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Research Article

Attitude, Knowledge, Association and Screening of Human Papilloma Virus-Immunoglobulin M Antibody in Young Females in Nnewi

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Abstract

Female children have been neglected as an important age group in the screening of HPV disease in Nigeria. A cross-sectional study on the attitude, knowledge, associations and sero-prevalence of HPV-IgM was carried out on 93 females of age 12 - 29 years in urban Nnewi town. Data was collected using questionnaire method and HPV IgM assessed using ELISA method. Result revealed that females aged 12 - 15 years had the highest sero-prevalence (12.0%). Majority of the respondents had low knowledge of HPV and cervical cancer (72.0%). The respondents from Enugu, Igbos, singles, and secondary school females had the highest sero-prevalence 9.7%, 16.1% and 16.1%, (18.6%) socioeconomically respectively, with a weak association between disease status among State of origin (X^2 = 12.556; P = 0.051). Those who were not in a relationship and were not sexually active had more positive status 9.7% (9/93) each. The respondents who had their first sexual debut before 13 and within 17 - 19 years had more positive status 3.9% each. Those who had 2 - 5 lifetime sex partners had the highest seroprevalence (7.8%), with no statistical significance amongst all, P > 0.05. There is an urgent need for the screening of females in Nnewi for HPV and as well as possible vaccination and education about the disease as stipulated by WHO.

Keywords: *Human Papilloma Virus; Immunoglobulin M; Attitude; Knowledge; Females; Nnewi*

Introduction

Human papilloma virus (HPV) is a sexually transmitted DNA virus that infects the skin and mucous membranes [1]. This preventable viral highly infectious disease can be acquired by contact with infected persons either transparently, through sexual contact or contact with body secretions. Though over 100 species exist, 40 types can infect genital area, cervix, vulva, vagina, anus, penis, scrotum, mouth and throat, while other types can cause common warts on hands, plantar of feet or other body parts [2]. The ultimate effect of these sexually transmitted strains is cervical cancer which is highly fatal in females causing up to 52% death annually and 24.8% in Nigeria with 14,089 cases diagnosed annually and 8,240 deaths [2]. According to Rampus., *et al*. [3], worldwide, the prevalence of HPV is ~12% with the frequency of HPV cases much higher in developing regions. Eastern Africa (33.6%) and the Caribbean (35.4%) have been noted to have the highest prevalence in 2012 [4] and although approximately 90% of HPV infections regress to a subclinical state within 2 years, a persistent infection may lead to cancer. WHO [5] stated that majority of the 100+ HPV strains are oncogenic and variants 16 and 18 alone cause 70% of cervical cancer cases. Cervical cancer is the most common cancer among women in developing regions and has 52% mortality rate. More than 270,000 women die per year from cervical cancer globally, with 85% of the deaths occurring in developing countries. Cervical cancer according to Bruni., *et al.* [6] is the second most prevalent cancer in 15 - 44 years aged Nigerians.

HPV IgM antibody in serum is an antibody that evaluates humoral immune response to HPV in serum of exposed individuals. The seropositive people become high-risk carriers and later if it persists could serve as a risk of developing cervical intraepithelial neoplasia (pre-cancer lesion of the cervix) which can progress to cervical cancer in 15 - 20 years. The rise in social media, advancement in technology, reduction in age of physical maturity due to improved living conditions, the current rate of societal crack-down in security as well as rise in insurgency, rape, forced sexual practices, and abuses have become common in this part of the country. Vulnerable pre-teens could be victims of sexual abuse especially in rural settings where they have to go to farms and streams, as well as cases of forced relationships by parents to well-to-do suitors in order to help with the family needs. These can increase chances of their being infected. In 2014 an estimate of about 23.7% of women in the general population in Nigeria were noted to harbor cervical HPV infection at a time WHO [2] and as the virus are ubiquitous with most women in the world at risk of probably being infected with at least one type of HPV during their sexual viruses life Akarolo., *et al*. [7], WHO introduced a three dose HPV vaccine for primary prevention of cervical cancer with 100% efficacy of prevention if taken prior to exposure of the virus [8]. In 2006, the Centre for Disease Control and Prevention (CDC) recommended HPV vaccine for both sexes of ages 11 and 12 or a 2 dose vaccine for 9 - 14 years prior to sexual exposure. This is because the vaccine has the highest efficacy if they have not acquired HPV infection and they also encouraged all regulatory bodies to recommend HPV vaccine to pre-teens and teens. Following these directives, about 58 countries introduced this vaccine in their National Immunization Programme (NIP) [9]. Unfortunately, Nigeria was one of the countries that rejected the uptake on several basis. Despite WHO's call for its adoption, there is a low rate of uptake due to lack of knowledge about HPV and the vaccine among mothers of eligible children as to their contribution to a choice to be vaccinated or not does not count [10]. Though the vaccine was licensed and introduced in Nigeria in 2009, its acceptance and uptake is still very low, with a range of between 0 and 49% being utilized by a few privileged population [11]. Studies in Nigeria by Ezem [12] found that despite the need for the vaccine, the cost for HPV vaccination per person is beyond what an average Nigerian can afford.

