Role of Platelets, Bacterial Vaginosis, Serum Interleukins and TNF In Genital Human Papillomavirus Infection Among Black Female Population in Nigeria

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Abstract

The study was aimed at evaluating the relationship between platelets count, bacterial vaginosis and immune response in cervical human Papillomavirus positive women in Bayelsa State. Cervical smears were collected from apparently healthy subjects attending clinics in Bayelsa State. Consensus primers GP5+/6+ and MY09/11 were used to detect HPV DNA in cervical smears and further subjected to multiplex PCR for molecular typing of HPV subtypes. Nugent method was used for scoring of bacterial vaginoses based on gram staining reaction. Interleukins were studied using sandwich ELISA kits. The prevalence of bacterial vaginosis among HPV positive, HPV negative and high-risk HPV was 35.9% and 18.2% and 51.7% respectively. Bacterial vaginosis expressed statistically significant relationship with high risk HPV (OR=9.64, P=0.002, 95%CI= 1.08-86.26).Interleukin 4 (IL-4), IL-6, IL-8, IL-10, IL-12, IL-17, IL-18, Tumor Necrotic Factor (TNF) and Interferon Gamma (INF-γ) were equally higher in HPV positive participants compared with negative participants and statistically significant. There exists a statistically significantly decrease in platelets count (227.8±57) of HPV positive participants compared with negative participants (259.3±57), (X2=6.8, P=0.01) as well as neutrophils count of 45.2±8.5 of HPV positive compared with negative participants (50.6±8.4), (X^2=2.9, P=0.01).White blood cell count, monocytes and packed cell volume were equally higher in positive cases compared with negative cases. Bacterial Vaginosis showed a strong relationship with high risk human Papillomavirus, indicating that a healthy vaginal flora will not only protect a woman from the acquisition of sexually transmitted viruses but also high-risk HPV. Interleukins participate in HPV infection and association interferon levels were depleted in HPV infected participants. Thrombocytes and neutrophils are cellular mediators in genital HPV infection. It is recommended therefore that women exposed to high-risk HPV should be screened for bacterial vaginosis, with concomitant estimations of levels of platelets as well as inflammatory cytokines.

Introduction

Estimated population of 47 million women aged 15 years and above are at risk of developing cervical cancer worldwide. There are reported rising incidence of cervical dysplasiain Nigeria according to the information center on HPV and cancer based on various

studies done in population in Nigeria [1]. Cell growth, function and differentiation, steering immunological response and inflammatory processes are initiated by certain biological molecules known as cytokines [2]. These cytokines and chemokines are secreted by keratinocytes which are either activated or suppressed by the immune system as they participate in human Papillomavirus infection. Certain cells like the dendritic cells, Langerhans cells, natural killer cells, and natural killer T-helper cells among others are also involved in promoting immune response against HPV infection [3]. A delicate balance exists between pro-inflammatory and anti-inflammatory cytokines and their disturbances as reported in many acute disease conditions like pyrexia, rheumatic disorders and cancers. In histological sections, cervical Papillomatosis is characterized by marked keratinocyte hyperproliferation, dense inflammatory infiltrate consisting of T-helper cells and neutrophils, vascular dilatation and proliferation.

The primary defect in Papillomatosis patients is believed to be abnormal thickening of epidermal cells [4]. Genetic variations in IL-6, IL-8 or IL-10 polymorphisms is associated with the risk of cervical cancer. Also, circulating levels of cytokines IL-2, IFN-a ß, IL-2, IL-8, and IL-10 are useful in identifying women at higher risk of developing cervical invasive cancer, LSIL or HSIL, and risk of metastasis. Circulating or tissue levels of IL-6, IL-8 and IL-10 serve as additional markers in the prognosis of patients with advanced stage disease and may help the decision-making processes [2]. Platelets disorders have been reported in certain viral infection like human immunodeficiency virus where surface glycoprotein gp120 causes an increase in megakaryocyte apoptosis in vitro due to increased TGFb and down regulation of the proliferation, inducing TNF super family member 13(TNFSF13). The envelope protein gp120 also interacts with CD4, which is expressed by immature megakaryocyte expressing CCR5 in infectious state [5]. Zapata, et al. [6] documented that in dengue virus infection, platelet production is impaired by suppression of megakaryopoiesis via infection of hematopoietic progenitor cells or indirectly via altered cytokine levels in the bone marrow due to impaired stroma cell function.

