MOLECULAR EPIDEMIOLOGY AND CHARACTERIZATION OF K1, K15P AND ORF75 GENES OF HHV-8 ASSOCIATED WITH HIV/AIDS KAPOSI'S SARCOMA IN PATIENTS AT KENYATTA NATIONAL HOSPITAL

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REG. NO: W80/96747/2014

THESIS SUBMITTED TO THE UNIVERSITY OF NAIROBI IN FULFILLMENT FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN TROPICAL AND INFECTIOUS DISEASES.

2020

DECLARATION

This thesis is my original work and has not been presented for the award of any other degree or to any other University.

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Title of the Work: Molecular epidemiology and characterization of K1, K15 (P) and ORF75 genes of HHV-8 associated with HIV/AIDS Kaposi's sarcoma in patient's at Kenyatta National Hospital.

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DEDICATION

I am grateful to the Almighty God for the blessings in undertaking this work. I would like to dedicate this thesis to my parents Mr. and Mrs. Ong'injo Demba, dear wife Dr. Sylviah M. Aradi, two sons Emmanuel Kyle Demba, Milan Lloyd Demba and my siblings Lyndon O. Demba, Golda A. Demba, Nelly A. Demba, Godfrey C. Demba and Nobert E. Demba for their unwavering support, patience and encouragement.

ACKNOWLEDGMENTS

I would like to thank my supervisors Prof. Walter Mwanda and Prof. Matilu Mwau for their invaluable support, continuous guidance and unlimited advice during the research work and thesis writing. They took their precious time to supervise me ensuring I complete my thesis.

I would like to thank the University of Nairobi, Institute of Tropical and Infectious Diseases for giving me a chance this pursue Ph. D degree.

To Kenyatta National Hospital (KNH), thank you for issuing me with ethical approval letter that enable me collect my data, in addition would like to extend my gratitude to Cytology department for allowing me to use their archived tissue blocks.

To Director KAVI- Institute of Clinical Research Prof. Omu Anzala and Biozeq laboratory specialist Mr. George Warui Gachuhi thanks for allowing me to use the Institute's molecular laboratory for my research.

To my Dad and Mum, thanks for setting me on this noble journey in the medical field and challenging me to go beyond the skies. You provided a foundation on which I have been able to advance in this challenging field.

Finally, a special thanks to my wonderful family, my wife, Dr. Sylviah M. Aradi, two sons Emmanuel Kyle Demba, Milan Lloyd Demba for their continued love, encouragement and support during this journey.

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LIST OF ABBREVIATIONS AND ACRONYMS

ACTG	AIDS Clinical Trial Group
AIDS	Acquired Immunodeficiency Syndrome
AIDS-Ks	Acquired Immunodeficiency Syndrome-Associated Kaposi's sarcoma
ART	Antiretroviral Therapy
cART	Combinedantiretroviral therapy
DNA	Deoxyribonucleic Acid
dsDNA	Double stranded Deoxyribonucleic Acid
ECs	Endothelial cells
ELISA	Enzyme-linked Immunosorbent Assay
EMMPRIN	Extracellular Matrix Metallo PRoteinase Inducer
H & E	Haematoxyline and Eosin
HIV	Human immunodeficiency Virus
HAART	Highly Active Antiretroviral Therapy
HHV-8	Human Herpes Virus 8
IFA	Immunofluorescent Assay
IL	Interleukin
IRIS	Immune Reconstitution Inflammatory Syndrome
KNH	Kenyatta National Hospital
KSHV	Kaposi's sarcoma herpes virus
KS	Kaposi's sarcoma
LANA	Latency-Associated Nuclear Antigen
m-TOR	mammalian Target of Rapamycin

MCD Multicentric Castleman's Disease

MMPS Matrix metalloproteinases

MSM Men who have sex with men (MSM)

ORF Open Reading Frame

OR Odds ratio

PBMCs Peripheral Blood Mononuclear Cells (PBMCs),

p53 Tumor protein

PCR Polymerase Chain Reaction

PBS Phosphate Buffer Saline

RB Retinoblastoma

RNA Ribonucleic Acid

UNITID University of Nairobi Institute of Tropical & Infectious Diseases

USA United State of America

VEGF-A Vascular Endothelial Growth Factor-A

VEGF Vascular Endothelial Growth Factor

WHO World Health Organization

OPERATIONAL DEFINATION

Alleles: Are variants forms of a given gene or copies of a gene that differs from each other.

Epidemiology: Is the branch of medicine which deals with the incidence, distribution, and possible control of diseases and other factors relating to health.

Characterization: Is the description that involves distinguishing traits or features of someone or something into kinds, classes or categories

Gene: Is a unit of heredity information made up of complex molecules that codes genetic information for the transmission of inherited traits.

Characterization of gene: Is distinguishing inheritable traits containing DNA sequences.

Molecular characterization: The DNA aberrations that are detected in a sample from a patient with the disease in question.

Genotyping: Is the process of determining difference in genetic makeup by examining DNA sequences and comparing with reference sequences.

Molecular epidemiology: A branch of epidemiology and medical science that focus on genetic typing at molecular level, to the etiology, distribution and prevention of disease across a given population.

Molecular genetics: It is the field of biology that studies the molecular structures and functions of genes, involving the DNA.

Phylogenetic analysis: It is to determine the hierarchial relationship that exists between species and the estimation of their divergence overtime based on their DNA sequence.

Phylogenetic tree: It is a branching diagram showing evolutionary relationship that exist among species based on their similarities and difference in genetic characteristics.

ABSTRACT

Background: Kaposi's sarcoma is a lifelong persistant infection in human once exposed. Immunosupression due to HIV sets foundation for Kaposi's sarcoma herpesvirus infection. Kaposi's sarcoma overcomes cellular intrinsic immunity to initiate viral protein expression. The strength of the current study is that it brings new knowledge in understanding the biology of KS disease based on the expression of KSHV genes K1 and K15P which are known to modulate immune responses during disease progression and ORF75 which enhances lytic viral replication.

Objective: To describe the molecular epidemiology and characterization of the selected HHV-8 genes associated with AIDS-KS at Kenyatta National Hospital, Kenya.

Materials and methods: This was a hospital based descriptive cross-sectional study. The quantitative approach of data collection, analysis and presentation was used. The cases were traced following histological reports diagnosed as KS or KS-like between January 2013 and December 2016. Consecutive sampling technique was used to pick the cases from Kenyatta National Hospital. In total, 81 cases that met the inclusion criteria were studied. The laboratory test used to study the cases was histology, polymerase chain reaction and sequencing. A comprehensive bio-medical data linking the blocks was obtained from the registry records. The Statistical Package for Social Sciences (SPSS) version 21.0 where Logistic regression analysis, chi-square and t-test was used. A p value less than 0.05 was considered statistically significance.

Results: Of the 81 cases studied, mean age was 39.3 (SD=9.5) years, range 19-63 years, mode 34 years, and median 38 years. Majority (48.2%) of the studied cases were in the age group of 30-39 years. Males were 46 (56.8%) and females 35 (43.2%). The ratio of males to females was 1:0.8. The mean difference in age between male and female cases was 2.9 years, t=1.3, p=0.10. The 81 studied cases, 49 (60.5%) had nodular KS lesions, while 19 (23.5%) and 13 (16.0%) were plaques, and patchy lesions respectively. Both K1 and K15P gene was demonstrated in 42 (91.3%) males and 30 (85.7%) females, OR=1.7(0.4-7.0), p=0.42. The ORF75 gene was identified in 28 (60.9%) males and 21 (60.0%) females, OR=1.0 (4.2-2.5), p=0.93.

Discussion: During the height of HIV and AIDS, KS gained public attention and the manifestation of the lesions caused a lot of stigmatization among the affected patients. The introductionof antiviral therapy globally led to reduction in the number of KS cases, although KS is stilled observed in people with well-controlled HIV infection. In the current study, Kaposi's sarcoma herpesvirus was highly prevalent in the studied cases who also had HIV. Although KS is associated with HIV and AIDS, KSHV has an infectious origin that is independent of HIV. A combination of KSHV and impaired host immunity causes Kaposi's sarcoma. The findings on age and gender are different from published reports on the molecular epidemiology and characterization of KSHV. In the current study, the cases that had Kaposi's sarcoma were young adults and male preponderance observed. The current study findings on molecular epidemiology and characterization showed that K1, K15P and ORF75 genes were predominantly among the male cases. The K1, K15P and ORF75 genes of Kaposi's sarcoma overcomes cellular intrinsic immunity to initiate viral protein expression and genome replication in a primary infection. The nucleotide sequences of K1, K15P and ORF75 gene were aligned to other HHV8 gene sequences which have been deposited in the NCBI data bank (http://www.ncbi.nlm.nih.gov).