Awareness and knowledge of the infection its prevention would stimulate demand and uptake of the vaccines. Increasing demand may drive the introduction of the vaccine into the National Immunization schedule thereby making the vaccine more affordable and accessible. This study is therefore carried out to assess the attitude, knowledge, association and screening of HPV-IgM antibody in young females in Nnewi, Anambra state, Nigeria.

The study was done to screen for the presence of Human Papilloma Virus-IgM antibody in serum and determine the attitude, knowledge, risk- associations of the disease in young females in Nnewi.

Materials and Methods

Study design

The study design was a cross-sectional study to screen for the presence of Human Papilloma Virus-IgM antibody in serum and determine the attitude, knowledge, risk- associations of the disease in young females in Nnewi.

Study area

The study was carried out at Nnewi, Anambra state in southeastern Nigeria.

Study population

The study population comprises of 93 young females (12 - 29 years) residing in Nnewi as at the time of study.

Sample size

The sample size was calculated using the formula by Naing., *et al.* [13]:

$n = Z^2 \times P\ (1\text{-}P)/d^2$

Where n = desired sample size.

P = Prevalence of (15%) as recorded in previous studies by Clarke., *et al*. [14]

Z = Confidence interval of 95% which is equivalent to confidence coefficient of 1.96

d = desired level of precision = 0.05

$n = Z^2 \times P\ (1\text{-}P)/d^2$

A total of 93 respondents were used for the study.

Inclusion criteria

- Sexually exposed and non-exposed females.
- Must be in the age range 12 - 29 years old.
- Must be residing in Nnewi town, Anambra State as at the time of study.
- Willingness to participate in the study.

Exclusion criteria

- Refusal to give consent.
- Males
- Females below 12 and above 29 years old.
- Residents outside Nnewi town, Anambra State.

Data collection: Data collection was collected using questionnaire which was filled out with the help of the participants who gave their consent.

Sample collection: Three (3 ml) milliliters of venous blood was collected aseptically by vein-puncture from the subjects and dispensed into a plain container for centrifugation at 10,000 rpm for 3 minutes. The serum separated were stored at - 4°C until analysis.

Method of assay: Screening for HPV (IgM) antibodies in serum was carried out using Enzyme-linked immunosorbent assay technique with kit procured from (Melsin Medical Company Limited, Changchun Jilin province, China). The optical density (O.D) was read within 15 minutes at 450 nm using a microtitre plate. The test was carried out according to manufacturer's instructions with cut-off value calculated with manufacturer's specifications. A negative HPV result was interpreted as any sample with an optical density value less than the calculated cut-off value and samples with an optical density greater than the calculated cut-off value were reported as positive for IgM to HPV.

Informed consent: Informed consent was sought and obtained from the subjects prior to sample collection after which the subjects were given the questionnaires.

Ethical consideration: Ethical approval was obtained from ethics committee of College of Health Sciences Nnamdi Azikiwe University in accordance with the Helsinki declaration by the World Medical Association on the ethical principles involving human subjects.

Method of data analysis: The results and data from the questionnaires were analyzed with percentages and presented as tables and figures. SPSS (Statistical Package for Social Science, version 20) was used for data analysis. Simple prevalence, chi-square analysis was used where necessary and level of significance was set at 95% at 0.05 confidence interval.

