

**LAGOS STATE UNIVERSITY 5TH FACULTY OF SCIENCE
(LASU FOSC) CONFERENCE, 2017**



ORAL PRESENTATION

**CO-INFECTION OF EPSTEIN-BARR VIRUS, HERPES SIMPLEX
VIRUS TYPES- 1 AND 2 IN PATIENTS WITH AND WITHOUT
SEXUALLY TRANSMITTED DISEASES IN LAGOS, NIGERIA**

Anjorin, AbdulAzeez. A. ^{1}, Oyefolu, Akeeb. O. B. ¹, Salu, Olumuyiwa. B. ², Akinyemi,
Kabir. O. ¹, Omilabu, Sunday. A. ²*

¹ Department of Microbiology, Lagos State University, Ojo, Lagos, Nigeria.

*² Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, Idi-Araba,
Lagos, Nigeria.*

**Correspondence: abdul-azeez.anjorin@lasu.edu.ng, +234 8020956357.*



INTRODUCTION

- Epstein-Barr virus (EBV), Herpes Simplex Virus (HSV) types-1 and 2 are ubiquitous and contagious, oncogenic, creeping DNA viruses (WHO, 2016).
 - in the family Herpesviridae.
- They establish lifelong latency in human causing acute and chronic severe diseases.
- According to the WHO, 2017, HSV-2 alone affects 356 million people globally, causing oral and genital diseases
 - while EBV causes infectious mononucleosis (Looker *et al.*, 2012; WHO, 2017).



INTRODUCTION



Etymology (*Greek Herpes- Creeping*).

There are 8 *Herpes Viruses* (HHVs) in that family infecting Human:

HHV- 1 (Herpes Simplex Virus 1)

HHV- 2 (Herpes Simplex Virus 2)

HHV- 3 (Varicella Zoster Virus)

HHV- 4 (Epstein- Barr Virus)

HHV- 5 (Cytomegalovirus)

HHV- 6 (Herpes Lymphotropic Virus)

HHV- 7 (Human Herpes Virus 7)

HHV- 8 (Kaposi)

INTRODUCTION





INTRODUCTION

- Herpesviruses are the most common viruses in humans infecting 80–90% of the global population (Slots, 2009).
- Infections in Africa and other developing areas are characterised by primary exposure in early childhood.
 - Due to certain cultural practices, than in the developed countries (WHO, 2016).
- While the exact status of EBV is usually determined by specific markers against viral capsid antigen (VCA),
 - transmission of EBV and HSV pose both economic and public health risks (Kriebs *et al.*, 2008).



STATEMENT OF PROBLEM

- The **major problem is that EBV and HSV shares common transmission pattern** of intimate contact with the fluid of an infected person (Bowden *et al.*, 2006).
- EBV and HSV infections and/ **co infections pose both economical and public health risk**, genital HSV is dangerous to neonate and women at child bearing age.
- Unfortunately, there is **dearth of epidemiological data** on EBV, HSV and their co-infection **in Nigeria**.



AIM OF STUDY

- Therefore, this study was instituted to investigate IgM marker to EBV, HSV-1 and 2 and their co-infection in patients.



METHODOLOGY

- **STUDY DESIGN, SAMPLE SIZE, LOCATION, PERIOD AND APPROVAL**

- We designed hospital-based, basic, cross-sectional study, based on the sample size bearing in mind estimation method:
 - $N = Z^2 p (1 - p) / d^2$, first developed by Kish, Leslie in 1965 where Z= reliability coefficient= 1.96 at 95% confidence interval.
- in order to generate epidemiological data for future prospective sentinel surveillance.
- This study was carried out among patients attending 5 public health institutions (Agbado-ljaye, Ebute-Metta, Ikorodu, Ikotun and Surulere) in Lagos.
 - Between June and October, 2016.
 - Ethical approval was obtained from CMUL/ LSMH while all patients consented.



METHODOLOGY

- **STATISTICAL ANALYSES**

- Socio-demographic distribution of patients were computed with Microsoft excel sheet using Windows 2013.
 - Based on patients demographics on marital status, all patients were grouped into 3, as either single, married or divorce.
- Chi-square and Fisher's exact tests were used to calculate P-values for comparable epidemiological parameters using Graphpad 5.0, San Diego, USA.
 - Level of significance was tested at 95% CI.
- A total of 90 blood samples were collected by venepuncture from patients
 - with and without sexually transmitted diseases.