Iannacone, et al. [7] and Pozner, et al. [8] reported that in Arena virus infection by either Lymphocytic Choriomeningitis Virus (LCMV) or Junin virus results in thrombocytopenia and decreased platelet responses in mice. Lutteke, et al. [9] reported that Hantavirus' directly interact with and activate platelets via GPIIb/IIIa'. Infection of megakaryocyte with Hantavirus induces the up regulation of Human Leukocyte Antigen (HLA) class 1 molecules on the megakaryocyte surface leading to cytotoxic T-lymphocyte-mediated destruction of megakaryocyte. Influenza and other viruses possess enzymes which can modulate platelet functions and promote neuraminidase which causes hydrolyses of the terminal sialic acid residues from host cell receptor thereby decreasing the life span of platelets, by targeting platelets for rapid clearance in the liver and spleen. Metcalfpate, et al. [10] and Singh, et al. [11]

documented that human parvovirus do not has the ability to reproduce itself in megakaryocyte, but rather triggers a downward activation in platelet count via platelet activation. Therefore, the need to establish the effect of genital HPV on platelets count, interleukins and bacterial vaginosis among women in Bayelsa State.

Materials and Methods

Study Population/ Sampling

Fifty apparently health persons attending two hospitals in Bayelsa State participated in the study. Snowball and convenient sampling were the sampling methods adopted. Cervical-vaginal smears samples collection was done under aseptic technique; cervical smears were collected using Cytobrush and disposable speculum and swab stick respectively. Smears were made and the remaining stored in phosphate buffer used for DNA extraction for HPV detection and identification. Also, 6mls of venous blood was equally collected from each participant and dispensed into EDTA containers for blood count and 4mls into plain containers for interleukins estimation.

Interleukins

The serum circulating interleukins (IL-4, IL-6,IL- 8, IL-10, IL-12, IL-17, IL-18), interferon gamma (INF-) and TNF mean concentration were assayed using reagents from Elabscience(R), a USA-based company and in accordance with the method of Nnodi, et al. [12]. The process required multi-step washing, rinsing and soaking. The ELISA kits use sandwich ELISA principle. The Standards sera and the test samples were added to appropriate micro ELISA wells impregnated with a capture antibody. Then a biotinylated detection antibody specific for the interleukin and avidin Horseradish Peroxidase (HRP)conjugate was successively added and incubated at 37^0c. Color (blue) was developed using substrate reagent to form enzyme-substrate complex. The enzyme-substrate reaction was terminated with a stop solution and appeared yellow in color. The Optical Density (OD) was measured using a spectrophotometer at 450nm using Opsys DYNEX technologies serial NO: PMRA-2976 ELISA micro plate reader. Optical Density (OD) was proportional to the serum interleukin concentration in each well.

The serum interleukin mean concentration was calculated comparing the optical density of the test serum sample with the standard curve. Standard working solution of different concentrations in serial dilution gradients (1000, 500, 250, 125, 62.5, 31.25, 15.63 and 0 pg. /ml were prepared. Then 100uL of each concentration was added into the two wells side by side and 100uL of each serum was added into duplicate wells side by side. The ELISA wells were covered and incubated for 90min. at 37^0C. Excess liquid on each well was removed without washing and immediately, 100uL of biotinylated detection antibody working solution was added into the wells, covered and gently mixed and incubated for another 1hr. at 37^0C. The wells were decanted and 350uL of wash

buffer added to each well, soaked for 2min, decanted and pat-dried followed by addition of 100uL of HRP conjugate working solution to each well, covered and incubated for 30min. at 37^0C. The wells were decanted and washed with 350uL wash buffer, soaked for 5min decanted pat dry for 5 times. Substrate reagent (90uL) was added to each well, covered and incubated for 15min. at 37^0C. with maximum protection from light. Thereafter, 50uL of the stop solution was added to stop the reaction. The optical density of each well was determined at once with a micro plate reader set to 450nm.