Conclusion: Molecular epidemiology and characterization results showed that K1, K15P and ORF75 genes of KSHV were highly present in the studied cases. The nodular morphological subtype of KS was also predominant.

Recommendation: Molecular technique should be adopted for the testing of KSHV because it permits better detection of the virus.

CHAPTER ONE

INTRODUCTION

1.1 Background

1.1.1 Molecular epidemiology and characterization of Kaposi's sarcoma herpes virus

Kaposi's sarcoma herpesvirus (KSHV) is an infectious cause of neoplasm that is also known as Human herpesvirus 8 (HHV 8) (Moody and Laimins, 2010). Molecular characterizations of Kaposi's sarcoma herpes virus that involve sequences of a whole-genome are very few. The first genome sequence to be produced was obtained from a Greek patient who had classic KS lesion (Rezaee *et al*., 2006). The three other whole genome sequences of Kaposi's sarcoma herpes virus to be characterized were produced from a patient who had KSHV with primary effusion lymphoma (PEL) cell lines (Yakushko *et al.,* 2011; Brulois *et al.,* 2012). The sixth Kaposi's sarcoma herpes virus genome to be characterized following successful sequencing was produced using IIIUmina next-generation sequencer and was obtained from an infected patient's plasma (Tamburro *et al.,* 2012). Several studies (Hayward and Zong, 2007; Leao *et al.,* 2013), have reported that it is a rare occurrence to witness co-infection by dissimilar human herpes virus 8 (HHV 8) genotype.

Analysis on highly variable regions by use of sequencing technique has permitted the identification of seven major subtypes or the genotypes of Kaposi's sarcoma herpes virus. Molecular epidemiology of these genotypes of KSHV includes genotype A, B, C, D, E, F, and Z with all showing distinct ethnic and geographical clustering (de Souza *et al.,* 2007; da Silva *et al.,* 2011; Borges *et al.,* 2012; Isaacs *et al.,* 2015). According to Boshoff and Weiss, (2001) different genotypes of KSHV are distributed worldwide but no record shows that they have

dissimilar pathogenesis. It was observed by Etta *et al.*, (2018) that in Africa, 29.3% of KSHV studies were conducted on genotypic analysis while 9.5% were on both sero-epidemiology and genotypic analysis. In addition, Etta *et al.*, (2018) recorded that approximately 38% of the countries in Africa had molecular epidemiological data on K1 genotypes A, A5, B, C, F and Z, while data on K15 genotypes comprised of P, M, and N at 23%. Molecular characterization of HHV 8 is by genetic variability and this is diagnostically done through the whole genome. The molecular epidemiology of KSHV that entails genotypic classification includes A, A5, B, C, D, E, F and Z on the hyper variable region of K1 gene while genotype P, M, and N are on the K15 gene (Isaac *et al.*, 2016).

According to Kajumbula *et al.*, (2006); Hayward and Zong, (2007); Kanno *et al.*, (2010); Ouyang *et al.*, (2014), sequence analysis of K1, classifies Kaposi's sarcoma herpes virus into seven main molecular types that are epidemiologically distributed as, A, B, C, D, E, F and Z. In addition, Ouyang *et al.*, (2014), noted that epidemiology of the KSHV strains are based on geographic local and ethnic background. Studies (Dupez *et al.*, 2006; Cassar *et al.*, 2007; Cassar *et al.*, 2010) on molecular epidemiology of KSHV mainly concentrated on the K1 region resulting to subtypes A, B, C, D, E showing geographical clustering. In addition, Cassar *et al.*, (2010) reported that the K1 region has two highly variable regions (VR1 and VR2) that are often targeted by the host immune system on the K1 protein. The molecular epidemiology of the K1 gene has been characterized to have five major subtypes that indicate geographical clustering.

Molecular epidemiological study on KSHV that was conducted in Cameroon by Bestem *et al.*, (2014) revealed that there was genetic diversity in subtype A5 of K1 gene but not B of K1.

However, Olp *et al.,* (2015), reported that both K1 and K15 genes are poor surrogates to be used for measuring diversity of a whole KSHV genome. A study conducted in Malawi using molecular techniques such as polymerase chain reaction (PCR) and sequencing, showed that subtype B, A2 and A5 was in circulation on blood and saliva (Beyari *et al.,* 2003). Only one study (Kasolo *et al.,* 1998) has reported the occurrence of genotype Z in Zambia among children. However, all other genotypes of HHV 8 have been reported in adults and children across the African continent. Alagiozoglou *et al.* (2000) noted that it was not clear why the epidemiology K15 genotype N was found to be exclusively restricted in Southern Africa (South Africa and Zambia). Zong *et al.,* (2002) also observed that the molecular epidemiology of KSHV recorded intra-genotypic variants in few countries in Africa but data on inter-genotypic recombination was found to be scanty. In Zambia, molecular characterization of KSHV was conducted by Olp *et al.,* (2015). In that study Olp *et al.,* (2015) sequenced 16 whole genome of Kaposi's sarcoma herpes virus from a KS patient. In addition, Olp *et al.,* (2015), observed that there was a low-level of genetic discrepancy in the central conserve region on the genome. Further to that, upon molecular characterization (Olp *et al.,* 2015) witnessed distinctness in the phylogenetic structure from the Zambian isolates compared to isolates from United States and Greece.

The magnitude of molecular epidemiology and characterization of KSHV gene has been comprehendible following successful genome sequences done by Yakushko *et al.,* (2011); Brulois *et al.,* (2012; Tamburro *et al.,* (2012) in the United State, Olp *et al.,* (2012) in Zambia and Awazawa *et al.,* (2017) in Japan. The association between the clinical advancement of Kaposi's sarcoma and the KSHV genotype still remains unknown (Mancuso *et al.,* 2008; Tornesello *et al.,* 2010; Kouri *et al.,* 2012; Cordiali-Fei *et al.,* 2015; Isaacs *et al.,* 2015). While

certain reports (White *et al.,* 2008; Mancuso *et al.,* 2008; Cordiali-Fei *et al.,* 2015) indicates that clinical advancement of Kaposi's sarcoma have been shown to be linked to HHV 8 genotype A, other studies (Tornesello *et al.,* 2010; Nascimento *et al.,*2005), did not arrive at the same results. Isaacs *et al.,* (2015) in South Africa for example, found out that subtype A5 and B were dominating, and distributed along the ethnic lines. Noteworthy, was AIDS-KS linked to A5 subtype which presented with extensive disease. In addition, Isaacs *et al.,* (2015) noted that both subtype A1 and A4 were connected with lower chances or poor risk of developing tumor extension, and that subtype A1 particularly was associated with lower possibility of involving the lower limbs.

In a study done by Elena *et al.,* (2003) on molecular epidemiology and characterization of Kaposi's sarcoma herpes virus from Russian patients, the K1 gene was sequenced and thirty strains were successfully obtained. These sequences and their phylogenetic analysis as revealed by Elena *et al.,* (2003) showed that the novel KSHV belonged to either subtype A or C and of note was subtype A variants gotten from acquired immunodeficiency disease syndrome associated Kaposi's sarcoma (AIDS-KS). Furthermore, Elena *et al.,* (2003), used PCR-based sub-typing for K14.1 and the findings showed that 23 out of the 32 were novel strains belonging to subtype P. In conclusion, Elena *et al.,* (2003), reported that there was no correlation between K1 molecular subtype and specific Kaposi's sarcoma (KS) type be it epidemic, classic or post-transplant, as well as between K1 gene and K14.1 molecular subtypes.