Result

Seroprevalence of human papilloma virus IgM antibodies according to age group

Human papilloma virus IgM antibodies were detected in serum samples from 15 (16.1%) of study participants. Children aged 12 - 15 had the highest prevalence 11 (12.0%), while age groups of 19 - 29 had the lowest prevalence 1 (1.1%). There was no significant difference among the three age groups sampled ($x^2 = 2.096$, P = 0.351).

Age range Years	Frequency n (%)	Positive HPV n (%)	Negative HPV n (%)	X^2	P value
12 - 15	52 (56.6)	11 (12.0)	41 (44.6)		
16 - 18	28 (30.2)	3 (3.0)	25 (27.2)	2.096	0.351
19 - 29	13 (13.2)	1 (1.1)	12 (12.1)		
Total	93 (100)	15 (16.1)	78 (83.9)		

Table 1: *Seroprevalence of human papilloma virus IgM antibodies according to age group.*

Key: HPV: Human Papilloma Virus; X^2: Chi square.

Distribution of level of knowledge and attitude towards human papilloma virus disease among the respondents

Females with low knowledge of the disease (HPV) had the greater frequency 67 (72.0%), while respondents with moderate knowledge of the disease had the lowest frequency 4 (4.3%). In relation to attitude, 87 (93.5%) of the respondents believed HPV is a big problem to women, 85 (91.4%) of them believe cervical cancer can cause death in women and that HPV is a serious disease, while 66 (71.0%) believe they are at risk of getting the infection.

Knowledge status	Frequency	Percentage
Low knowledge	67	72.0
Moderate knowledge	4	4.3
High knowledge	22	23.7
Total	93	100.0
Attitude towards cervical cancer		
Believe they can be easily infected	66	71.0
Think cervical cancer is a big problem	87	93.5
Think HPV is a serious disease	85	91.4
Think cervical cancer causes death	85	91.4

Table2: *Distribution of level of knowledge of human papilloma virus disease and attitude towards cervical cancer among the respondents.*

Key: HPV: Human Papilloma Virus

Distribution of the association between HPV status, personal, demographical and socioeconomic variables

Single women had the highest prevalence 15 (16.1%) with no significant association among marital status and HPV infection, ($X^2 = 0.096$; P = 0.234). Highest status was observed in women from Enugu state 9 (9.7%), while the lowest were from Ekiti, Lagos and Abia

states, 0 (0.0%) with a weak association between disease statuses and states of origin (X^2 = 12.556; P = 0.051). Ibo females 15 (16.1%) recorded the highest prevalence (X^2 = 0.0596; P = 0.440). People who attained secondary school level had the highest prevalence 11 (18.6%) while the lowest were drop-outs and illiterates 0 (0.0%), (X^2 = 1.354; P = 0.852) in table 3a.

Variables	Size (n)		Positive		Negative		X^2	P- value
	N	%	N	%	N	%		
Marital status								
Single	80	86.0	15	61.4	65	69.9	2.096	0.234
Living together	1	1.1	0	(0.0)	1	(1.1)		
Married	12	12.9	0	(0.0)	78	(83.9)		
Demographic								
State of origin								
Anambra	24	25.8	2	2.2	22	23.7		
Enugu	23	24.7	9	9.7	14	15.1		
Ebonyi	26	28.0	3	3.2	23	24.7		
Imo	12	12.9	1	1.1	11	11.8	12.556	0.051
Lagos	2	2.2	0	0.0	2	2.2		
Ekiti	1	1.1	0	0.0	1	1.1		
Abia	5	5.4	0	0.0	5	6.4		
Ethnic group								
Igbo	90	96.8	15	16.1	75	80.6	0.596	0.440
Yoruba	3	3.2	0	0.0	3	3.2		
Socioeconomic								
Educational level								
None	3	3.2	0	0.0	3	3.2		
Primary	1	1.1	0	0.0	1	1.1	1.354	0.852
Secondary	59	63.4	11	18.6	48	51.6		
Tertiary	29	31.2	4	13.8	25	26.9		
Drop-out	1	1.1	0	0.0	1	1.1		

Table 3a: *Association between HPV and marital status, demographical, and socioeconomic variables.*
Key: HPV: Human Papilloma Virus; X^2: Chi Square.

Students had the highest positive status 15 (16.1%) and were the only occupational groups affected, (X^2 = 3.169; P = 0.674), while those living in self-contained houses had the highest status 5 (5.4%) and those squatting the lowest, 0 (0.0%), with no significant association (X^2 = 1.619; P = 0.805) in table 3b.