Blood Count

Blood count of the participants was assayed using automated hematology analyzer in accordance with the method of Schapkaitz and Levy [13]. The blood count analysis was based on the principle of electrical impedance. The impedance changes as the blood passed through the orifices. The change in impedance is proportional to cell volume, resulting in cell count and volume. The impedance analysis returns blood count in three parts white blood cell differentials (granulocytes, lymphocytes and monocytes) but unable to distinguish between similarly sized granular leucocytes: eosinophil, basophils and neutrophils. The procedure involves passing 2ml of venous blood collected from each participant into EDTA containers into an orifice attached to the machine. The EDTA blood container was vortexed and passed through a tiny orifice in the automated hematology analyzer and the machine sucked an aliquot of the blood sample in the container and within 30 sec. results were displayed on the screen and were printed.

Bacterial Vaginosis

The methodologies of Nugent, et al. and Mendoza, et al. [14,15] were adopted. The principle of the reaction is based on Gram staining reaction. A score of 0 to 3 large gram-positive rods was considered normal, 4 to 6 rods was classified as abnormal vaginal flora (or intermediate) and 7 to 10 was indicative of bacterial Vaginosis. A sterile swab stick was used to collect the samples from the vagina of the participants to make a thin smear. The smears were air-dried and protected from contaminants and then fixed in absolute ethanol for 15min. The fixed smears were flooded with crystal violet stain for 1min and rapidly washed away with distilled water. They were drained and flooded again with Lugol's iodine for 1min, decolorized rapidly (for 2secs) with acetone and washed immediately with clean water and covered with safranin stain for 2min. The excess stain was washed off with water, allowed to drain and air-dried and examined microscopically first with X40 objective to check staining and then X100 oil immersion objectives to report bacteria rods, yeast cells, pus cells and epithelial cells.

Multiplex PCR Protocol

Seventeen (17) HPV genotypes (6, 11, 16L 16U, 18, 30 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66) were studied using the method of Nistiwaki, et al. [16] in a single tube multiplex PCR. Multiplex polymerase chain reaction is a technique by which different samples of DNA are amplified in one single reaction and used for detection and identification of large number of mutation or organisms depending on their molecular size. This process amplifies DNA in a sample using multiple primers, 2X Master Mix and a temperature-mediated DNA polymerase in a thermal cycler. Optimization of the primers was carriedout to allow the primers work at the same annealing temperature. The PCR mixture included 2x multiplex master mix (Taq polymerase, dNTPs, $MgCl_2$) forward and reverse primers at concentration of 0.1μl each and 3.0ul of the extracted DNA. Nuclease-free water of 6.92μl was used to make up to the PCR mixture of a final volume of 30μl. Each PCR was carried out in a DNA thermal cycler (Gene Amp PCR system 9700, Singapore) in the following conditions; initial denaturation step at 95°C for 5 min, 40 cycles of denaturation step at 94°C for 30 sec, annealing at 65°C for 60 sec and initial extension at 72°C for 90sec with final extension at 72°C for 5min. The PCR products were analyzed by electrophoresis on a 2% agarose gel stained with ethidium bromide with band sizes estimated by comparison with 100bp molecular marker (Quick-load DNA molecular ladder, NECO England Biolabs Inc.).