METHODOLOGY

- **LABORATORY ANALYSES**

- The assay was performed at the Virology Research Laboratory, Department of Microbiology, Lagos State University
 - under a locally designed Biological Safety Cabinet.
- The samples were analysed for IgM specific markers against
 - EBV-VCA
 - HSV-1 and 2
 - Using Enzyme Immunoassay (Dia.pro, Italy).
- Analysis was carried out following the manufacturer's procedure. Briefly (figure i),

Samples were diluted with specimen diluent in ratio 1: 101, mixed and vortexed.

100ul each of negative control, calibrator in duplicate, and positive control were dispensed into proper wells, leaving a blank.

100ul samples were added into the sample wells. All were checked to be blue coloured before incubating at +37°C for 60min.

100ul of Ag/Ab immunocomplex was added and covered with the sealer. All were checked to be red coloured, except the blank.

The microplate was again incubated at +37°C for 60min, after which, it was thoroughly washed.

100ul Chromogen/Substrate mixture was added into each well including the blank well before incubating at 18-24°C for 20minutes.

100ul sulphuric acid was dispensed into all the wells which turned the positive control and positive samples from blue to yellow.

The colour intensity of the solution in each well was measured at 450nm filter (reading) and at 620nm. **>10arbU/ml= positive**



RESULTS

- The demographic characteristics of the patients showed mean age of patients to be 31.5 years (median age, 30 years; range 18-50 years)
 - including 28 males and 62 females, giving female preponderance with male: female ratio of 1 : 2.21.
 -
- The most prominent age group was 21- 30 years with 41 (45.6%) number of patients recruited.



RESULTS

- The results revealed that 51 (56.7%) of the patients were positive for EBV, HSV-1 and 2, comprising of:
 - 34 (37.8%) to EBV,
 - 12 (13.3%) to HSV-1 and 2, and
 - remarkable co-infection of EBV, HSV-1 and 2 detected in 5 (5.6%) of the patients.
- Statistical testing showed a significant difference ($p < 0.05$) between EBV, HSV-1 and HSV-2 seropositivity.
- Fifty (50) percent of the individuals positive to HSV types-1 and 2 had a common clinical manifestation of painful urination.