Variables	Size		Positive		Negative		X^2	P-value
	F	%	F	%	F	%		
Occupation								
None	5	5.4	0	0.0	5	5.4		
Student	79	84.9	15	16.1	64	68.8		
Trader	6	6.5	0	0.0	6	6.5	3.169	0.674
Health worker	1	1.1	0	0.0	1	1.1		
Trader	1	1.1	0	0.0	1	1.1		
Hair dresser	1	1.1	0	0.0	1	1.1		
Living condition								
Self-contained	34	36.6	5	5.4	29	31.2		
Flat	18	19.3	3	3.2	15	16.1		
Squatting	4	4.3	0	0.0	4	4.3	1.619	0.805
One room	15	16.2	2	2.2	13	14.0		
Others	22	23.7	5	5.4	17	18.3		

Table 3b: *Association between HPV status and socioeconomic variables.*
Key: HPV: Human Papilloma Virus X^2: Chi Square.

In relation to sexual characteristics and symptoms, those not in a relationship 9 (9.7%), had no sexual contact 9 (9.7%), are not sexually active 9 (9.7%), had first sexual intercourse between <13 and 17- 19 years old 2 (3.9%), had 2-5 sexual partners in their life time 4 (7.8%), had sex partners in the past 6 months 4 (7.8%), had 1 - 6 sex contact in the past six month 5 (9.8%), had not been rapped 5 (9.6), had never been diagnosed of sexually transmitted disease 30 (58.8%), had never been sexually molested 11 (11.8%), never received blood transfusion 13 (14.0), and had no warts 15 (16.1%) had the highest positive status, with no statistical significance among them, P > 0.05 in table 3c-3e.

Variable	Size		Positive		Negative		X^2	P- value
	N	%	N	%	n	%		
In a relationship								
Yes	45	48.4	6	6.5	39	41.9	0.504	0.478
No	48	51.6	9	9.7	39	41.9		
Ever had sexual contact								
Yes	57	61.3	6	6.5	51	54.8	3.417	0.065
No	36	38.7	9	9.7	27	29.0		
Sexually active								
Yes	50	53.8	6	6.5	44	47.3	1.363	0.243
No	43	46.2	9	9.7	34	36.6		
Age at FSI								
< 13 years	9	17.9	2	3.9	7	13.7		
14 - 16	21	41.2	1	2.0	20	39.2	2.407	0.492
17 - 19	11	21.6	2	3.9	9	17.6		
> 20	10	19.6	1	2.0	9			
Number of LSP								
1	20	39.2	1	2.0	19	37.3		
2 - 5	24	47.8	4	7.8	20	39.2	1.480	0.477
6 - 10	7	13.7	1	2.0	6	11.8		

Table 3c: *Association between HPV status sexual behavioral characteristics.*

Key: HPV: Human Papilloma Virus; FSI: First Sexual Intercourse; LSP: Lifetime Sex Partner.

Age range Years	HPV result n (%)	Positive HPV n (%)	Negative HPV n (%)	X^2	P value
Sex partners in the past 6 months (n)					
0	10 (19.6)	1 (2.0)	9 (17.6)		
1	27 (52.9)	4 (7.8)	23 (45.1)	0.942	0.918
2	9 (17.6)	1 (2.0)	8 (15.7)		
> 2	5 (9.8)	0 (0.0)	5 (9.8)		
Sex contact in the past 6 months (n)					
0	11 (21.6)	1 (2.0)	10 (19.6)		
1 - 6	28 (54.9)	5 (9.8)	23 (45.1)		
7 - 24	4 (7.8)	0 (0.0)	4 (7.8)	2.677	0.613
25 - 54	3 (5.9)	0 (0.0)	3 (5.9)		
> 54	5 (9.8)	0 (0.0)	5 (9.8)		
Ever been raped					
Yes	9 (17.3)	1 (1.9)	8 (15.4)		
No	48 (82.7)	5 (9.6)	38 (73.1)	0.002	0.965
Ever been diagnosed of STI					
Yes	16 (31.4)	1 (2.0)	15 (29.4)		
No	35 (68.6)	5 (9.8)	30 (58.8)	0.683	0.409

Table 3d: *Association between HPV status and symptoms and sexual characteristics.*

Key: STI: Sexually Transmitted Infection; HPV: Human Papilloma Virus.