Result

Serum interleukins mean concentration was high in women with cervical HPV and statistically significant, compared with HPV negative women. Interleukin 4 (IL-4), IL-6, IL-8, IL-10, IL-12, IL-17, IL-18 while Tumor Necrotic Factor (TNF)) and interferon gamma (INF-) were equally lower in HPV positive participants compared with negative participants and was statistically significant in either reduction or increases in their mean concentration as seen in (Tables 1 & 2). Also, bacterial vaginosis expresses statistically significant relationship with high risk HPV (OR=9.64, P=0.002, 95%CI= 1.08-86.26) but no statistically insignificant relation with HPV DNA positive women. Furthermore, blood count (Red blood cells, White blood cells count, platelets count, mean cell hemoglobin concentration, hemoglobin, packed cell volume, percentage neutrophils, lymphocytes and monocytes among HPV positive participant and HPV negative participants were studied. There exists a statistically significantly decrease in platelets count (227.8±57) of HPV positive participants compared with negative participants(259.3±57), (X^2=6.8, P=0.01) as well as neutrophils count of 45.2± 8.5 of HPV positive compared with negative participants (50.6±8.4), (X^2=2.9, P=0.01). Again, there was no statistical relationship in mean red blood cells (4.4±0.5 vs. 4.5±0.5) of HPV positive and HPV negative subjects. Mean cell volume (84.7±6.2 vs. 86.8±6.6), % lymphocytes (44.4±7.3 vs. 45.0±8.3) in HPV positive compared with negative participants showed no significant association. The white blood cell count, monocytes and packed cell volume were equally higher in positive cases compared with negative cases.

Table 1: Multiplex Primers Synthesis.

S/no	HPV Primer	Forward and Reverse primer sequence	Size (bp)	Length	GC %	Tm⁰C
1	pp x6/F	GCTAAAGGTCCTGTTTCGAGGCGGCTA	263	27	55.56/55.56	69.16/69.16
	PPx6/R	GGCAGCGACCCTTCCACGTACAAT		24	58.33/58.33	67.98/67.98
2	pp x 11/F	GCGTGTTTTGCAGGAATGCACTGAC	472	25	52/52	66.22/66.22
	pp x 11R	TGCGTCTTGTTTGTCCACCTTGTCC		25	52/52	66.22/66.22
3	pp x 16L/F	CGCACAAAACGTGCATCGGCTACC	217	24	67.08/67.08	58.33/58.33
	pp x16L/R	TGGGAGGCCTTGTTCCCAATGGA		23	56.52/56.52	66.33/66.33
4	PPx 16U/F	TCCTGCAGGTACCAATGGGGAAGAGG	397	26	57.69/57.69	69.32/69.32
	PP x16U/R	TGCCATACCCGCTGTCTTCGCTTT		24	54.17/54.17	66.28/66.28
5	PPx18/F	AACAGTCCATTAGGGGAGCGGCTGGA	187	26	57.69/67.69	69.32/69.32
	PP x18/R	TGCCGCCATGTTCGCATTTG		21	57.14/57.14	64.52/64.32
6	ppx30/F	ACGTAGACGAAAACGGGCCTCTGCT	249	25	60/60	69.5/69.5
	ppx30/R	GGCCTAGCAGGGGATGCGTCCACA		24	66.67/66.67	71.4/71.4
7	Ppx31/F	GCGGTCCAAACGCTCTACAAAACGCACT	360	28	53.57/53.57	69.01/69.01
	PPx31/R	GCAGGGGCACCAACATCAACAATTCCA		27	51.85/51.85	67.64/67.64
8	pp x33/F	ACACAGAGGCAGCCCGGGCATTGTTT	139	26	57.69/57.69	69.32/69.32
	ppx33/R	CACGGGTTTGCAGCACGATCAACA		24	54.17/54.17	66.28/66.28
9	ppx35/F	CCATAACATCGGTGGACGGTGGACAGG	434	27	59.26/59.26	70.88/70.88
	PP x35/R	CCATTACATCCCGTCCCCTCCCCTTCA		27	59.26/59.26	70.68/70.68
10	PP x39/F	CCGACGGAGTGTCCCTGGACCATCTTA	229	27	59.26/59.26	70.68/70.68
	PP x39R	CCAGCGTTTTTGGTTCCCTTACCCCGTA		28	53.57/53.57	69.01/69.01
11	PP x45/F	TGTTGGACATCACACCTACCGTGGA	205	25	52/52	66.22/66.22
	PP x 45/R	TCCGTACCTGACCCAGAAGATGCAA		25	52/52	66.22/66.22
12	pp x51/F	CAACTAGCAACGGCGATGGACTG	299	23	56.52/56.52	66.33/66.33
	pp x51/R	CTGCTTCGCGGGCTGACTAGAA		22	59.09/59.09	66.4/66.4
13	pp x 52/F	GGTGTTGGTGCTGGTGCTTTTGCTA	517	25	52/52	66.22/66.22
	PP x52/R	CAGTTACAGGGGGACGAATGGTGGA		25	56/56	67.87/67.87
14	pp x56/F	TGTTGTTTTTCCGCCATTTTGTACATGCAACC	330	32	40.63/40.63	65.93/65.93
	PP x 56/R	TGGCCTACATAGTGTATTCTGCAAGCCAAAAC		32	43.75/43.75	67.21/67.21
15	pp x58/F	ACCACCGAGGCCACCAACAACGAAAGT	128	27	55.56/55.56	67.21/67.21
	PP x58/R	CGTGGTCTACTGTCCACGGCGCAGTCT		27	62.96/62.96	72.2/72.2
16	pp x59/F	CCGAGCAAGACACCTAAGACAGCAACG	169	27	55.56/55.56	69.16/69.16
	PP x59/R	TCGGAGTCGGAGTCAGGTAATTGCT		25	52/52	66.22/66.22
17	PP x 66/F	GCGGGCGGCTCCTACCTCTTCCTCTTC	277	27	66.67/66.67	73.71/73.71
	PP x66/R	CCACCTAACCTGACACACACTGCCCAAGG		29	58.62//58.62	71.69/71.69

