Moreover, homologous PPR attenuated vaccine is highly recommended to be used to protect against virulent virus challenge in the country for control of PPR.

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Full Length Research Paper

Cutaneous infectious diseases in Tunisian adolescents

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Few studies have been completed in adolescent's cutaneous diseases. The aim of this work is to assess the adolescent epidemiological profile of the cutaneous infectious diseases and its evolution between 1997 and 2007. We have performed a comparative retrospective study on all adolescent outpatients attending Charles Nicolle's Hospital Dermatological Department on 1997 and 2007 and focused on cutaneous disease. The total number of outpatients was 9254 and 11343 on 1997 and 2007, respectively. Among this population, we counted 1155 teenagers on 1997 (12.4%) and 1176 teenagers on 2007 (10.63%). Comparatively, the results revealed that there were no significant differences in terms of age and gender between 1997 and 2007. For both years, the majority of cutaneous infections were of viral origin, followed by fungal dermatosis, bacterial dermatosis and finally parasitic dermatosis. We have noted a major modification in the skin infections profile: a decrease in bacterial skin diseases frequency ($P < 10^{-3}$), and an increase in warts ($P = 0.02$) and scabies ($P = 0.014$). Meanwhile, sexually transmitted diseases (STDs) including venereal warts have also considerably increased especially in teenage females ($P = 0.059$).

Key words: Warts, adolescents, pityriasis vesicular

INTRODUCTION

Adolescents' health is an increasing concern over the last decades around the world and also in our country - Tunisia. The United Nations organisation report in 2003 defined adolescence as the period ranging between 10 and 19 years old, and concluded that we dispose of little knowledge about adolescents compared to other age groups (Fund of the Nations United for the population, 2003). The socioeconomic development and the epidemiological transition observed in our country have an impact on the health of the population and particularly of adolescents. Adolescence is a period of life with its own unique characteristics. Dermatological affections are the second consultation motives for children and teenager after ophthalmological affections (Bruijnzeels et al., 1998) with an impact on adolescent's health-related

quality of life (Golics et al., 2009; Smith, 2001). Cutaneous infections and infestations are common problems in childhood and adolescence (Campbell and English, 2011). In Tunisia, cutaneous infections are the main infections in childhood (Kharfi et al., 2008) and in old people (Souissi et al., 2006).

The aim of this work is to assess the adolescent epidemiological profile of cutaneous infectious diseases through a retrospective hospital attendance study. We analyze the differences in consultation motives between 1997 and 2007.

MATERIALS AND METHODS

The study took place in the Capital of Tunisia, a north African country that has recently maintained a rapid economic development and so has reached a high level of development (ranked 94th out of 187 on the Human Development Index scale in 2011 (United Nations Development Program, 2011)

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The target population was adolescents, according to the definition of adolescence by the United Nations Organisation (Fund of the Nations United for the population, 2003).

We reviewed all adolescent outpatients who have attended Charles Nicolle's Hospital Dermatological Department during 1997 and 2007. We used a digitized data base. The data is updated daily by a specialized personnel. The coding of the data is realized by a doctor after each consultation. The studied variables for each patient were the age, gender and diagnosis. Thereafter, we only considered the patients with an infectious disease, and proceeded to a classification according to the microbial origin of the dermatosis, on four groups: bacterial, fungal, viral or parasitic disease. All mycological, parasitic and certain bacterial infections have been confirmed by a biological test. We treated separately sexual transmissible diseases (STDs). Data were analysed using SPSS 11. Analysis was realized first, by groups of disease, and then in details for each infectious dermatosis; we studied the distribution by gender in terms of absolute and relative frequency. We finally performed a comparative study on the results between 1997 and 2007, using the Pearson's Chi-square test and Fischer's exact test for the comparison of percentages, and Student's test for the comparison of means. The significance level was set at 0.05, using bilateral tests. This comparison seemed to be possible since the Department of Dermatology in Charles Nicolle's Hospital covers both regions.

The methodology of our study conformed to ethical principles applicable to any shape of medical search for the statement of Helsinki of the World Medical Association (Statement of Helsinki of the world medical Association, 2011).

RESULTS

In 1997, 1115 adolescents, among whom 520 were males and 635 were females, have consulted our department. The mean age of patients was 15.3 ± 2.7 years. We diagnosed 1254 dermatosis. We identified an infectious disease 525 times, which represents 41.8% of all dermatosis during this year.

In 2007, 1176 adolescents, 523 males and 653 females, were consulted. Their mean age was 15.0 ± 2.8 years. A total of 1430 diagnosis were made, 539 were infectious diseases, representing 37.7% of all dermatosis during this year.