Size Parameter	HPV result n (%)	Positive HPV n (%)	Negative HPV n (%)	X^2	P value
Ever been sexually molested					
Yes	19 (20.4)	4 (4.3)	15 (16.1)		
No	74 (76.6)	11 (11.8)	63 (67.7)	0.428	0.513
Ever been transfused					
Yes	10 (10.8)	2 (2.2)	8 (8.6)		
No	83 (89.2)	13 (14.0)	70 (75.3)	0.124	0.725
Presence of Warts					
Yes	2 (2.2)	0 (0.0)	2 (2.2)		
No	91 (97.8)	15 (16.1)	76 (81.7)	0.393	0.531

Table 3e: *Association between HPV status and symptoms and sexual characteristics.*
Key: *HPV: Human Papilloma Virus.*

Discussion

Antibodies to Human Papilloma Virus (HPV-IgM) were detected more in children aged between [12-15] years old in this study (12.0%). This is probably because of early exposure to the infection as a result of either early indulgence in sexual activity or exposures via the mother or other sources. Living in urban or rural setting could also be contributory factors. In our society today, there is a rise in the current rate of societal crack-down in security and morals as well as rise in insurgency, rape, displacements, forced sexual practices and abuses. They could have been vulnerable victims of sexual abuse especially in rural settings increasing their chances of being infected. Low knowledge about this infection may also contribute to its prevalence among this age group as most of these children in the study setting could have had no awareness of the disease or its preventive measures and so may have no knowledge about its acquisition route. Most of them at that age are also prone to unsteady relationships and they could be at their debut for sexual relationship, thereby getting them exposed to this infection. Possible persistence of maternal antibodies in young age in these children also can contribute to the positive status of HPV IgM antibody within this age range. It was however lowest in the oldest groups 19 - 29 years old 1 (1.1%), maybe from chance, due to geographical location, they were protected during sexual intercourse or were not exposed to transmission route. Since the specie types were not specified, a possibility that there could be low-risk HPV genotypes exist and non-sexual types have been noted to affect females in all age groups. LaCour [15] analyzed HPV infection in USA in 2003 - 2004 and in females aged 14 - 19; he obtained a prevalence of 35%, higher than that observed in the study area. Difference in both findings across age range could be due to difference in sexual behavior across geographical regions. There is also a possibility of exposure before vaccination age-range. Differences in prevalence have been shown to be associated with differing geographical location and age [16]. In their study, they also noted that HPV infection decreased with increasing age in females similar to observations in the present study, from a peak prevalence in younger women (< or = 25 years of age). A total of 16.1% obtained in this research in the total population sampled. The apparently low prevalence observed in the area of study in this research compared to other works, could be as a result of sensitivity of the kit, geographical differences, life style factors, and genetic constitutions of the affected groups, as well as incubation period at the time of study in the individuals. This percentage is lower when compared with (26.3%) reported in Ibadan, Nigeria by Okolo., *et al.* [17] and 40% reported in southern Mozambique [18].

Majority of the subjects in the study area had low level of knowledge of the infection (72.0%). Lack of awareness of the disease in the study area may be because of relationship status or because the government and healthcare providers have did not really create awareness of the disease probably because of the controversy surrounding the acceptance of the screening of this disease and since Nigeria did not adapt to the low age vaccination recommended by WHO, this may be a reason why a greater population had no knowledge about the disease. They consequently lacked the educative programme and awareness which could be constitutive reasons to the low level of awareness. Others could be the level of literacy in the study area and personal interest to know. This finding is similar to that from University

teaching hospital, Federal Capital Territory, Abuja, where small proportion (40%) of respondents knew about the disease [19]. A similar finding carried out by Khadka., *et al*. [20] in Nepal, India indicated low knowledge (15.5%) of HPV infection.

The highest observation in attitude towards HPV and cervical cancer (93.5%) in the general population in this research in believing that cancer is a big problem for women and due to low knowledge about the infection, some of them believe they cannot be easily infected could be because Nigeria had for a long time created awareness about cancer and in people's mind-set, associates it with sure death and pain could be reason for the high awareness. This is similar to community studies carried out in Lagos and Ibadan by Ezenwa., *et al*. [21] and Odetola., *et al*. [22] respectively, where it was discovered that knowledge of cancer of cervix was very high, while knowledge of HPV disease in the study in Ibadan was very high, but low in Lagos. In a similar study by Ezat., *et al*. [23] in Kuala Lumpur, Malaysia, over 50% of the respondents were aware of cancer of cervix and HPV disease.