RESULT...1



EPIDEMIOLOGICAL DATA ON VIRAL AETIOLOGIES, CO-INFECTION AND SEX PARAMETER

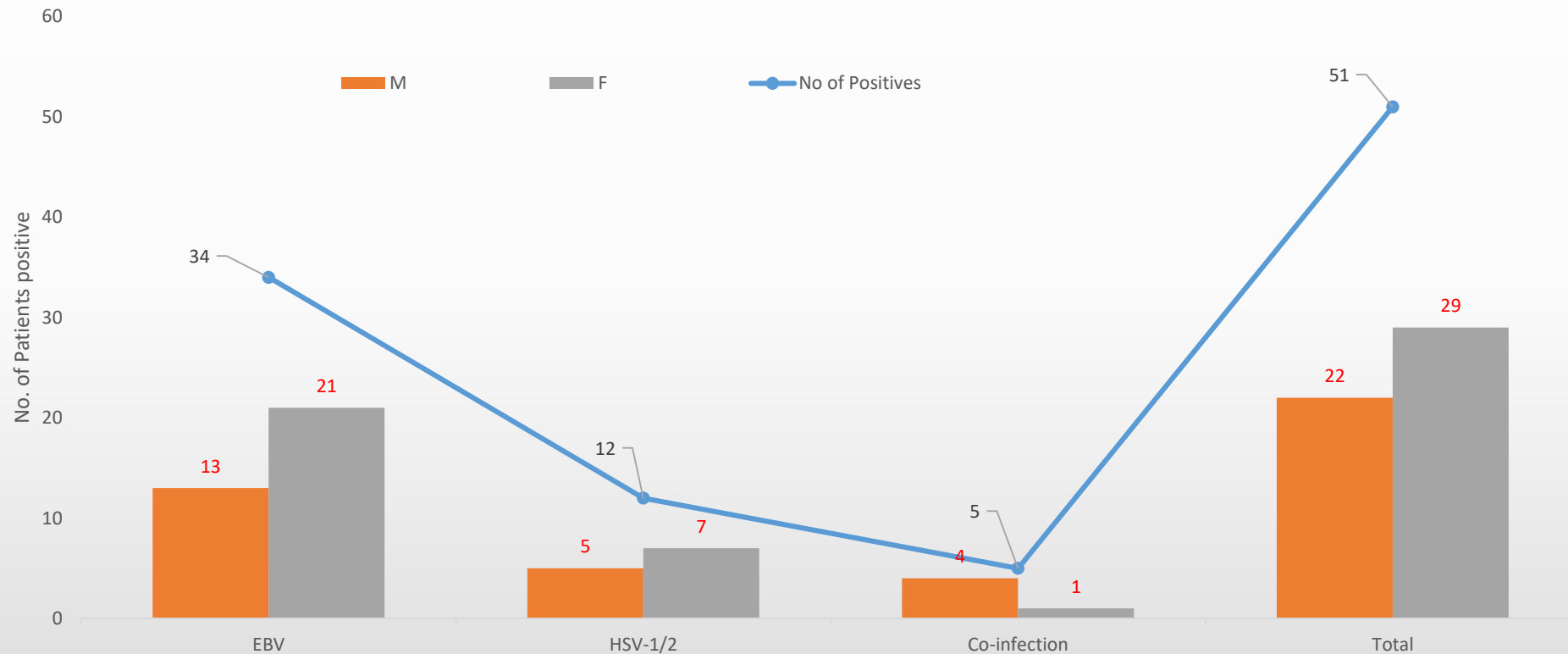
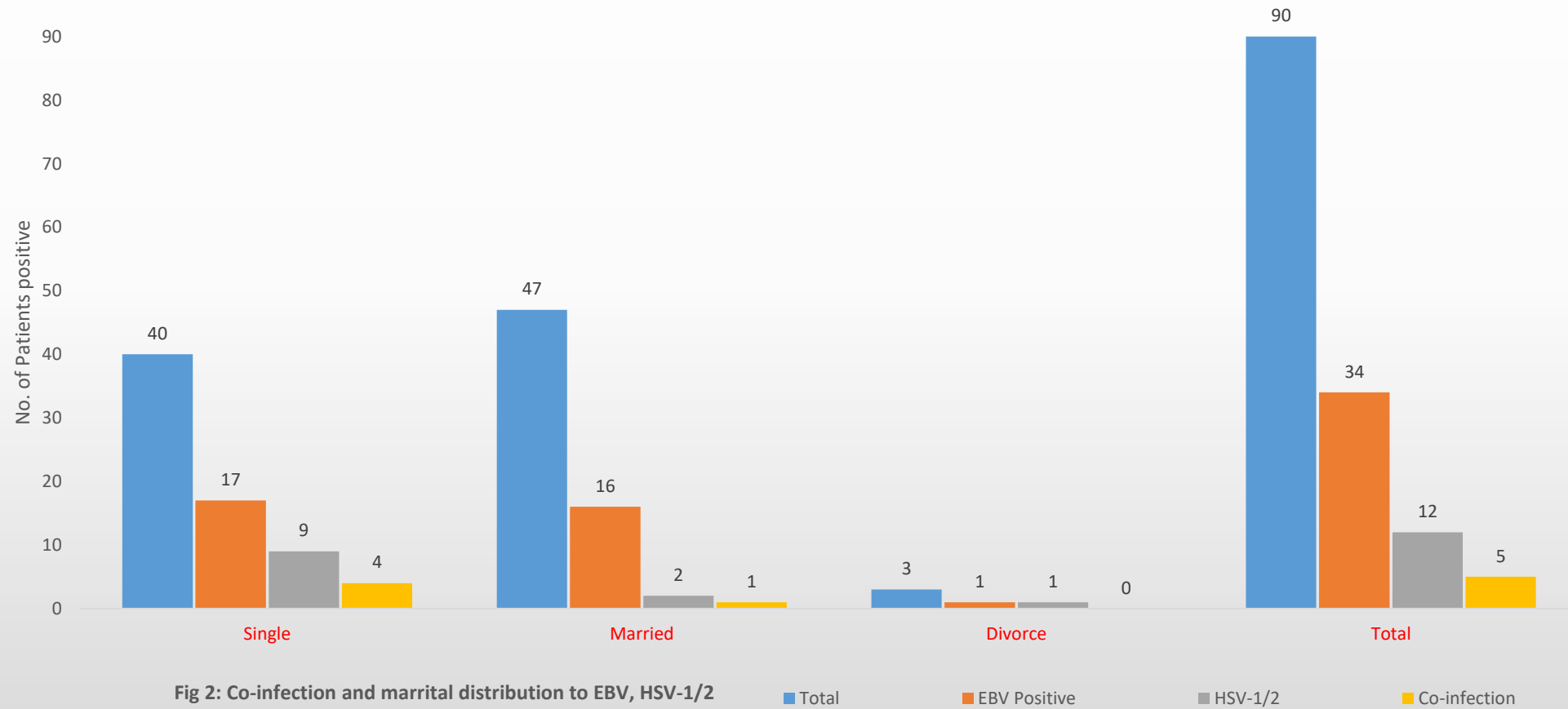