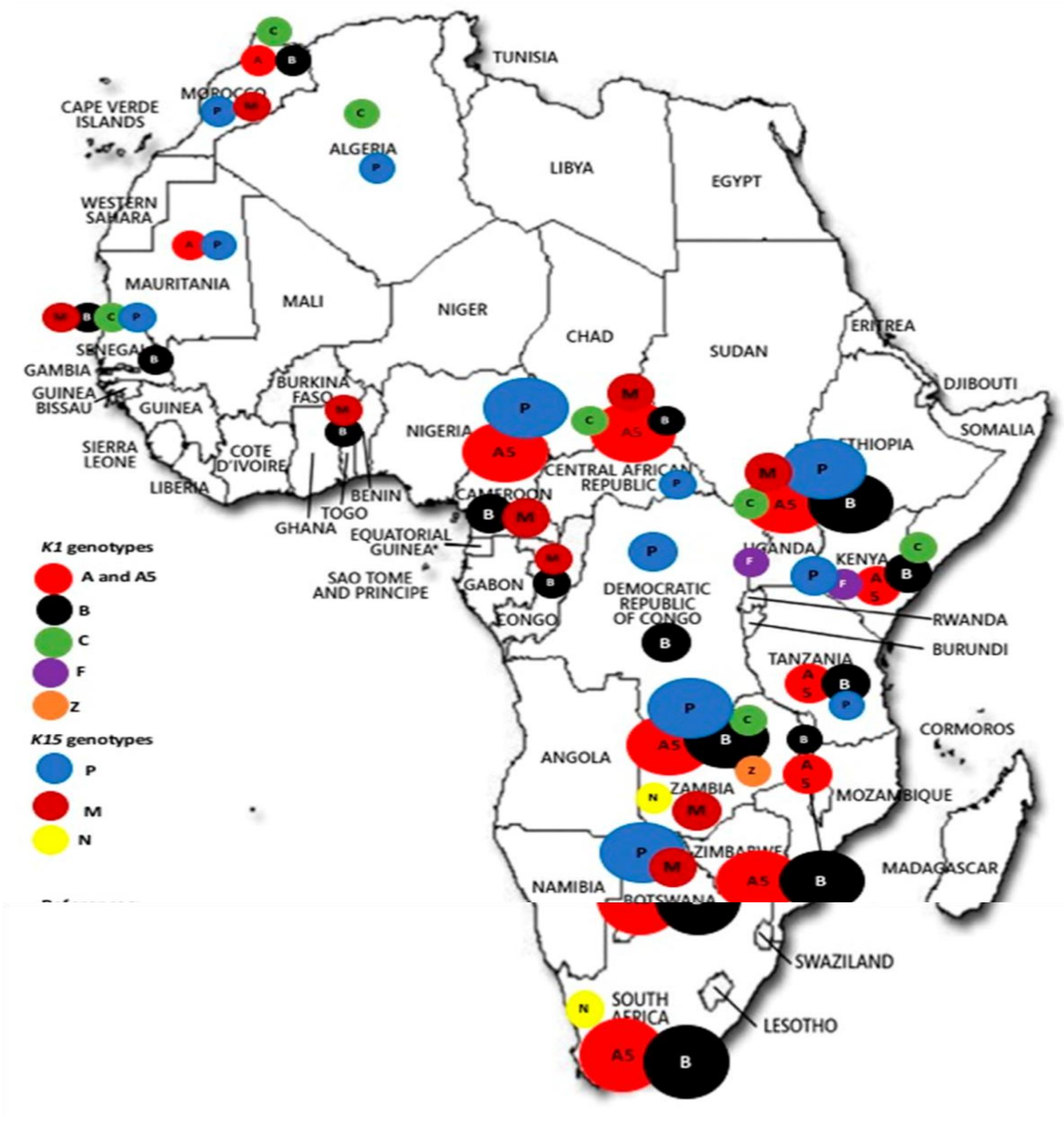


Figure 1.1: Molecular epidemiology of HHV 8 genotypes in African countries from 1998-2017 (Etta *et al*., 2018).

In Ghana after successful molecular characterization, Gobbini *et al.*, (2012) proposed addition of a new genotype (HHV 8-G for Ghana) to the current nomenclature. The successful genotyping of HHV 8 DNA was detected from blood donors whose bootstrap had constituted a 97% distinction. Molecular epidemiology and characterization by Zong *et al.*, (2007) reported that

four (B, N, Q and R) subtypes of KSHV have been found in sub-Saharan Africa. In addition to the molecular epidemiology, Kajumbula *et al.,* (2006) noted that the HHV 8 subtype B was predominant in Africa whereas F was detected in Uganda among the Bantu tribe. Studies (Whitby *et al.,* 2004; Mancuso *et al.,* 2008) on molecular pathogenesis had shown that it was still unclear as to whether the different genotypes of HHV 8 might have different pathogenic and cancer genetic properties linked with the different rates of disease development. To add-on molecular epidemiology and characterization, Tozetto-Mendoza *et al.,* (2016), noted that the distribution of HHV 8 genotypes A, B, C and F in HIV AIDS patients from Brazil was indistinguishable when comparison was made between non-KS and KS group. The K1 of HHV 8 has been categorized into six subgroups (A-F), subtype B and A5 dominating in Africa (Katz and Abera, 2015). The K1 gene encodes for trans-membrane signaling molecule. It was thought that K1 gene play a vital role in the lifecycle of KSHV, prompting cellular activation of endothelial cell immortalization and works synergistically with HIV-1 Tat to stimulate tumor formation (Yao *et al.,* 2015; Zhang *et al.,* 2016).

Inadequate proof existed to a certain that Kaposi's sarcoma herpes virus subtypes may correlate with a particular epidemiological type of KS or a more antagonistic disease (Isaac *et al.,* 2016). The HHV 8 has been shown not to be a ubiquitous virus unlike the Epstein- Bar both whom belong to the γ-herpes virus group (Dedicoat and Newton, 2003). Hayward and Zong, (2007) established that there were genetic variations of HHV 8; they further mapped out the ethno-geographic circulation of the major clades. Figure 1.1 shows molecular epidemiology of HHV 8 genotypes in African countries from 1998-2017 (Etta *et al.,* 2018). The K15 gene is encoded by

3 terminus of the Kaposi's sarcoma herpes virus, in addition, sequences of K15 have additional classification as P, M or N allele (Hayward and Zong, 2007). Furthermore, Figure 1.2 displays the epidemiological proportion of genotype K1 (figure 1.2 A) and K15 (figure 1.2 B) in African countries (Etta *et al.*, 2018). It was recorded as shown in figure 1.2 A that, K1 genotype B was the most prevalent followed by A5. Figure 1.2B displays K15 genotype where P was found to the most prevalent than M in African countries from the year 1998-2017 (Etta *et al.*, 2018).

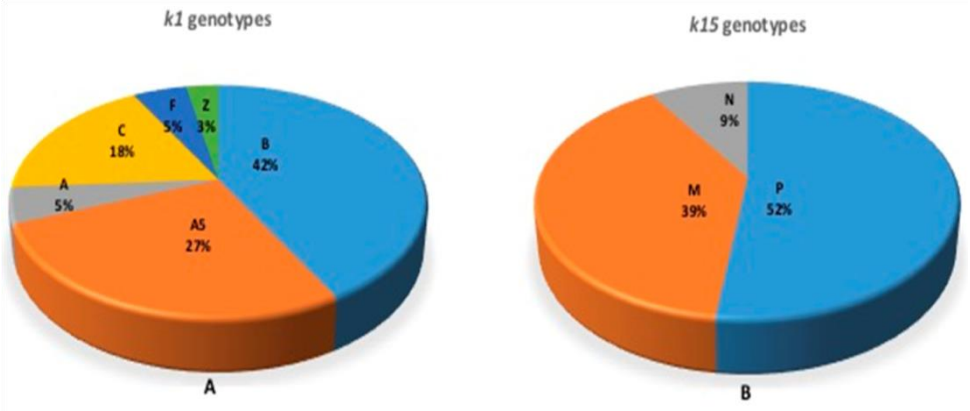


Figure 1.2: Epidemiological proportion of K1 and K15 genes of HHV 8 in Africa (Etta *et al.*, 2018).