The viral skin disease was the main aetiology of infectious dermatosis for both years (15.7% on 1997 and 16.9% on 2007), followed by fungal infections (13.5% on 1997 and 13.6% on 2007), bacterial infections (10.8% on 1997 and 4.7% on 2007) and finally parasitic disease (1.9% on 1997 and 2.5% on 2007). Table 1 summarizes all the infectious dermatosis.

The viral skin disease was the main aetiology of infectious dermatitis for both years, followed by fungal infections, bacterial infections, and finally, parasitic disease (Table 1).

Viral pathology was the first reason for consultation for both years. It has represented 37.5% of infectious dermatosis in 1997 and 44.7% in 2007. This pathology was more frequent among female adolescents mainly in

1997 (sex-ratio = 0.75 in 1997 and 0.99 in 2007) with no significant difference. The different clinical features of viral infections are reported in Table 2.

Fungal disease was the second cutaneous infection for both years. It has represented 13.5% and 13.6% of infection dermatitis, respectively in 1997 and 2007, without any significant difference ($P = 0.90$). The fungal infection was diagnosed among 83 males and 86 females in 1997 (sex-ratio = 0.96) and among 96 males and 99 females in 2007 (sex-ratio = 0.96). The different fungal cutaneous infections are reported in Table 3.

Dermatophytosis were predominant during both years with a trend towards an increase ($P = 0.06$). Glabrous skin seat ranked the first among dermatophytosis (79.7% in 1997 and 68.2% in 2007), followed by nail seat (13.5% of dermatophytosis in 1997 and 25.5% in 2007) and dermatophytosis of the scalp (6.76% of dermatophytosis in 1997 and 6.36% in 2007). Toes folds were the most frequent localisation in glabrous skin (64.4% in 1997 and 42.7% in 2007). For scalp lesion, trichophytic ringworm was the most frequent for both years (4 cases in 1997 and 5 cases in 2007).

Yeast mycosis represented 6.5% of all dermatitis in 1997 and 5.9% in 2007, without any significant difference ($P = 0.48$). Pityriasis vesicular was the main yeast mycosis for both years (95.2% of yeast mycosis in 1997 and 94% in 2007), followed by mucosal candidiasis (1.2% of yeast mycosis in 1997 and 3.6% in 2007) and onychia candidiasis (3.7% of yeast mycosis in 1997 and 2.4% in 2007).

Bacterial infections occupied the third place for both years (10.8% in 1997 and 4.7% in 2007). We noticed a significant decrease of bacterial dermatosis in 2007 ($P < 10^{-3}$), in specific and non-specific germs infections in both sexes.

Non-specific germs infections were the main bacterial infections for both years (6.4% of all dermatitis in 1997 and 2.7% in 2007, $p < 10^{-3}$), followed by Staphylococcal infections (3.9% in 1997 of all dermatosis and 2.2% in 2007, $p = 0.016$). Tuberculous dermatitis was rare (1 case in 1997 and 1 case in 2007).

Parasitic infections represented 1.9% in 1997 and 2.5% in 2007 of all dermatosis. They were more frequent in 2007 without significant difference ($P = 0.29$). Scabies was the most frequent parasitic skin infections for both years (0.9% of all dermatosis in 1997 and 2% in 2007) with a significant increase in 2007 ($P = 0.014$). The rate of leishmaniasis was stable (0.8% in 1997 and 0.5% in 2007). Only 3 cases of pediculosis were observed in 1997 (0.2%) and no one in 2007.

STDs were more frequent in 2007 (1.2%) than in 1997 (0.7%), with no significant difference ($P = 0.21$) but sex-ratio dropped from 1.25 in 1997 to 0.54 in 2007. We noted a significant increase of venereal warts (44.4% of

Table 1. Infectious dermatosis in adolescents.

Infections	1997 (n = 1254)		2007 (n =1430)		P
	Number	%	Number	%	
Viral infections	197	15.7	241	16.9	0.42
Fungal infections	169	13.5	195	13.6	0.90
Bacterial infections	135	10.8	67	4.7	<10 ⁻³
Parasitic infections	24	1.9	36	2.5	0.29

Table 2. Viral epithelial tumors.

Tumor	1997 (n = 1254)		2007 (n = 1430)		P
	Number	%	Number	%	
Warts	120	9.6	172	12.0	0.041
Verrucous papilloma	25	2.0	15	1.1	0.044
Molluscom contagiosum	8	0.6	14	1.0	0.33
Cutaneous herpes	4	0.3	5	0.3	-
Chickenpox	7	0.5	6	0.5	-
Zona	1	0.08	5	0.4	0.22
Eruptive fever	4	0.3	0	-	0.32

Table 3. Fungal infections.