Note: Primary Source: NCBI gene bank. (http//www.ncbi.nih.gov/genbank). Inqaba biotechnical industries (pty) Hartfield, South Africa. GC%= guanine-cytosine percentage expressed as minimum and maximum, Tm= melting temperature. R= reverse primer, F= forward primer.

Table 2: Relationship between interleukins expression and cervical HPV infection among participants studied.

parameters	Hpv negative (n=11)	Hpv positive n= (39)	t- test	df	p. value
IL-4	3.0±0.067	3.9±0.120	6.6	48	<0.0001***
IL-6	4.8±0.061	14.0±0.037	78	48	<0.0001***
IL-8	13.0±0.027	36.0±0.035	420	48	<0.0001***
1L-10	2.5±0.058	16.0±0.100	110	48	<0.0001***
IL-12	25.0±0.054	37.0±0.046	110	48	<0.0001***

IL-17	7.7±0.034	8.6±0.068	12	48	<0.0001***
IL-18	33.0±0.044	36.0±0.035	35	48	<0.0001***
IFN -ɣ	0.98±0.043	0.46±0.005	59	48	<0.0001***
TNF	26.0±0.026	11.0±0.046	29	48	<0.0001***

Note: IL=interleukins, IFN- γ=interferon gamma, TNF= Tumour necrotic factor, HPV =cervical human papillomavirus, value expressed as Mean±SD in pg/ml, **statistically significant differences observed, p< 0.01.