Fig 1: Co-infection and sex distribution to EBV, HSV-1/2

RESULTS...2



EPIDEMIOLOGICAL DATA ON VIRAL CO-INFECTION AND MARRITAL STATUS



RESULTS...3



EPIDEMIOLOGICAL DATA ON VIRAL CO-INFECTION AND AGE CLASSICAL INTERVAL

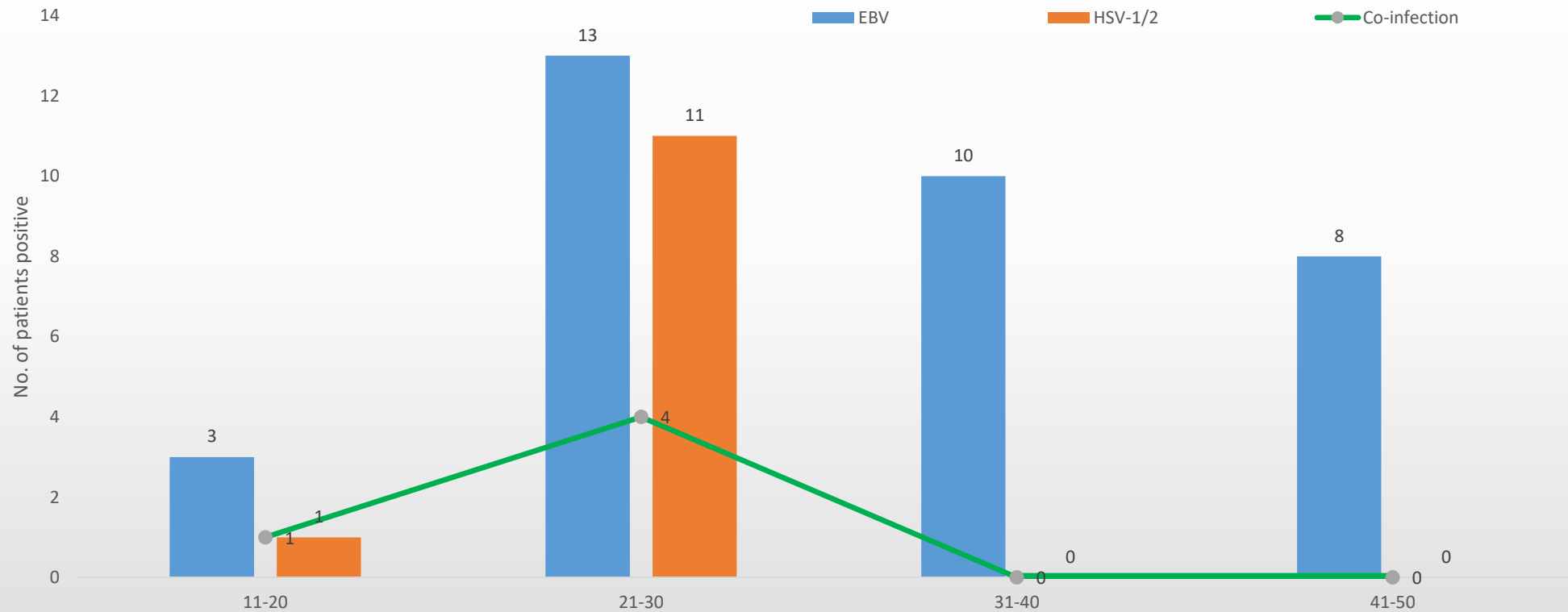


Fig 3: Co-infection with Age distribution to EBV, HSV-1/2

RESULTS...4



EPIDEMIOLOGICAL DATA ON VIRAL CO-INFECTION AND PATIENTS CLINICAL PRESENTATIONS

Table 1: Clinical Signs and symptoms observed in seropositive patients to EBV, HSV-1/2 in Lagos, Nigeria