Infection	1997 (n = 1254)		2007 (n =1430)		P
	Number	%	Number	%	
Dermatophytis	74	5.9	110	7.7	0.067
Yeast mycosis	82	0.5	84	5.9	0.48

STDs in 1997 and 94.1% in 2007, $p=0.010$).

Five cases of urethritis were observed in 1997 and one case of syphilis in 2007. Urethritis was not observed in 1997, and Syphilis was not observed in 2007. No case of VIH infection was observed.

DISCUSSION

In fact, our study consists on a retrospective analyse about cutaneous infections observed among adolescents in our department, in order to appreciate the differences in consultation motives between 1997 and 2007. The age bracket that we studied is often subsumed in one or the other period of life (children or adults). Few studies have focused on adolescents.

Through a review of the literature, infectious diseases remain the leading skin disorders. Infectious pathology was dominated by viral dermatosis followed by fungal,

bacterial and parasitic (Parthasaradhi and Al Gufai, 1998; Elpern, 1985; Doe, 2001).

In Tunisia, Kharfi (Kharfi et al., 2008) found that viral dermatosis is predominant in children (11% of all dermatosis), and Souissi et al. (2006) noticed viral infections in 6.8% in an old population.

Our study identified the predominance of viral dermatosis for both years, 15.7% in 1997 and 16.9% in 2007. Viral infections frequency among adolescents seems more important in children than elderly population in Tunisia. Furthermore, most studies have noted the progression of warts among cutaneous viral infections (Goh and Akarapanth, 1994). In a French study realised in 2000, warts represented 13.6% of all dermatosis and 58 to 70% of warts affected persons aged between 10 and 14 years (Lukasiewicz et al., 2002). In our study, warts have significantly increased between 1997 and 2007.

Problems related to fungal infections differ based on

regions and socioeconomic (Piérard et al., 2000; Anand and Gutpa, 1998; Dagnew and Ewing, 1991) factors. Athlete's foot is an emerging problem in developed countries while scalp ringworm is the most frequent mycosis in developing countries (Havlickova et al., 2008, Gibbs, 1996; Onayemi et al., 2005).

In Netherland, the incidence rate of fungal cutaneous infections in children less than 17 years old has increased; it was 25.7 in 1987 and became 35.2/1000pers/year in 2001 (Robbert, 2006). In the study of Tunisia, fungal pathology was dominant in old people (19.5% of dermatosis) with a predominance of onychia (Souissi et al., 2006), and was less frequent than in pediatric population (9.9% of dermatosis), ringworm has 10.7% dominance (Kharfi et al., 2008).

Our study showed the frequency of fungal disease, it constituted 13.5% of dermatosis in 1997 and 13.6% in 2007. It also showed some particularities among adolescents, like the predominance of pityriasis versicolor in this population, the persistence of ringworm in adolescents, and the scarcity of nail involvement. According to literature, the highest frequency of onychomycosis is observed in old population. Onychia seems to be an old patient's pathology (Souissi et al., 2006; Tuncel and Erbagci, 2005).

Our study identified a significant decrease of bacterial dermatosis in 2007. They represented 10.8% in 1997 and 4.7% in 2007. This decrease seems to be real even though its estimation in hospital is sometimes biased by the management in general or pediatric medicine, especially since it has been found in other studies in Tunisia (Souissi et al., 2006). The auto medication evaluated at 12.4% in Tunisia also contributes to the under-estimation of this dermatosis (Mokhtar et al., 1997). We can explain this decrease by the improvement of living standard. Concerning parasitic infections, a study on the epidemiological profile of cutaneous pathology realised in the governorate of Assiout in Egypt between 1994 and 1996, showed a clear predominance of parasitic infections that constituted 27.4% of all (Abdel-Hafez et al., 2003). Pediculosis was the most frequent parasitosis (19.4%) and represents 9.6% of dermatosis in male adolescents in Saudi Arabia (Bahamdan et al., 1996).

In Brazil, a cross-sectional study of cutaneous pathology in a rural region in the north, published in 2005 showed that pediculosis was present in 43.4% of the studied population; scabies was diagnosed in 8.8% of this population. Children aged between 10 and 14 years were the most affected (Heukelbach et al., 2003). In Netherland, the incidence rate of parasitic dermatosis in children less than 17 years was evaluated at 6.7% in 1987 and 7% in 2001 per 1000 persons/year.