In table 3a, singles had the highest prevalence (16.1%). This could be because they had they constitute mostly of the younger age group and were the highest number in sample size, or because they are still searching and therefore hang out with friends, creating chances of sexual exposure. Thompson., *et al.* [24] noted that an interest in the HPV vaccine was influenced by relationship status since young female adults represent a unique group that may face distinct barriers to HPV vaccination. Again, Piepenbrink., *et al.* [25] noted that women who were dating were more likely to perceive a high - risk for HPV compared to women in a committed relationship. Respondents from Enugu state had the highest positive status (9.7%) probably because of their lifestyle and geographical location. The Igbos had the highest positive status (16.1%) probably because almost all the subjects were Igbos. Highest prevalence of HPV infection was also observed in respondents with secondary school education (18.6%) and in students (16.1%), and were the only occupational groups affected, ($X^2 = 3.169$; $P = 0.674$). This could be because they are in a sexual experimental age, or due to early indulgence in sexual activity, lack of condom use, constant contact with each other sharing items as they grow together or due to lack of awareness of the disease. This result is consistent with previous research by Naucler., *et al.* [26] but in contrast with a report by Marrazzo., *et al.* [27] who showed that the HPV infection rate decreased with increasing level of education, and Bernard., *et al.* [28] associated lower education and higher poverty increased penile, cervical, and vaginal invasive cancer incidence rates. Students had the highest positive status among all the occupational status (16.1%), which may be as a result of low knowledge about the infection, communal manner of life and sexual frivolities. No statistical significance was observed ($X^2 = 3.169$; $P = 0.674$). Those living in self-contained houses and others had the highest positive status (5.4%) maybe as a result of chance, with no statistical difference among living condition ($X^2 = 1.619$; $P = 0.805$) in table 3b.

In relation to sexual characteristics and symptoms, those not in a relationship (9.7%), with no sexual contact (9.7%) and are not sexually active (9.7%), had never been diagnosed of sexually transmitted disease (58.8%) or never been sexually molested (11.8%) or rapped (9.6%) had more positive HPV IgM antibodies in the present research. Reason could be dependent on the infective strain type, since some HPV types are transmitted through body contact, horizontally and trans-placental and not necessarily through sexual contact, like the cutaneous types. More so, antibody persistence could be a contributory factor. HPV DNA has been detected at various sites in non-abused children [29].

Those who had [29] first sexual intercourse between < 13 and 17 - 19 years old (3.9%), had the highest prevalence in the present research because at that first debut, they may be careless and unprepared and may not have worn condoms. Again those who had 2 - 5 sexual partners in their life time (7.8%), had sex partners in the past 6 months (7.8%) and had 1 - 6 sex contact in the past six month (9.8%) had the highest status because sexual intercourse is the most effective and surest way of contacting the infection and having many sex partners increases ones chance of being infected. These are in line with other findings by Nejo., *et al.* [30] who found younger age (< 18) at sexual debut, polygamy and divorcees to be associated significantly with HPV infection in females in a Nigerian study.

Finally, those who never received blood transfusion (14.0) and had no warts (16.1%) had the highest positive status, with no statistical significance among all mentioned above, $P > 0.05$. This could be because HPV can be contacted through many other sources other than blood route and genital warts is a characteristic of some strains of the virus and could depend on types of oncogenic HPV infection types, latency period or duration of the infection [29] frequency of exposure and its absence may not necessarily mean the infection is absent

but may be a sign of remission. Dareng., *et al*. [31] found HIV infection and number of sexual partners in the past year as important risk factors for prevalence and incidence of genital warts in Nigeria.

Conclusion

In conclusion, young female children 12 - 15 years old in Nnewi are at risk of HPV IgM infection and since there is low knowledge and poor attitude of the disease in the area, it is recommended that there is an urgent need for government intervention programme, awareness, genetic type screening and vaccine adoption in the area to avert possible spread and increase in chances of cervical cancers in females in the area.

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