Molecular characterization analysis conducted on KSHV from Zambia revealed that there was segregation of the sequences from those in the Western region regardless of the fact that the study included the highly variable genes K1 and K15 (Olp *et al.*, 2015). In addition, Olp *et al.*, (2015), reported that four genes within the conserved region of the whole KSHV genome contained polymorphism. However, they did not contribute to the uniqueness of the Zambian KSHV whole-genome phylogenetic structure. Three allele of K15 have been identified by Poole *et al.*, (1999), as P, M and N, of note is the little variation within the allele group but great

deviation across the alleles. Olp *et al.,* (2015), successfully characterized and identified the N allele from the Zambian samples even though the N allele is highly deviated from the M and P allele. Olp *et al.,* (2015), successfully extracted the DNA of Kaposi's sarcoma herpes virus from human biopsy and the sequence results revealed that there was prominent phylogenetic clustering between the Zambian genomic sequences and those from Western countries. In addition, Olp *et al.,* (2015), observed that the whole-genome of Kaposi's sarcoma herpes virus had greater diversity than what was previously valued.

It was reported by Tiffany *et al.,* (2008) that the K1 gene of the Kaposi's sarcoma herpes virus has a distinctive geographical distribution. Furthermore, upon molecular characterization, Tiffany *et al.,* (2008), noted that genetic diversity in K1 gene subtype B and subtype A5 existed in Zimbabwe. To conclude the study, Tiffany *et al.,* (2008), reported that there was no significant link between the subtypes of K1 gene and the clinical or demographic features witnessed in cases of AIDS-associated Kaposi's sarcoma. The findings of Tornesello *et al.,* (2010), that was done from DNA samples collected in Cameroon, Kenya and Uganda revealed that majority of HHV 8 ORF 26 variant were of R and B subtype. Molecular epidemiology of KSHV as stated by Tornesello *et al.,* (2010), showed that subtype A/C were obtained from European and North European tumor and genotyping of K14/15 loci revealed that P subtype was predominant. In conclusion, Tornesello *et al.,* (2010), reported that AIDS- associated KS showed a significant linkage of the Human herpes virus 8 variants in a specific population which has not changed throughout the AIDS epidemic.

Betsem *et al.,* (2014) reported that, in Cameroon they were able to conduct good phylo-geographic studies on the subtypes epidemiology and polymorphism because it is occupied by a mass of ethnic groups that are divergent in history origin. White *et al.,* (2008) reported that the subtype B strain have gathered more non-synonymous mutations compared to strains from A5 group. In the sub-Saharan Africa, subtype B1-4 and clade A5 were observed to be predominant (Kasolo *et al.,* 2007; Tornesello *et al.,* 2010; White *et al.,* 2008). The molecular characterization in Morocco revealed that, HHV 8 K1 gene belonged to subtype C, furthermore elevated genetic diversity within subtype C was also recorded (Dupez *et al.,* 2006). Molecular studies by Alagiozoglou *et al.,* (2000) and Fouchard *et al.,* (2000) focused on genetic variability and polymorphism of HHV 8 ORF 75 and ORF26 from KS specimens.

The molecular epidemiological studies and the importance of genetic variability of K1 gene have also been published (Biggar *et al.,* 2000; Kadyrova *et al.,* 2003 and Kazanji *et al.,* 2005). Molecular characterization studies by Meng *et al*., (2001); Zong *et al.,* (2002); Whitby *et al*., (2004), identified five subtypes of the K1 gene as A, B, C, D and E. In addition, the molecular epidemiology by Meng *et al*., (2001), Zong *et al.,* (2002) and Whitby *et al*., (2004) recorded that HHV 8 B strain was mainly in Africa, subtype A and C were found in Europe and European-descent residents of from United States of America where us the subtype D was confined to Pacific population. Studies by Meng *et al*., (2001), Zong *et al.,* (2002), and Whitby *et al*., (2004) noted that subtype E was present among Brazilians, French's Guiana and Ecuador. Kaposi's sarcoma herpesvirus has diverse molecular subtype D variants which were found to be endemic among Ni-Vanuatu people in Australia (Olivier *et al.,* 2007). To add-on, Olivier *et al.,* (2007)

stated that most K1 genes isolated were almost identical to Polynesian strains; however, a few had shown clustering features with Australian strains.

According to Hayward and Zong, (2007); Tornesello *et al.,* (2010), the molecular epidemiology and characterization of KSHV subtypes, defined by K1 gene have definite geographical pattern and are distributed globally. Furthermore, Hayward and Zong, (2007); Tornesello *et al.,* (2010), reported that subtypes A and C are from Europe, Asia and United State whereas subtype B and A5 are predominantly from Africa and from Pacific Islands the rare subtype D is exclusively found. Previous reports by Hayward and Zong, (2007); Tornesello *et al.,* (2010), stated that HHV 8 subtype B and A5 are confined to Africa, Kasolo *et al.,* (2007); Zong *et al.,* (2007); White *et al*., (2008), have further reported that they found subtype B and A5 outside Africa.

Cassar *et al.,* (2010), similarly showed a diverse distribution of HHV8 among the KS patients in Peru. Genotypic distribution of AIDS-associated Kaposi's sarcoma herpes virus in Brazil indicated that subtype A, B, C and F were indistinguishable when comparison was made between KS and non-KS group (Tania *et al.,* 2016). It was suggested by Tania *et al.,* (2016); Tozetto-Mendoza *et al.,* (2016), that genotype B might be linked with better tumor prognosis compared to other subtypes. Kourí *et al.,* (2012), evaluated the distribution of Kasposi's sarcoma herpes virus from 1991 to 2009 and linked the variations of the KSHV subtypes from Cuban samples. Kourí *et al.,* (2012), reported that the expansion of subtype B in Cuba might have been due to change in human behavioral pattern.

In Xinjiang, China, a molecular epidemiology and characterization study conducted on the frequency of KSHV genotype from AIDS-KS individual revealed that, strains of genotype A was more than genotype C (Ouyang *et al.,* 2014). Still, it is not well understood whether different KSHV genotype have dissimilar pathogenic and tumorigenic features linked with diverse pathogenesis (Mancuso *et al.,* 2008). Genotype A of KSHV have been shown to be speedily evolving to classic Kaposi's sarcoma (Mancuso *et al.,* 2008). Compared to genotype C, Matteoli *et al.,* (2012), was able to detect genotype A, predominantly A1 variants from saliva which was considered to have high viral load.

It is worth mentioning Hbid *et al.,* (2005); Mseddi *et al.,* (2005) considered the Maghreb region (Northwest Africa or Northern Africa) as highly endemic for KS. Zong *et al.,* (2002) postulated that stain C of KSHV might be a primordial than the C viruses. They further hypothesized that the C branch originated from the Middle Eastern/North African area whereas the current C strain might be from European and North Asian population. Sajad *et al.,* (2017), reported that among the Iranian general population, HHV 8 of genotype C was dominant particularly subgroup C00. In addition, Sajad *et al.,* (2017), stated that, genotype A was common among HIV infected individuals with or without Kaposi's sarcoma. Low prevalence of KSHV has been reported in Iran as well as Western countries (Jalivand *et al.,* 2011), nevertheless, high frequency regions are linked to South America and Africa. Jalivand *et al.,* (2012), further reported that genotype C and A are widely spread in Iran with genotype C being prominent among Iranian population.

Molecular epidemiological and characterization studies (Kanno *et al.,* 2010; Ramos *et al.*, 2011), have shown that certain genotypes are dominant whereas others are more frequent. Genotype A was observed to be frequent among AIDS-associated Kaposi's sarcoma and genotype C was prominent among classic Kaposi's sarcoma patients. Both genotype A and C have been reported to be common in Western countries, whereas, genotype C seems to be more prevalent in Middle East and Asia (Zhang *et al.,* 2008; Kanno *et al.,* 2010; Tornesello *et al.,* 2010). Certain molecular characterization studies (Kanno *et al.,* 2010; Ramos *et al.,* 2011), have shown that genotype C is common among classic Kaposi's sarcoma whereas genotype A is dominant in HIV infected individuals. Studies (Mancuso *et al.,* 2008; Ramos *et al.,* 2011), have also shown that genotype A of HHV 8 was specially distributed at the beginning of AIDS pandemic among the HIV positive patients. High level of genetic diversity and clustering subtypes has been displayed by the genome of Kaposi's sarcoma herpes virus (Hayward and Zong, 2007). In addition, Hayward and Zong, (2007) reported that chimeric genomes and the theory that dissimilar genomes of KSHV segments have dissimilar evolutionary histories.

