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

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Abbreviations: TNF: Tumor Necrotic Factor, LCMV: Lymphocytic Choriomeningitis Virus, OD: Optical Density, HRP: Horseradish Peroxidase, BV: Bacterial Vaginosis

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 vaginosis 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), (X²=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 dysplasia in 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 Papillomatisos is
characterized by marked keratinocyte hyperproliferation, dense
inflammatory infiltrate consisting of T-helper cells and neutrophils,
vascular dilatation and proliferation.

The primary defect in Papillomatisos 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-α,
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 TGFβ 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.
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
GPⅠb/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-γ) 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 anti body. Then a biotinylated detection
antibody specific for the interleukin and avidin Horseradish
Peroxidase (HRP)-conjugate was successively added and incubated
at 37°C. 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°C. 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°C. The wells were decanted and 350uL of wash
buffer added to each well, soaked for 2 min, decanted and pat-dried followed by addition of 100μL of HRP conjugate working solution to each well, covered and incubated for 30 min at 37°C. The wells were decanted and washed with 350μL wash buffer, soaked for 5 min, decanted and pat-dried for 5 times. Substrate reagent (90μL) was added to each well, covered and incubated for 15 min at 37°C with maximum protection from light. Thereafter, 50μL of the stop solution was added to stop the reaction. The optical density of each well was determined at once with a microplate reader set to 450 nm.