Discussion

Pro-inflammatory and anti-inflammatory cytokines expressions were observed in this present study. This present study observed a statistically significant increase in the various interleukins studied, IL-4, IL-6, IL-8, IL-10, IL-11, IL-18, and TNF while IFN and IL-12 showed a statistically significant increase expression among HPV positive subjects compared with HPV negative participants. Previous reports have shown a relationship between interleukins and some disease conditions and therefore the need to evaluate their presence or absence in cervical HPV. Increased circulating IL-4 and IL-10 as observed in the current study among HPV positive women is in concordance with previous works [17-19]. Shekari, et al. [20] had linked IL-4 (Rp1/Rp2) gene polymorphism to double the risk of cervical cancer in which case HPV was a major risk factor in their study. Furthermore, Serum IL -10 was statistically significant in women with HPV infection compared with negative subjects in our present study and is in agreement with Feng, et al. [21] who reported that increase in serum levels of IL-10 was observed in women with advanced cervical cancer and high-grade cervical intraepithelial neoplasia. Local levels of IL-10 in cervico-vaginal secretions were observed to have increased in women with HIV and HPV associated with cervical intraepithelial neoplasia(CIN) [22-24] as well as IL-10 polymorphism have been reported and correlated with some immunological disorders and cervical cancer [20,25]. Elevated serum levels of serum IL-6 was reported in the current study among women with cervical HPV infection compared with HPV negative participants. Several authors have reported high circulating IL-6 in both pathological and physiological conditions [26]. Also, Bustamam, et al. [27] had reported IL-6 relationship with cervical carcinogenesis and cervical intraepithelial neoplasia (CIN) which preceded to cervical cancer in an animal model. He reported a significant correlation between local cervico-uterine and serum levels of IL-6 with increasing grades of CIN and metastasis. High levels of IL-6 have been observed in cervico-vaginal washings and in serum of individuals with intraepithelial neoplasia and cancer of the cervix in accordance with Tavares-Murta, et al. and Tjiong, et al. [28,29]. Similarly, serum circulating IL-8 level shows increased mean concentration in women with HPV infection in our present study. High levels of IL-8 have been reported in previous works related to lung, colorectal, breast, brain, liver, bone and gynecological cancers [30-32]. Also, Wu, et al. [2] documented that patients with cervical cancer whose cervical biopsy expresses IL-8 were likely to have lymph node metastasis. Baker, et al. [33] also documented increased plasma levels of circulating IL-8 in elderly women with persistent HPV infection. Interleukin-12 (IL-12) appears to be immuno-protective in cervical HPV in the present study. The present study reported a statistically significant reduction in the level of IL-12 expression in HPV positive participants compared with negative participants. Yang, et al. [18] reported that reduced expression of IL-12 in cervical biopsy specimens from invasive cancer cases. Souza, et al. [34] also reported higher serum level of IL-17 expression in LSIL patients as seen in the present study. Liu, et al. [35] has established an association between Tumor Necrotic Factor (TNF) polymorphism with increased risk of cervical cancer. Interestingly TNF-a gene polymorphism can increase or decrease the susceptibility to cervical cancer depending on whether it is TNF-a- allele or TNF-a- is altered [36] wealso observeda decreased circulation of TNF among HPV positive participant. Parmar and Plantains, [37] has reported on the immunoprotective nature of interferon and that they can be defective and deficient in cervical cancer. The present study observed a defective interferon among HPV positive women as seen in (Table 3). According to Boccardo, et al. [38] interferon has been evaluated in experimental and clinical studies for immune function capabilities. IFN therapy has been used in patients with cervical intraepithelial neoplasia to cure or arrest of the cervical lesion progression [38,39]. HPV interfere with the protective action of IFNs at several levels and allow escape of HPV virus from immune degradation or clearance [3, 35, 40]. Gillet, et al. [41] optioned that Bacterial Vaginosis (BV) is characterized by a depletion of Lactobacillus species and a concurrent overgrowth of anaerobic bacteria and the presence of potentially pathogenic bacteria which are most frequently detected in the vaginal tract. In our present study the prevalence of bacterial vaginosis among HPV positive, HPV negative and high-risk HPV were 35.9% and 18.2% and 51.7% as shown in Table 4 and Table 5 respectively and agrees with previous works. Pamela, et al. [42] has reported a 31.5% prevalence of bacterial vaginosis among indigenous Paraguayan and is in accordance with our finding in HPV infection. Also, previous studies have reported 47.2%, 43.7% and 15.2% respectively [43-45] respectively. Furthermore, the present study recorded a statistically significant relationship between high risk HPV and BV (P<0.02; OR =9.46; 95% CI, 1.08-86.26) and lack of statistically significant relationship between HPV in general and BV(P<0.46; OR= 2.5; 95% CI=,0.47-13.4). Gillet, et al. [41] performed a meta-analysis, in which twelve eligible studies were selected to review the association between BV and HPV infection and a strong relationship was established [41]. Also, in the work of Nardis, et al. [46] the overall estimated odds ratio showed a positive association

between BV and cervical HPV infection (OR= 1.43; 95% CI=1.11-1.84). This magnitude of association between Bacterial Vaginosis (BV) and HPV infection has varied in epidemiological studies and still remains controversial, yielding a whole lot of conflicting results and ranging from absence of any association [45] to a clear positive relationship [41] in accordance with our findings.