In our study, parasitic infections have occupied the

fourth rank of infectious dermatosis for both years without any significant difference between 1997 and 2007 but have revealed a significant increase of scabies in 2007. This increasing of scabies has been noticed in other studies in Tunisia (Kharfi et al., 2008; Souissi et al., 2006).

For STDs, adolescence constitutes a high risk factor of sexual infections. According to our study, STDs represented 1.0 and 1.3%, respectively, in 1997 and 2007. These frequencies are low in comparison with literature data. We suppose that there's an under estimation of STD's frequency in adolescents. Our results are biased by the management of this type of infections in other structures.

STD predominated in male in 1997 (sex-ratio = 1.25). In 2007, we noticed a feminine predominance. The review of literature reveals the same tendency of STDs getting higher in female population. Concerning the increasing of venereal warts, many studies have noticed it (Parent and Binet, 1998).

Conclusion

The findings of this study support that cutaneous infections among adolescents have probably changed during the period between 1997 and 2007. We have particularly noted the increase of simple and venereal warts, scabies and sexual disease in female population. A significant decrease of bacterial pathology was clear and a high frequency of pityriasis versicolor was assessed. This study shows the importance in screening the core evolution of STDs in order to improve the sexual education scheme and to develop a prevention plan on the other.

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Full Length Research Paper

Knowledge and awareness on HIV/AIDS among blood donors: A Study at Rajshahi, Bangladesh

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Human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) is the most devastating disease that mankind has ever faced. For being neighboring country of India, Bangladesh has been highly vulnerable to HIV infection. Though Bangladesh continues to maintain low HIV prevalence status, however the infection rate is on the rise at Rajshahi Division. Some studies have been carried out on the knowledge about HIV/AIDS among some risk groups, but studies on blood donor's awareness about HIV/AIDS are rarely found. This study aims at investigating the knowledge and awareness about HIV/AIDS among the blood donors at Rajshahi Metropolitan of Bangladesh. In this study, primary data was used. Data reveals that, though about 89% blood donors knew it is transmitted through blood transfusion, only 1% of them had undertaken blood test before donation, which is alarming. The odds of having preliminary and preventive knowledge fell with the increase in age of the respondents. Besides these, blood donors who were educated and who were in service were found more aware about the HIV/AIDS than the others. Strict rules and regulations should be maintained regarding blood screening. Overall people should be encouraged to use condoms and abide by the religious rules to avoid HIV/AIDS infection. Both, Government and Non-Government organizations should augment their educational and focused group discussion (FGD) programs on HIV/AIDS knowledge.

Key words: Human immunodeficiency virus (HIV), acquired immune deficiency syndrome (AIDS), blood donors, awareness, focused group discussion (FGD).

INTRODUCTION

AIDS stands for acquired immune deficiency syndrome. The human immunodeficiency virus, which is commonly called HIV, is a virus that directly attacks certain human organs, such as the brain, heart, and kidneys, as well as the human immune system. The immune system is made up of special cells, which are involved in protecting the body from infections and some cancer. HIV damages the natural defense system in human body and the state is called AIDS.

HIV/AIDS is the most devastating disease that mankind has ever faced. HIV does not survive well outside the

body. It can not be transmitted through casual daily contact. Mosquitoes and other insects do not transmit HIV. HIV can be spread by sexual contact with an infected person, by sharing needles and/or syringes and/or other injecting equipments or, less commonly (and now very rarely in countries where blood is screened for HIV antibodies), through transfusion of infected blood or blood clotting factors. Babies born to HIV infected women may become infected before or during birth or through breastfeeding after birth.

After the first detection of AIDS in 1981 in New York City and San Francisco, this disease in Bangladesh was first found in 1983. Since the first detection of HIV in Bangladesh, the rate of infection has not increased in comparison to our neighbors (for example, India). Given that the overall prevalence of HIV remains below 1%

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(0.7%). Bangladesh continues to maintain low prevalence status (National HIV serological surveillance, Bangladesh, 9th round, 2011). But it is clear that this situation may not continue if the risky behaviors that increase vulnerability cannot be contracted among the high-risk group, vulnerable group and general population. Needle sharing among drug users occurs at alarmingly high rates (77%) in central region (National HIV serological surveillance, Bangladesh, 4th round, 2002). of Bangladesh. In a study, Rahman et al. (1998) revealed that only 18% Commercial Sex Workers (CSW) in Bangladesh heard about AIDS and 8% knew that it can be transmit by sexual contact. AIDS was believed to be curable by 5% of the total respondents. A vigorous AIDS awareness campaigns for the CSWs and their clients is necessary to prevent an explosive epidemic in Bangladesh. In another study, Kumar et al. (2002) concluded that awareness about HIV/AIDS infection, transmission and preventive measures is the essential thing for the people in the countries of developing world, especially for women who are engaged in commercial sex works and those of socially and deprived groups. Indeed, there is a host of factors that have rendered the country highly vulnerable to a surge in the epidemic. These include the overall poverty levels, the documented risk behaviors including needle sharing, growing sex work, population mobility, persisting gender disparity and inequality, low levels of general awareness and knowledge about HIV/AIDS among general population, and unscreened blood transfusion.