Historically, genotype B of HHV 8 is thought to have appeared in Africa continent 100,000 years ago whereas D/E strains originated from Pacific Islands approximately 70,000 years ago. Additionally, genotpe A and C appeared in Europe and Northern Asia and later expanded into America over 35,000 years ago (Hayward and Zong, 2007). In the course of studying the genetic diversity of HHV 8 K1 gene using molecular epidemiology, five subtypes of K1 (A-E) associated with geographic origin of the samples were identified. Furthermore, subtype D has been reported among occupants from Western Pacific region (Hayward and Zong, 2007). Molecular characterization studies on K1 genetic variability was also done in Italy, Greece,

Israel and Saudi Arabia by (Zong *et al.,* (2002); Davidovici *et al.,* (2001). Kouri *et al.,* (2007), reported a case of KSHV subtype B from a Cuban sample, apparently, this patient had lived in Mozambique where it is alleged that the patient had heterosexual contact with the native of Mozambique.

1.1.2 Sero-Epidemiology of Kaposi's sarcoma herpesvirus

The epidemiology of HHV 8 worldwide has been shown to be uneven but it tends to trail the countries that have reported high prevalence of HHV8 (Fontana *et al.,* 2014). A conspicuous difference in the sero-prevalence of HHV 8 across different parts of the world can be clearly seen in the general population (de Sanjose *et al.,* 2009). When a correlation analysis was done between HHV 8 sero-prevalence and the risk of developing Kaposi's sarcoma, it was found that men who have sex with men (MSM) were at a higher risk. Furthermore, it was not clear whether the findings would be the same among heterosexual people (Bhutani *et al.,* 2015). According to Curado *et al.,* (2007), Kaposi's sarcoma is infrequently reported to the Cancer registry, apart from cancer registry of Colombia where 0.2 case/100,000 women and sub-Saharan Africa with 20 cases/100,000 women.

Mosam *et al.,* (2008), observed that the age-specific distribution pattern for KS among the females was identical to the HIV pattern. These findings were with regards to age-specific distribution pattern. Further to that, Mosam *et al.,* (2009), reported that, from 1983 to 2006, the age standardized incidence rate of KSHV in South Africa has increased 20-fold in men and 50-fold in women.

About 84% of KS occurs globally (Olp *et al.,* 2015) and both KSHV and KS are endemic in Sub-Saharan Africa (Lisa *et al.,* 2011; Olp *et al.,* 2015). Kaposi's sarcoma herpes virus has been studied globally (Schwartz *et al.,* 2008; Gabni and Whitby, 2009) since it was first discovered in 1994 (Chang *et al.,* 1994). The distribution of KSHV worldwide is heterogeneous and the epidemiological determinants are unalike depending on the endemicity level (Pica and Volpi, 2007). The epidemiology of human herpes virus infection is globally distributed, with a high endemicity ranging between 30-70% in sub-Sahara Africa, intermediate at 5-20% in the Mediterranean and as low as less than 5% in Northern Europe, USA, South-east Asia and Japan which are also a reflection of KS and HIV prevalence (Enbom *et al.,* 2002; Duker and Rezza, 2003).

1.1.2.1 Sero-prevalence of HHV 8 distribution in Europe, Asia and America

In Europe and United States, Kaposi's sarcoma is the commonest cancer among HIV individuals even after the introduction of antiretroviral therapy which was believed to be widely accessible (Shiels *et al.,* 2011; Yanik *et al.,* 2013; Yanik *et al.,* 2013; Gopal *et al.,* 2014). In European population, subtype A1 -4 and subtype C are known to be predominant (Duprez *et al.,* 2006) and some are also found in Asia (Zhang *et al.,* 2008; Cassar *et al.,* 2010). In the United States and Europe, the DNA of HHV 8 have been detected in high rate from saliva among men (Phipps *et al.,* 2014). The sero-prevalence of KSHV in the United State was found to be ranging from 1.5%-7%. This followed alteration that were done on the cut-off point of the detection method that was adopted (Engels *et al.,* 2007). In addition, Engels *et al.,* (2007), stated that there was no clear link between KSHV infection and other sexually transmitted infections. Engels *et al.,* (2007) reported that the sero-prevalence of HHV8 in USA was at 6%. In consistent with Engels

et al., (2007), Anderson *et al.* in 2008 reported that USA has a low sero-prevalence of HHV 8 in children but significantly elevated in certain adult population such as Men who have sex with men (MSM). Sero-prevalence of HHV 8 in the general population of Europe and United State is low at <4% (Engels *et al*., 2007; Mesri *et al.,* 2010).

In comparison to the 1980s and early 1990s, the incidence of KS in Western countries has rapidly reduced to less than tenth of what it used to be. Five years after the onset of AIDS, cumulative incidence was measured and the results showed that in USA recorded a reduction from 14.3% in 1980-1989 to 1.8% in 1996 to 2006 (Simard *et al.,* 2011). It was noted that roughly 29% of all the patients who took part in the KS studies in the USA between the years 1996-2007 had > 300 CD_4 T cells/μl and the HIV plasma viremia was below detection (Krown *et al.,* 2008). The sero-prevalence of KSHV in the United States of America has been reported to be 15-40 folds higher among homosexuals than in the overall population (Anderson *et al.,* 2008).

The sero-prevalence of HHV 8 is less than 5% in Europe and ranges between 50-70% in sub-Saharan Africa (Gobbini *et al.,* 2012). In Germany, five deaths attributed to KS was reported to have arisen from cohort of 230 KS cases diagnosed between the years 2000 and 2014, this was noted in HIV centers (Meyer *et al.,* 2016). The sero-prevalence of KSHV among HIV cohort in Italy was 60% (Suligoi *et al.,* 2003). The sero-prevalence of Kaposi's sarcoma in Spain or Greece was found to be lowest at 6%-8% while in Italy it was highest at 20.4% (Valdarchi *et al.,* 2007; Zavitsanou *et al.,* 2007).

Kaposi's sarcoma herpes virus is not ubiquitous like other viruses belonging to herpes viridae family. The sero-prevalence of KSHV in Asia, West Europe and North America has been reported to be approximately 1-10% (Zang *et al.,* 2014; Ghezeldasht *et al.,* 2015). Biryahwaho *et al.,* (2010) reported that KSHV was endemic in Africa and Mediterranean region with a varied prevalence from 30-50%. Disparity across geographical regions was not found to be significant, however, KSHV infection was considered to be low in America, Asia and Europe with an exception of Mediterranean regions (Tornesello *et al.,* 2010; Stiller *et al.,* 2014; Zhang and Wang, 2017). In Italy and Central part of Africa, KSHV was found to be 50% in the general population and in Western countries MSM were almost exclusively affected (Krown *et al.,* 2008). Kaposi's sarcoma herpes virus of the subtype B is predominantly existing among individuals from African origin (White *et al.,* 2008), to add-on, Hayward and Zong, (2007) reported a case of subtype B from a Mexican female patient who had AIDS and was living in Miami.

Among the elderly population in the Mediterranean, the prevalence of KSHV is intermediate. Furthermore, the sero-prevalence of KSHV was predominant among the people who had grown up in small communities or had the experience of working in the plantations particularly those that had contact with soil. As hypothesized by Mbulaiteye *et al.,* (2005); Ascoli *et al.,* (2006); Whitby *et al.,* (2007), there is possibility that such exposure might affect KSHV replication resulting to an elevated likelihood of infection or sero-conversion.

The sero-prevalence of KSHV in Central Italy was two rimes higher in the rural areas, particularly among the farmers (Valdarchi e*t al.,* 2007). Further to that, it was also reported that the sero-prevalence of KSHV was 4.7 fold higher among the inhabitants of Sardinia who are mostly in the rural than in the Sardinian emigrants in the mainland of Italy habituating the urban region (Angeloni *et al.,* 2006).