Signs/ Symptoms	No. of cases	EBV pos.	HSV-1/2 pos.	Total pos.	Co-infection
Blister	18	4	2	6	1
Painful urination	16	12	6	18	3
Sensation/Itching	19	7	1	8	-
Vaginal discharge	8	3	2	5	-
Rashes	8	4	0	4	-
Painful groin	5	2	1	3	1
Others	14	2	0	2	-
Total	94	34	12	46	5



DISCUSSION

- Again, EBV and HSV types 1 and 2 are viruses of serious public health importance. Unfortunately, there is rarity of information in Nigeria on this menace.
- The finding of 51 (56.7%) seropositivity to EBV, HSV-1 and 2 calls for serious concern.
- 37.8% (n=34) to EBV with a wide variation to 7.7% (n= 5) in Amazon, Brazil reported by Guimarães et al., 2012.
 - But this is comparable to studies in 8 other countries in Africa and other continents with
 - 75% (n= 15) in Sudan; 40% (n= 8) in USA; 80% (n= 16) in UK;
 - 71% (n= 12) in Sweden; 70% (n= 14) in Norway; 45% (n= 9) in India;
 - 35% (n= 7) in Sri Lanka; and 22% (n= 4) in Yemen.
 - Sample type and detection method and other factors may however contribute to the differences.



DISCUSSION

- We reported a monotypic infection of HSV-1/2, 13.3% (n= 12).
- This is higher than that of previous study by Oke *et al.* (2012) on HSV-2 in the same location of Lagos that reported a prevalence rate of 9.7%.
 - It shows an increment of approximately 4% in the prevalence rate within 4 years with a given annual increment of 1% which is worrisome.
 - Elsewhere in Nigeria, Abdulfatai *et al.* (2013), reported that 99.2% of people living in Kaduna metropolis had past infection against HSV-1
 - Okonko and Tochi (2015) showed that 99.4% of the pregnant women attending antenatal clinic were seropositive for HSV-1 IgG.



DISCUSSION

- In other countries, in Amazon, Brazil, Guimarães et al., 2012 reported HSV-1, 7.7% ; HSV-2, 16.9% (n= 11).
 - In Sudan, 15% (n= 5) was reported by Jalouli *et al.*, 2012.
 - Also, in developed countries, 55% (n= 11) in Sweden; 12% (n= 2) in Norway; and 20% (n= 4) in the UK.
 - While in developing countries, 10% (n= 2) was reported in Sri Lanka; and 11% (n= 2) in Yemen.



DISCUSSION

- Co-infection causes more severe and debilitating effects thereby compounding the health status of the individuals.
 - Notably for haematologic abnormalities: mononucleosis, thrombocytopaenia and anaemia.
- Remarkable co-infection of EBV, HSV-1 and 2 was detected in 5.6% (n=5) of patients.
 - This result is higher than that of the study by Sinniah *et al.*, 1993 that reported 1 co-infection in Singaporean and British patients.
 - But much lower than that of Rodrigues *et al* (2010) who reported a prevalence of HSV and EBV of over 30%.



DISCUSSION

- Also, previous studies by Jalouli *et al.*, 2012 reported co-infection of EBV and HSV of:
 - 30% (n= 6) in the UK;
 - 12% (n= 2) in Sweden; and
 - 15% (n= 3) in Norway.



CONCLUSION

- This study bared **recent infection** of EBV, HSV-1 and 2, and their **co-circulation** in patients which may further worsen the disease state of the individuals **especially in** those having sexually transmitted diseases.
- We therefore **recommend routine screening of these viruses** in our public health centres.



***LAGOS STATE UNIVERSITY 5TH FACULTY OF SCIENCE
(LASU FOSC) CONFERENCE, 2017***