In many countries, it has been observed that, once an HIV epidemic begins among Injection Drug Users (IDU), it is unlikely to remain limited in that population (Cohen, 2004; Panda, 2000). This is especially true in Bangladesh, since behavior surveillance data show that IDU are not at all an isolated population. Drug injector's sells blood and highly involved in other risk behaviors. HIV prevalence among IDUs has increased to 5.3% in 2011 (National HIV serological surveillance, Bangladesh, 9th round, 2011). From 4% in 2002 (National HIV serological surveillance, Bangladesh, 4th round, 2002) which is a threat for blood receivers, as blood screening system is very poor in Bangladesh. According to the Federal Centers for Disease Control and Prevention (CDC), about 6% infection among women occurs through blood transfusion, which is threatening to the future generation. Bangladesh professional donor should have knowledge about HIV/AIDS. Since Bangladesh is a developing country, most of the people do not have enough knowledge about the importance of blood test before blood donation. A limited number of blood donors have knowledge about HIV/AIDS in Bangladesh.

Though it is encouraging that, HIV prevalence has declined over the years among population at risk in some geographic areas of Bangladesh, however in many areas

especially in Rajshahi Division, high and rising HIV prevalence was recorded (National HIV serological surveillance, Bangladesh, 9th round, 2011). The rise of infection at Rajshahi has put the city into the category of vulnerable area. Some researchers (Rahman et al., 1998; Khan et al., 1997) have studied the knowledge about HIV/AIDS among some risk groups, such as patients with sexually transmitted disease, pregnant woman at antenatal clinics, commercial sex workers, long distant truck driver and overseas workers. But the studies on awareness about HIV/AIDS of blood donors are very rare. The purpose of this study is to focus the knowledge and awareness scenario on different aspects of HIV/AIDS among the blood donors at Rajshahi Metropolitan (divisional city) of Bangladesh.

MATERIALS AND METHODS

This study is based on primary data. The data was collected under the authority of the Department of Population Science and Human Resource Development of Rajshahi University, Bangladesh. To collect the data, purposive sampling technique was applied. Interview method has been used to collect information from the respondents. A total of 160 blood donors of Rajshahi Metropolitan had been interviewed and their information was recorded. The period of data collection was 3rd March to 28 April in 2008. Among the total respondents, only 21% were female and 52% were married. Only 18% blood donors were under 21 years of age, while 4% were above age 40 years. 88% donors were Muslim and 64% were village dweller. It was observed that, 49% blood donors did not have any income, as most of them were student and only 2% had income over 20000 Tk/month (about US \$ 250). 12% donors were illiterate, while 7% were with post graduation. Among the respondents, 34% were student, 16% were agriculture worker, and more interestingly 13% were house wife.

In this study, descriptive statistical techniques have been applied to examine the differentials. Besides the descriptive statistics, multivariate analytical technique (logistic regression model) has also been used to investigate the determinants of knowledge and awareness about HIV/AIDS. The statistical analyses have been performed by the software SPSS. In this study, two sets of variables are selected as dependent variable, which are variables relating preliminary knowledge of HIV/AIDS (which includes whether knows the name of HIV/AIDS, agree that AIDS is a fatal disease, AIDS is not a contagious disease and knowledge about the medium of HIV/AIDS infection), and variables relating preventive knowledge of HIV/AIDS (includes the knowledge about the means of preventing HIV/AIDS infection). The variables relating preliminary knowledge and preventive knowledge were further categorized into two groups depending upon the extent of knowledge, the categories are sufficient and insufficient knowledge. The classification was made by subjective judgment. For both the variables, if the 90% of the answers of the respondent were found correct then the respondent was considered having sufficient knowledge, otherwise he was considered to have insufficient knowledge. Independent variables used in the analyses are age, sex, education, occupation, possession of Radio/TV, condom's impact and religious rule's impact. In case of independent variable, classifications for bivariate analysis differ from that of multivariate analysis, where in multivariate analysis; independent variables include lower number of classes than that in bivariate analysis. This is done to make the

variation of impact of different classes more clear and apparent, and to facilitate interpretability.