According to Wan *et al.,* (2018), reported that in China, individual factors such as race, age, immune status, origin of KSHV infection showed no impact on the development of KS. A study by Zhang *et al.,* (2012) restricted in China, noted that the sero-prevalence of HHV 8 was varied in different geographical sub-regions and ethnic groups and that there was a three times chances of HHV 8 sero-conversion in HIV infected compared with HIV uninfected individuals. In China the sero-prevalence of KSHV differs between regions of the country and was noted by, Mei *et al.,* (2007); Fu *et al.,* (2009); Wang *et al.,* (2011); Cao *et al.,* (2014) to be between 7.3% and 16.1% in adults living in different provinces. Precisely in Xinjiang province of China, Wang *et al.,* (2011); Cao *et al.,* (2014) reported that the sero-prevalence of AIDS associated KSHV was quite high, however, the reason for such geographical and population difference remains to be addressed.

1.1.2.2 Sero-prevalence of HHV 8 distribution in Africa

In Africa, the prevalence of Kaposi's sarcoma herpes virus is generally reported as high (Malope-Kgokong *et al.,* 2008). Kaposi's sarcoma herpes virus differs greatly across Africa; this has been attributed to cofactors that are correlated to geographic or environmental features that

might influence the risk of infection (Biryahwaho *et al.,* 2010). Herpes viruses are known to be ubiquitous universally except KSHV which is predominantly in sub-Saharan Africa (Nalwoga *et al.,* 2015). Biryahwaho *et al.,* (2010) noted that the dissimilarities in the sero-positivity of KSHV across regions of small-area within countries in African remain unknown. In sub-Saharan Africa, Kaposi's sarcoma is the most prevalent neoplasm in men and the prevalence continues to be elevated among people living with HIV/AIDS (Johann and Dirk, 2017).

The sero-prevalence rate of Kaposi's sarcoma herpes virus ranges from 20%-80% among adult populations in Africa and Mediterranean regions, less than 10% in the United States and Northern Europe (Stolka *et al.,* 2014). According to de Souza *et al.,* (2007), the sero-prevalence of Kaposi's sarcoma herpes virus in non-Amerindians increases with age and reported that more than 70% of children in that population were positive for KSHV. The striking pattern noted by de Souza *et al.,* (2007), was consistent with de Sanjose *et al.,* (2009), who noted that in Africa, KSHV infection occurs in childhood. In contrast, de Sanjose *et al.,* (2009), reported that sero-prevalence of KSHV is quite low in most Asian countries.

In African continent, Mbondi-Wonje *et al.,* (2013) recorded that HHV 8 is ubiquitous and with increased incidence of AIDS associated Kaposi's sarcoma among HIV/AIDS population. Elevated incidence of KS in Africa has been attributed to HIV infection however; the incidences are mitigated with the accessibility of combined antiretroviral treatment (Facciola *et al.,* 2017). In Ghana, sero-prevalence of HHV 8 is high (65.6%) in HIV and AIDS patients compared to 23.7% in HIV sero-negative (Adjei *et al.,* 2008). Sub-Saharan Africa and Mediterranean

countries have been recorded to have high prevalence of Kaposi's sarcoma infections (Ghaninejad *et al.,* 2009). Very low prevalence of HHV 8 are found in Europe and United State of America (Mwakigonja *et al.,* 2008; Ablashi *et al.,* 2002), this has been attributed to availability, accessibility and widespread use of antiretroviral drugs (Krown *et al.,* 1997; Maskew *et al.,* 2011). Gay men were reported to have the highest prevalence of KSHV in USA in the beginning of 1980's and this later declined radically (O' Brein *et al.,* 1999). In Africa the malignant tumor affecting the skin that is linked to KS is prevalent due to HIV endemic (Olp *et al.,* 2013). Studies by Butler *et al.,* 2011; Dollard *et al.,* 2010 revealed that the prevalence of HHV 8 has reached 50% in sub-Saharan Africa

In sub-Saharan Africa and South Africa, high sero-prevalence of KS is attributed to HIV/AIDS (Jemal *et al.,* 2012; Parkin *et al.,* 2014). Other studies (Dollard *et al.,* 2010; Chokunonga *et al.,* 2013; Wabinga *et al.,* 2014; Robey and Bower, 2015; Rogena *et al.,* 2015; Semeere *et al.,* 2016; Chokunonga *et al.,* 2016) in sub-Saharan African indicates that KS continues to encompass a significant percentage of total cancer burden in with 24% in Mozambique, 27% in Uganda and 23% in Zimbabwe. In South African, study done by Malope-Kgokong *et al.,* (2008) reported that Kaposi's sarcoma herpes virus infection varied from 35% to 49% across municipalities in one province.

In central Africa KSHV is highly prevalent in Cameroon (Tornesello *et al.,* 2010; White *et al.,* 2008). Betsem *et al.,* (2014) reported that in Cameroon, the prevalence of KSHV was increased with age and the findings were comparable to those found among Central and East Africa as

documented by Tornesello *et al.,* (2010). In Cameroon, KSHV sero-positivity is mostly prevalent in two major population found in Southern and Eastern part of the country (Betsem *et al.,* 2014). Study conducted in Cameroon by Stolka *et al.,* (2014), reported that the sero-prevalence of Kaposi's sarcoma herpes virus was about 80% among HIV positive individuals; this was considered as a high prevalence from the general population. High prevalence of HHV 8 has been reported in both HIV infected and uninfected among Cameroonians by Mbondji-Wonje *et al.,* (2013).

The sero-prevalence of HHV 8 has been reported to vary based on geographic region and sub-population, for example Uganda is considered an endemic region for KS with a sero-prevalence of up to 50% from the general population according to Biryahwaho *et al.,* (2010); Butler *et al.,* (2011). Butler *et al.,* (2009), notable supported the hypothesis that was earlier stated that in Uganda, KSHV occurred before adulthood and in South Africa it occurred in adulthood. Butler *et al.,* (2009), reported that the sero-prevalence of HHV 8 was high among children in Uganda compared to South African children. In Entebbe and Kampala Uganda, the sero-prevalence of Kaposi's sarcoma herpes virus in children was noted by Pfeiffer *et al.* (2010); Butler *et al.* (2011); Wakeham *et al.* (2013), to be increasing on ascending trend as the age increases. In Uganda where KSHV is considered endemic, Lin *et al.,* (2008), hypothesized that other factors present outside and within the environment or household might have influenced the risk of acquisition of KS by children and among factors mentioned included geographic region that had volcanic soil, parasitic infection especial schistosomiasis, biting flies and or plants constituents.

In Uganda, there is possibility that KSHV subtypes might have evolved and paved way for major diversification we see today in sub-Saharan Africa in addition there is possibility that horizontal transmission of KSHV subtypes might have broken up the vertical lineages of the virus that is passed down among the population (Kajumbula *et al.,* 2006). Biryahwaho *et al.,* (2010) noted that in Uganda, KSHV sero-positivity showed a significant variation by age, sex and geographical area in addition these findings were inversely related to the level of formal education attained by the subjects. In Uganda, the sero-positivity of HHV 8 does not follow the HIV pattern, this implies that the pattern is similar to the one witnessed during the pre-AIDS era (Bunnell *et al.,* 2008; Mermin *et al.,* 2008). The sero-positivity of KSHV in Uganda was linked to low socioeconomic status, the use of surface water and or a standpipe of a private tap water that was being used communally (Mbulaiteye *et al.,* 2005). Uganda and Tanzania have reported high cases of Kaposi's sarcoma infection even before the HIV and AIDS pandemic (Buonaguro *et al.,* 1996; Cook *et al.,* 1999). Variants of Kaposi's sarcoma herpes virus are heterogeneously distributed in varied geographic regions (Tornesello *et al.,* 2010). East Africa is traditionally known to have a high prevalence of KS (van Bogaert, 2012).