Table 3: Association between bacterial vaginosis and HPV DNA.

Nugent score	HPV+	HPV-	0R	p value	95% CI
0-3(N)	20(51.3)	08(72.7)	0.39	0.3	0.09-1.7
4-6(IN)	04(10.3)	01(9.0)	1.14	1	0.11-11.4
7-10(BV)	14(35.9)	02(18.2)	2.5	0.46	0.47-13.4
Total	39(100)	11(100)			

N = Normal smear, IN = Intermediate smear, BV = Bacterial Vaginosis, HPV+ = Human Papillomavirus positive, HPV- = Human Papillomavirus negative, OR = Odds Ratio, 95% CI = 95% Confidence Interval

Table 4: Association between Bacterial Vaginosis and High-Risk HPV.

Nugent score	HR-HPV+	HPV-	OR	p value	95% CI
0-3(N)	8(33.3)	8(72.7)	0.26	0.14	0.05-1.2
4-6(IN)	03(12.5)	01(9.0)	0.46	0.58	0.07-3.3
7-10(BV)	13(54.7)	02(18.2)	9.64	0.02*	1.08-86.26
Total	24(100)	11(100)			

N = Normal smears, IN = Intermediate smear, BV = Bacterial Vaginosis, HR-HPV+ = high risk human Papillomavirus positive, HPV- = Human Papillomavirus negative, OR = Odds Ratio, 95% CI = 95% Confidence Interval, * Significant association observed between Bacterial Vaginosis and High Risk HPV, p< 0.05.

Table 5: Full blood count of participants studied.

Haematological parameters	HPV positive	HPV negative	X2	p.value	0.05
Red Blood Cell count (103/mm)	4.4±0.5	4.5±0.5	0.4	0.5	ns
White Blood count (106/mm)	6.5±1.2	5.5±1.6	2.6	0.1	ns
Platelets count	259.3±57	227.8±57	6.8	0.001	***
Mean Cell Haemoglobin (pg)	27.3±2.5	27.2±2.6	1	0.74	ns
Mean Cell Volume	84.7±6.2	86.8±6.6	1.1	0.3	ns
MeanCell Haemoglobin Con.(%)	32.2±1.2	32.6±1.4	0	0.87	ns
Haemoglobin (g/dl)	12.87±1.5	12.3±2.1	0	0.88	ns
PCV (%)	39.5±4.3	38.5±4.1	0.4	0.49	ns
Neutrophils (%)	50.6±8.5	45.2±8.3	2.9	0.001	***
Lymphocytes (%)	44.4±7.3	45.0±8.3	3	0.08	ns
Monocytes (%)	9.5±4.5	6.3±2.6	0.6	0.42	ns

Full blood count evaluated in our current study showed a statistically significant reduction in platelets among HPV positive subjects as well as neutrophils (Table 5) Thrombocytopenia in response to viral infections is often multifactorial. Thrombocytopenia in viral hepatitis is caused by platelet specific glycoprotein antibodies [47] and immune complexes form bound to platelet surface to cause platelets destruction [48]. Coagulation, inflammation, and platelet activation play a role in HCV-induced decrease of platelet count. HCV also indirectly affects megakaryopoiesis [48,49]. The mechanism of platelets destruction in HPV might be linked to inflammatory response. In conclusion bacterial Vaginosis showed a strong relationship with high risk human Papillomavirus, indicating that a healthy vaginal flora will not only protect a woman from the acquisition of sexually transmitted viruses but also high-risk HPV. Interleukins participate in HPV infection and association interferon levels were depleted in HPV infected participants. Thrombocytes and neutrophils are cellular mediators in genital HPV infection. It is recommended therefore that women exposed to high-risk HPV should be screened for bacterial vaginosis, with concomitant estimations of levels of platelets as well as inflammatory cytokines.

Conflict of interest: There is no conflict of interest

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