In multivariate analysis, logistic regression model (Cox, 1972) has been applied. This model can be used to identify the risk factors as well as to predict the probability of success e.g. probability of developing a disease as a function of the particular risk factor. This probability can serve as an index of risk for a given disease or for not responding to certain treatment. The logistic regression has become the standard method for finding the relationship between the qualitative outcome variables and a set of explanatory variables. Then the logit transformation is defined to be

$$z_i = \text{logit}(p_i) = \log\left(\frac{p_i}{1-p_i}\right) = x_i \beta$$

Where β , a regression coefficient and this equation are known as the logit model that relates the independent variables to the transformation of p_i .

Taking these probabilities (p and $1-p$) as the basis of analysis, some functions are considered that transform the scale (0, 1) for the probabilities on to the real line. This function is known as link functions or response function. The logit model can be written as

$$\text{logit}(p_i) = \log\left[\frac{p_i}{1-p_i}\right] = X_i \beta = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \dots + \beta_k X_{ki}$$

$$\text{Where } p_i = \Pr(Y_i = 1 | x_i) = [1 + \exp(-X_i \beta)]^{-1} \exp(-X_i \beta)$$

RESULTS AND DISCUSSION

To study the blood donor's knowledge and awareness about HIV/AIDS, both, bivariate and multivariate analytical techniques have been used. To dissect the knowledge and awareness scenario and their pattern, descriptive statistical technique has been applied, and to spot the significant covariates and their degrees of impact on the knowledge and awareness of blood donors about HIV/AIDS, Logistic Regression analysis has been used. The results and discussion have been presented in the following sections.

Knowledge and awareness about HIV/AIDS

Main objective of this paper is to investigate the blood donor's knowledge and awareness about HIV/AIDS. Several questions were tried to capture their knowledge and awareness which have been summarized in Table 1.

From the table, it is observed that 97% blood donors heard the name of AIDS and 76% knew it is caused by HIV virus, whereas 3 and 24% respectively never heard the name of AIDS and do not know that it is caused by HIV virus. 94% blood donors knew it is fatal and 89% knew that it is transmitted through blood transfusion. But the worst thing is that, 94% blood donors did not test their blood before donating blood; only 1% did the test.

89% knew that HIV/AIDS transmits through sexual intercourse with more than one partner, 87% knew it is transmitted through sharing needle with others, 66% knew it may spread from pregnant mother to her child and 52% knew it may transmit through breast feeding.

Regarding preventive knowledge, 8% knew that it may be prevented by vaccine, 89% knew it can be prevented by using condom during sexual intercourse. 93% believed by increasing mass awareness and 92% believed by abiding religious rules, HIV/AIDS can be prevented.

Level and pattern of awareness and knowledge of the blood donors by background characteristics

Table 3 shows that, 67 respondents (41%) were found to have sufficient preliminary knowledge about HIV/AIDS and 109 respondents (68%) having sufficient knowledge about preventive measures of HIV/AIDS. Respondents of age group 21 to 25 years were most aware about both preliminary (22 out of 67; 33%) and preventive (35 out of 109; 32%) knowledge about HIV/AIDS. It was observed that the awareness decreases with the increase in age. Male respondents were found more aware about preliminary (57 out of 67; 85%) and preventive knowledge (89 out of 109; 81%) than its female counterpart. A positive relationship has been observed between educational level and awareness as in Table 2.

It was observed that, those who are student and service holder are more aware about HIV/AIDS. Those having Radio/Television were more conscious about the preliminary and preventive knowledge about HIV/AIDS. Higher percentage is observed to have sufficient knowledge about HIV/AIDS for those who know that using condom and abiding by religious rules can prevent HIV/AIDS than those who do not know.

Given the descriptive statistics and information of the different explanatory variables above, which covariates are likely to affect knowledge and awareness of blood donors comes into question? To identify the influential factors, Logistic regression analysis was performed and the result has been presented in the next section.

Logistic regression analysis

The logistic regression analysis is aimed at identifying the important contribution of variables that have an influence on the preliminary and preventive knowledge of blood donors. A variable was considered significantly associated with having knowledge, if its P value was below 0.10. The results of the analyses are shown in Table 3. From the analysis, we observed that, respondents of age group 20 to 24 years are 2.2 and 1.2

Table 1. Percentage distribution of respondents by selected categories having knowledge and awareness about HIV/AIDS.