In Tanzania HHV 8 has a prevalence of 50% among the healthy population whose subjects were the blood donors; however, there is poor documentation on patients with or without tumors (Massambu *et al.,* 2003). Caterino-de-Araujo *et al.,* (2010) noted that in Africa Mozambique is one of the countries that have been documented as an endemic for HHV 8 infection due to HIV and AIDS epidemic. The sero-prevalence of KSHV among HIV –positive patients is more uniform, in Uganda the sero-prevalence of Kaposi's sarcoma was 56% (Shebl *et al.,* 2011) and in South Africa it was 48% (Maskew *et al.,* 2011). Among the highest incidences of KS in the

world, Equatorial Africa earns the name “KS Belt”, this was notable before the HIV (Dollard *et al.,* 2011). Uganda is in the “KS Belt”, whereas Zimbabwe and South Africa being outside the Belt (Dollard *et al.,* 2011). Cases of Kaposi’s sarcoma have been reported by Ngalamika *et al.,* (2014); Bohlius *et al.,* (2014) in Zambia and South Africa respectively despite reports that epidemic KS has been decreasing due to availability and accessibility of anti-retroviral therapy used by HIV positive individuals.

In Kenya, Mwanda *et al.,* (2005) noted that the clinical pattern of Kaposi’s sarcoma in HIV sero-positive patients had indications of lymphadenopathy. The leading cause of AIDS associated neoplasm in Kenya is Kaposi’s sarcoma (Rogena *et al.,* 2015). In Gabon, the sero-prevalence of HHV 8 was found to be uniformly high (Capan-Melser, *et al.,* 2015). Adjei *et al.,* (2008), stated that in Ghana, a significantly high (65.6%) sero-prevalence of HHV 8 was detected in HIV positive blood donors compared to 23.7% HIV negative blood donors. Ghana and Cameroon are among the countries in African that have been reported to have endemic sero-prevalence of KSHV (Volpi, 2004; Edelman, 2005). Ablashi *et al.,* (2002) observed that KS infection among the adolescents had low sero-prevalence of 56% in Egypt, 8% in Cameroon and between 19-26% in Uganda.

Avelleira *et al.,* (2006); Malope *et al.,* (2007); Minhas *et al.,* (2008) noted that there was no difference in the sero-prevalence of KSHV that is linked to HIV infection in Zimbabwe and South Africa. In a study done by Caterino-de-Araujo *et al.,* (2010) in Mozambique revealed that there is possibility of an increase in HHV 8 in the endemic regions. Approximately 45% (24/53)

of countries in Africa have data on the sero-prevalence of HHV 8 and it ranges from 2% among children in Eritrea to 100% in Central African Republic and Morocco recording enormous group of individual with Kaposi's sarcoma (KS) (Etta *et al.,* 2018). Etta *et al.,* (2018) further noted that there was no significant difference in the sero-prevalence of KS across the regions in Africa; however, a significant level of infection was noted in children who had Human Immunodeficiency Virus (HIV).

According to Etta *et al.,* (2018), the sero-prevalence of HHV 8 across the African countries were distributed as follows; Southern Africa (14.0-90%), Central Africa (17.4-100.0%), West Africa (14.0-83.1%), East Africa (2.0-93%) and North Africa (0.0-92%). Africa and Eastern Mediterranean Basin reported highest endemic KS prevalence even before the HIV epidemic (Dollard *et al.,* 2010). Figure 1.3 displays the sero-prevalence of HHV 8 observed in one population in African countries from 1998-2017 (Etta *et al.*, 2018). When comparing the sero-prevalence of KSHV in sub-Saharan Africa and Europe, there is need to observe caution because high incidences of KS in sub-Saharan Africa are perhaps due to inability to access health care resulting from late diagnosis and treatment (IeDEA, 2014).

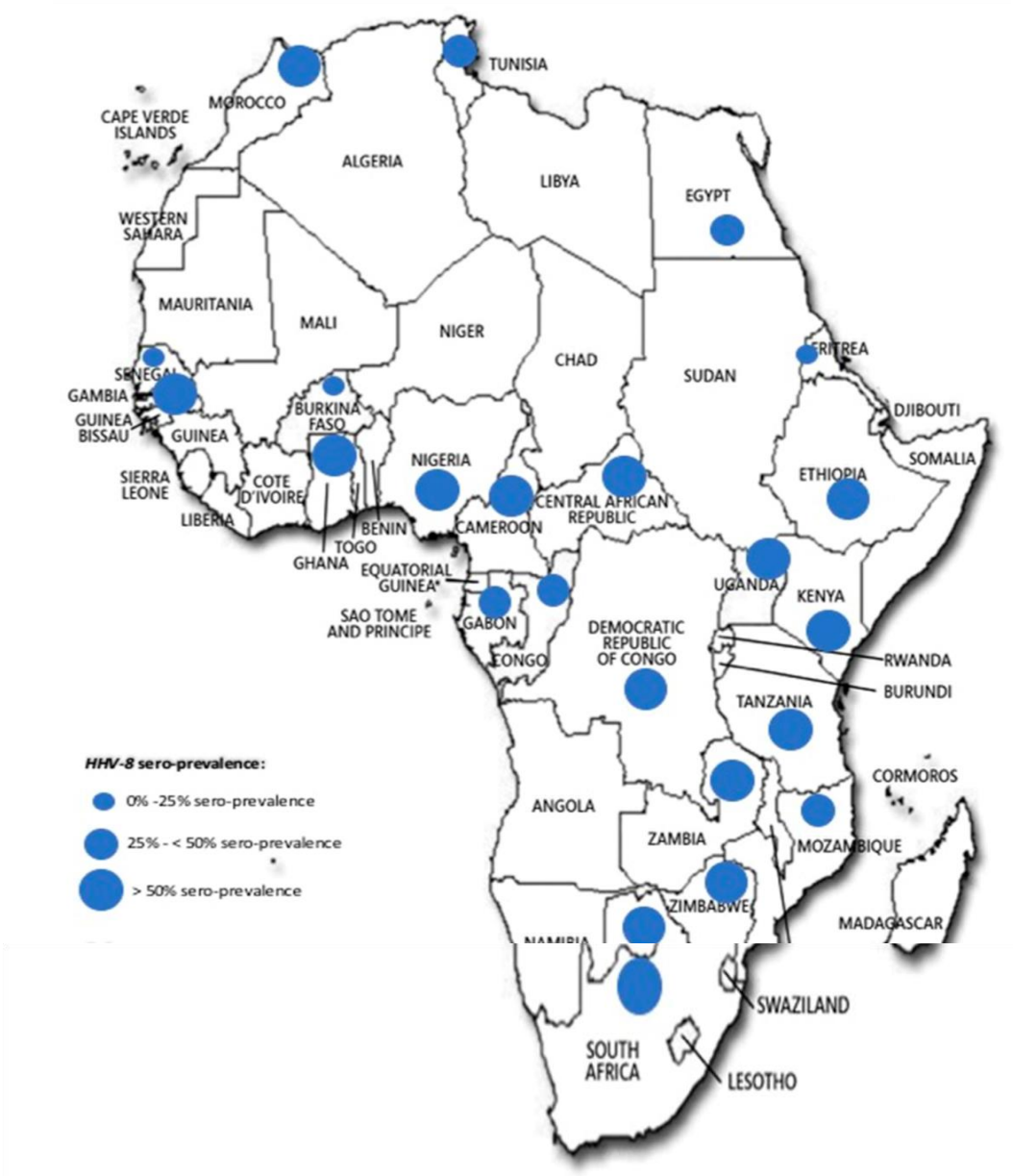


Figure 1.3: Sero-epidemiology of HHV 8 in African countries (Etta *et al*., 2018).

1.2 Problem statement

Mwanda *et al.*, (2005) reported that KS was ordinarily found among HIV patients in Kenya. Other studies (Mwakigonja *et al*., 2008; White *et al*., 2008; Dollard *et al*., 2010; Kumar *et al*.,

2013) have also showed that the KS belt in Africa covers, Kenya, Uganda, Tanzania, Zimbabwe and South Africa.

The genes of HHV 8 have been perceived to play a role in the pathogenesis of KS. The K1 and K15 genes for instance, has been attributed to the interaction between host immune response, furthermore, both genes have been detected in cells during late latency period and are also linked to viral neoplasia (Cho *et al.*, 2008). At the right end of HHV -8 genome K15 gene is located and occurs in two alleles as P (Predominant) and M (minor) (Choi *et al.*, 2000). The ORF75 gene has been proven to help in lytic replication and enhancement of virus pathogenesis in the host cells (Van Skike*et al.*, 2018).