**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 2 ml 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 15 min. The fixed smears were flooded with crystal violet stain for 1 min and rapidly washed away with distilled water. They were drained and flooded again with Lugol’s iodine for 1 min, decolorized rapidly (for 2 secs) with acetone and washed immediately with clean water and covered with safranin stain for 2 min. 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 carried out to allow the primers work at the same annealing temperature. The PCR mixture included 2x multiplex master mix (Taq polymerase, dNTPs, MgCl₂) forward and reverse primers at concentration of 0.1μl each and 3.0μl 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 90 sec with final extension at 72°C for 5 min. The PCR products were analyzed by electrophoresis on a 2% agarose gel stained with ethidium bromide with band sizes estimated by comparison with 100 bp molecular marker (Quick-load DNA molecular ladder, NEOC 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 participant (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), (X²=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 % | Tm°C |
|------|------------|-------------------------------------|----------|--------|------|------|
| 1    | pp x6/F    | GCTAAAGGTCCTGTTGGACGCGGCTA         | 263      | 27     | 55.56/55.56 | 69.16/69.16 |
|      | PP x6/R    | GGCAGGACCCCTCCCACTGCAAAT           | 24       | 58.33/58.33 | 67.98/67.98 |
| 2    | pp x 11/F  | GGAGTTTGGAGGCAAATGCGACTGAC         | 472      | 25     | 52/52 | 66.22/66.22 |
|      | pp x 11/R  | TGCTCTTGGTGGACGCTAATGAGGGCGG       | 25       | 52/52 | 66.22/66.22 |
| 3    | pp x 16L/F | GGCAGAACGCTCAGATGGGGGAAGGG         | 217      | 24     | 67.08/67.08 | 58.33/58.33 |
|      | pp x 16L/R | TGGAGGGGATCCGGCTTTCCTAAATG         | 23       | 56.52/56.52 | 66.33/66.33 |
| 4    | PP x 16U/F | TCTGCGAAGTCAAGATGGGGGAAGGGAGGG    | 397      | 26     | 57.69/57.69 | 69.32/69.32 |
|      | PP x 18/F  | AAAGTCTATTGGGAGGAGGGAGGGTGGGA     | 187      | 26     | 57.69/57.69 | 69.32/69.32 |
|      | PP x 18/R  | TGGCCGCTATTGGGAGGAGGGTGGGA        | 21       | 57.14/57.14 | 64.52/64.32 |
| 5    | pp x30/F   | AGTACAGAACAGAGGGCGGCTCTGCTTCT     | 249      | 25     | 60/60 | 69.5/69.5  |
|      | PP x30/R   | GTCGCTACAGAGGGCGGCTCTGCTTCT       | 24       | 66.67/66.67 | 71.4/71.4  |
| 6    | Ppx31/F    | GGGCTCAAAAGGCTCTACTAAAGGCCACT     | 360      | 28     | 53.57/53.57 | 69.01/69.01 |
|      | Ppx31/R    | GCAGGCGACCAAAACATGAAATCTTCCCA     | 27       | 51.85/51.85 | 67.64/67.64 |
| 7    | pp x33/F   | ACACAGAGGCGACGACGGCGGATGTGTTT     | 139      | 26     | 57.69/57.69 | 69.32/69.32 |
|      | PP x33/R   | GACGGCGTCTAGACGACGACGACGACGACT    | 24       | 54.17/54.17 | 66.28/66.28 |
| 8    | pp x35/F   | CATAAACATCGTGAGGGGAGGGCTAGG       | 434      | 27     | 59.26/59.26 | 70.88/70.88 |
|      | PP x35/R   | CCCTACTGACAGGAGGAGGAGGAGGAGG     | 24       | 59.26/59.26 | 70.68/70.68 |
| 9    | Ppx35/F    | GGGCTCAAAGAGGCTCTACTAAAGGCCACT    | 360      | 28     | 53.57/53.57 | 69.01/69.01 |
|      | Ppx35/R    | GCAGGCGACCAAAACATGAAATCTTCCCA     | 27       | 51.85/51.85 | 67.64/67.64 |
| 10   | pp x39/F   | CACAAGGCGACGACGGCGGCTTTCTTCTTTT  | 229      | 26     | 57.69/57.69 | 69.32/69.32 |
|      | PP x39/R   | GCAGGCTTTGGATTGCTTACCTCCTGGGA     | 24       | 54.17/54.17 | 66.28/66.28 |
| 11   | Ppx45/F    | TTCTTGCAACATCACCTAAGCGTTGGA       | 205      | 25     | 52/52 | 66.22/66.22 |
|      | Ppx 45/R   | TCTGCTTACAGGAGGAGGTCAAAGGAGG     | 24       | 52/52 | 66.22/66.22 |
| 12   | pp x51/F   | CAACCTGACAGGACGACGGCTTTCTTCTT    | 299      | 23     | 56.52/56.52 | 66.33/66.33 |
|      | PP x51/R   | CTCTGCAGGTGTTGCGGAGTACTGAGA       | 22       | 59.09/59.09 | 66.4/66.4  |
| 13   | pp x 52/F  | GTTGTTGAGTGTGTTGTTGTTGTTGTTGTA   | 517      | 25     | 52/52 | 66.22/66.22 |
|      | PP x52/R   | CAGTACAGGAGGACGAGTTGAGGG        | 25       | 56/56 | 67.87/67.87 |
| 14   | pp x56/F   | TGTGTTGTTGTTGCGCATTGTTATGCACTCACCA   | 330      | 32     | 40.63/40.63 | 65.93/65.93 |
|      | PP x 56/R  | TGCCCTACATAGTGTATCTGGACGCAAAAC   | 32       | 43.75/43.75 | 67.21/67.21 |
| 15   | pp x58/F   | ACCAGGACCGGACCCACAAACAGCAAGGAT   | 128      | 27     | 55.56/55.56 | 67.21/67.21 |
|      | PP x58/R   | CAGGGCTACTGCTAGGCGGCGGCTTCTT    | 27       | 62.96/62.96 | 72.2/72.2  |
| 16   | PP x59/F   | CGAGCAAGACACACAGACAGAAGG         | 169      | 27     | 55.56/55.56 | 69.16/69.16 |
|      | PP x59/R   | TGCAATGAGGGAGCTAGGTTGCTTCT      | 25       | 52/52 | 66.22/66.22 |
| 17   | PP x66/F   | GGGGCGGTCCTCTATCTCCTCTGCTTCT    | 277      | 27     | 66.67/66.67 | 73.71/73.71 |
|      | PP x66/R   | CCACCTAATCGACACGACTGCCCCAAGG    | 29       | 58.62/58.62 | 71.69/71.69 |

Note: Primary Source: NCBI gene bank. (http://www.ncbi.nlm.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*** |
| IL-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*** |
| Cytokine | Mean ± SD | P Value |
|----------|-----------|---------|
| IL-17    | 7.7 ± 0.34 | <0.0001*** |
| IL-18    | 33.0 ± 0.04 | <0.0001*** |
| IFN-γ    | 0.89 ± 0.04 | <0.0001*** |
| TNF      | 26.0 ± 0.02 | <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]. Shokari, 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 cervicovaginal 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 cervicovaginal 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 observed a 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- | OR  | 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       | 0.11-1.14 |
| 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±6.5      | 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 platelet 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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