Questions	No.	Age (%)
Did you listen the name HIV/ AIDS?		
Yes	153	95.6
No	7	4.4
Do you know the name of virus which causes AIDS?		
Yes	122	76.3
No	31	19.4
No answer	7	4.4
Is AIDS a fatal disease?		
Yes	151	94.4
No	2	1.3
No answer	7	4.4
Does HIV transmit through blood transfusion?		
Yes	142	88.8
No	3	1.9
Doesn't know	8	5.0
No answer	7	4.4
Before donation did you test blood?		
Yes	2	1.3
No	151	94.4
No answer	7	4.4
Does HIV/AIDS transmit through sexual intercourse with more than one male or female?		
Yes	143	89.4
Doesn't know	10	6.3
No answer	7	4.4
Does HIV/AIDS transmit through sharing same needle with others?		
Yes	139	86.9
No	1	0.6
Doesn't know	13	8.1
No answer	7	4.4
Does HIV/AIDS transmit from pregnant mother to her child?		
Yes	107	66.9
No	3	1.9
Doesn't know	43	26.9
No answer	7	4.4
Does HIV/AIDS transmit through breast feeding?		
Yes	83	51.9
No	4	2.5
Doesn't know	66	41.3
No answer	7	4.4

Table 1. Contd.

Have any vaccine to prevent HIV/AIDS?			
Yes		12	7.5
No		74	46.3
Doesn't know		66	41.3
No answer		8	5.0
Do you know that HIV/AIDS can be prevented by using condom?			
Yes		143	89.4
Doesn't know		10	6.3
No answer		7	4.4
Do you think that HIV/AIDS can be prevented by mass awareness?			
Yes		148	92.5
Doesn't know		5	3.1
No answer		7	4.4
Do you think that HIV/AIDS can be prevented by abiding religious rules?			
Yes		147	91.9
Doesn't know		6	3.8
No answer		7	4.4

Rajshahi field data, April, 2008.

Table 2. Percentage distribution regarding sufficient and insufficient preliminary knowledge and preventive knowledge about HIV/AIDS of the respondents.

Characteristics	Preliminary knowledge about HIV/AIDS		Preventive knowledge about HIV/AIDS	
	Sufficient knowledge (%)	Insufficient knowledge (%)	Sufficient knowledge (%)	Insufficient knowledge (%)
Age (in years)				
<21	16 (55.2)	13 (44.8)	23 (79.3)	6 (20.7)
21-25	22 (38.6)	35 (61.4)	35 (61.4)	22 (38.6)
26-30	13 (40.6)	19 (59.4)	23 (71.9)	9 (28.1)
31-35	10 (55.6)	8 (44.4)	14 (77.8)	4 (22.2)
36-40	5 (29.4)	12 (70.6)	11 (64.7)	6 (35.3)
40 ⁺	1 (14.3)	6 (85.7)	3 (42.9)	4 (57.1)
Sex				
Male	57 (44.9)	70 (55.1)	89 (70.1)	38 (29.9)
Female	10 (30.3)	23 (69.7)	20 (60.6)	13 (39.4)
Education				
Illiterate	2 (10.5)	17 (89.5)	7 (36.8)	12 (63.2)
Primary	7 (23.3)	23 (76.7)	13 (43.3)	17 (56.7)
Secondary	9 (31.0)	20 (69.0)	17 (58.6)	12 (41.4)
Higher Secondary	17 (51.5)	16 (48.5)	28 (84.8)	5 (15.2)
Graduate	24 (63.2)	14 (36.8)	33 (86.8)	5 (13.2)
Post-Graduate	8 (72.7)	3 (27.3)	11 (10)	0 (0)
Occupation				
Student	33 (60.0)	22 (40.0)	46 (83.6)	9 (16.4)

Table 2. Contd.

Agriculture	3 (12.0)	22 (88.0)	10 (40.0)	15 (60.0)
Service	20 (69.0)	9 (31.0)	25 (86.2)	4 (13.8)
Business	7 (23.3)	23 (76.7)	18 (60.0)	12 (40.0)
Housewife	4 (19.0)	17 (81.0)	10 (47.6)	11 (52.4)
Having Radio/TV				
Yes	40 (39.2)	31 (53.4)	61 (59.8)	10 (17.2)
No	27 (46.6)	62 (60.8)	48 (82.8)	41 (40.2)
HIV/AIDS can be prevented by using condom				
Yes	57 (40.1)	85 (59.9)	95 (69.3)	42 (30.7)
No	10 (55.6)	8 (44.4)	14 (60.9)	9 (39.1)
HIV/AIDS can be prevented by abiding religious rules				
Yes	61 (43.6)	79 (56.4)	99 (71.2)	40 (28.8)
No	6 (30)	14 (70)	10 (47.6)	11 (52.4)
Total	67 (41.9)	93(58.1)	109(68.1)	51(31.9)

Rajshahi field data, April, 2008.

times more likely to have preliminary and preventive knowledge about HIV/AIDS respectively than the respondent of below 20 years of age. But the odds fell with the increase in age. In the analysis, male respondent received 2.5 and 1.3 times higher odds than its female counterpart to have preliminary knowledge and preventive knowledge respectively about HIV/AIDS. Respondents with primary and secondary education and higher education received higher odds than the illiterate respondents. Service holders were 100 and 83% more likely to have preliminary and preventive knowledge respectively about HIV/AIDS than the others. Respondents had radio/TV were more likely to have knowledge about HIV/AIDS than those did not have any radio/TV. Persons knew that HIV/AIDS can be prevented by using condoms were 5.17 and 2.91 times more likely to possess preliminary and preventive knowledge about HIV/AIDS. Among those who knew that HIV/AIDS can be prevented by abiding by religious rules received higher odds of having preliminary and preventive knowledge than those who did not know.

Conclusion

HIV/AIDS is one of the deadliest disease that does not have any treatment, thus to get rid of the catastrophe, the only way out there is its prevention. To prevent the spread of the disease, knowledge and awareness among

the high risk group about it is indispensable. This study attempts to examine the knowledge and awareness of blood donors about HIV/AIDS. The matter of hope is that, 89% blood donors know that HIV/AIDS is transmitted by blood transfusion, but the worst thing is that only 1% undertook blood test before donation, which is frustrating. A sizeable magnitude knows that it can be prevented by using condom (89%) and by abiding religious rules (92%). In this study, age, sex, education, occupation, Radio/TV possession and knowledge about preventive role of condom and religious rules have been deemed to be important determinants of knowledge about both preliminary and prevention of HIV/AIDS. Interestingly, the awareness decreases with the increase of age, while all other variables are found positively related with awareness. To increase awareness in a particular population segment, focused group discussion (FGD) is the most effective tool and considerable emphasize should be given to FGD. Undoubtedly education is the most important determinant of any sort of awareness, and so in this study. GOs and NGOs should augment their educational program containing knowledge about HIV/AIDS, throughout the country.

Finally, radio and TV are the most popular medium of recreation, programs relating preliminary and prevention knowledge about HIV/AIDS should be increased in radio and TV. Besides these, overall People should be encouraged to use condoms and abide by the religious rules by the popular media. Now under the HAPP 98 safe

Table 3. Multivariate logistic regression estimates of relative odds of having preliminary knowledge and preventive knowledge about HIV/AIDS.

Characteristics	Relative risk (odd ratio)	
	Having preliminary knowledge about HIV/AIDS	Having preventive knowledge about HIV/AIDS
Age (in years)		
<20 (RC)	1.00	1.00
20-24	2.22 ^{***}	1.23 ^{***}
25-29	1.06 ^{**}	1.39 ^{**}
30-34	1.82 ^{**}	2.19 ^{**}
35 ⁺	1.23 ^{***}	2.05 ^{***}
Sex		
Female (RC)	1.00	1.00
Male	2.51 ^{**}	1.31 ^{**}
Education		
Illiterate (RC)	1.00	1.00
Primary and Secondary	1.30 ^{***}	1.68 ^{***}
Higher	1.86 ^{***}	2.29 ^{***}
Occupation		
Others (RC)	1.00	1.00
Service Holder	2.03 ^{***}	1.83 ^{***}
Having Radio/TV		
No (RC)	1.00	1.00
Yes	2.63 ^{***}	1.72 ^{***}
Condom can prevent HIV/AIDS		
Doesn't Know (RC)	1.00	1.00
Know	5.17 ^{**}	2.91 ^{**}
Abiding religious rules can prevent HIV/AIDS		
Doesn't Know (RC)	1.00	1.00
Know	2.84 ⁺	3.20 ⁺

Rajshahi field data, April, 2008; RC=Reference Category, * = P<0.10, ** = P<0.05, *** = P<0.01.

blood transfusion centers are screening the blood for HIV and 5 other diseases. Government of the country should pay special attention to the blood screening system. Strict rule should be established to screen the blood before taking that from the donors. The blood screening service should be expanded to Thana (sub-district) level and the numbers of the screening centers should be increased in metropolitan cities like Rajshahi.

As HIV/AIDS is an incurable disease, massive steps should be taken to prevent it through building awareness among the blood donors. Some recommendations regarding increase in knowledge and awareness level have been pointed out above which could be considered by the policy makers to improve the scenario.

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A microscopic view of human skin, showing the texture of the epidermis and a large red blood cell in the lower right corner. The image is framed by a thick red border.

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