In Kenyatta National Hospital (KNH), the diagnosis of KS is based on clinical presentation and Hematoxylin and Eosin staining of tissue biopsy. Clinical diagnosis poses a risk of misdiagnosis as the patient's lesion might be mistaken for other reactive neoplastic vascular proliferations (Dittemer *et al.*, 2012). In addition, the patient's in this study had dark skin pigmentation, and in another study conducted on dark- skinned patients, KS had been confirmed to mimic a number of non-KS like dermatological conditions (van Bogaert, 2012). van Bogaert, (2012), further stated that the patients who presented with violaceous skin lesions were likely to be considered a suspected case of KS which is difficult to demonstrate in dark-skinned individuals.

1.3 Study justification

High occurrences of KS have been reported in East Africa mainly Uganda and Tanzania (Buonaguro *et al.,* 1996; Cook *et al.,* 1999). Mwanda *et al.,* (2005) stated the need to watch out on clinical pathological cases of KS with the advent of antiretroviral drugs. The leading AIDS neoplasm in Kenya is Kaposi's sarcoma, therefore, there is need for physicians managing HIV/AIDS patient to be on the lookout (Rogena *et al.,* 2015). Kaposi's sarcoma patients throughout the world have shown great improvement when they are put consistently on chemotherapy and antiretroviral drugs (Mwanda *et al.,* 2005). Rogena *et al.,* (2015) further stated the need for early screening programs for the neoplasm in HIV/AIDS patients.

Understanding the histopathology of KS and use of molecular technique to analyze the subtypes has led to designing better treatment regimen (Pantanowitz *et al.,* 2010). Among the East Africa HIV infected patients Amerson *et al.,* (2016) emphasized the need to abandon the use of only clinical diagnosis of KS and augment it with pathologic diagnosis.

According to the WHO guideline of 2015 and the current Kenya AIDS strategic Framework-KASF 2014/15-2018/19 developed by National AIDS Control Council, it is becoming a challenge to get cases of patients presenting with typical manifestation of Kaposi's sarcoma because the guideline recommends initiation of ARVs upon confirmation that one is HIV positive. Herein, this study sought to characterize AIDS associated KSHV using molecular technique.

1.4. Research questions

1. What is the demographic and anatomical site distribution of AIDS-KS among the studied cases from Kenyatta National Hospital, Kenya?
2. Do the selected genes of HHV-8 associated with AIDS-KS exist among the studied cases from Kenyatta National Hospital, Kenya?
3. Is there an association between the targeted genes of HHV8, demographic, anatomical site location, histological and morphological sub-types of KS among the studied cases from Kenyatta National Hospital, Kenya?
4. What is the molecular epidemiology and characteristic patterns of the selected genes of HHV-8 associated with AIDS-KS among the studied cases from Kenyatta National Hospital, Kenya?

1.5 Objectives

1.5.1 Broad objective

To examine the molecular epidemiology and characterization of the selected HHV-8 genes associated with AIDS-KS from Kenyatta National Hospital, Kenya.

1.5.2 Specific objective

1. To investigate the demographic and anatomical site of AIDS-KS among the studied cases.

2. To identify the histological and morphological types of KS among the studied cases.

3. To detect the selected genes of HHV-8 associated with AIDS-KS among the studied cases.

4. To analyze the association between the targeted genes of HHV8, demographic, anatomical site location, histological and morphological types of KS among the studied cases.

1.6 Utility of the study

Till date, vaccine development efforts against Kaposi's sarcoma herpesvirus are going (Wu *et al.,* 2012). Pantanowitz *et al.,* (2010), stated that designing a better treatment for KSHV is pegged on understanding the histopathology and molecular characteristics of the virus. The treatment and preventive measures against KSHV can be well understood on the basis of understanding the viral pathogenesis, mechanism of viral replication, mode of viral infection and transmission. The K1 and K15P are studied in the context of viral immune escape mechanism, whereas ORF75 plays a role in viral lytic replication.

This being the first study describing the epidemiological and molecular characterization genotype K1, K15P & ORF75 of HHV-8 in Kenya. The observation made from the studied cases showed that the selected genotypes were highly prevalent in our Country. There is need for the Ministry of Health to develop guideline for testing KSHV and while at it, embrace the use of molecular technique. The molecular testing has been observed to be sensitive even on cases that were reported as KS like using histology. The use of molecular method for testing KSHV was able to give a conclusive result.

.

CHAPTER TWO

LITERATURE REVIEW

2.1 The biology of Human herpes virus 8 Gene

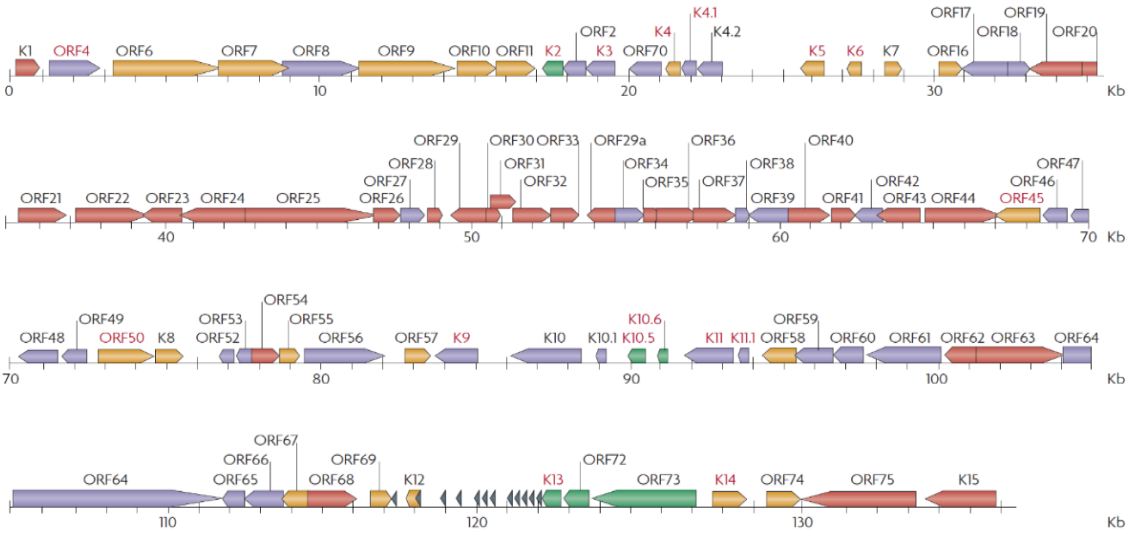
Kaposi's sarcoma is caused by human herpes virus which belongs to herpesviridae family (Altuğlu *et al.,* 2016). Human herpesvirus is a DNA virus that has an icosahedral capsid with a capsomere. The capsid is surrounded by tegument which is a protenious coat (Owen *et al.,* 2015). A whole Human herpes virus 8 genome (figure 2.1) has been documented to be about 165 Kb and 149 DNA containing coding information (Sasco, 2010).

Human herpes virus 8 is a dsDNA, gamma-herpes and of virinae sub-family (Desrosiers *et al.,* 1997). The genome of HHV 8 contains a minimum of 100 open reading frames (ORF) out of which 4 to 75 of them are known to be homologous to other herpes viruses (Raab-Traub, 2012). Kaposi's sarcoma herpes virus belongs to the gamma-herpes virus and it encodes for more than 84 proteins that plays a role in viral replication and host-virus interaction (Dittmer and Damania, 2013). The genome for Kaposi's sarcoma herpes virus encodes more than 80 open reading frames (ORF) and many human gene homologs strongly signifying that KSHV stimulates certain type of cell proliferation that might be linked with tumorigenesis (Thomas *et al.,* 2011; Yei and Gao, 2011). Arias *et al.,* (2014), stated that the genome map for Kaposi's sarcoma herpes virus has changed since it was first described, with the explanation that GK18 sequence reveals 86 genes of which twenty-two is alleged to encode for immune-modulatory protein.

A temporary and epigenetically expression of KSHV genes are usually in the early stages of KS infection. For maturation of viral capsid, herpes viruses depend on homodimeric protease (Gable *et al.,* 2014). Kaposi's sarcoma virus infects and continue to keep up in the endothelial and B lymphocytes expressing different gene patterns (Jha *et al.,* 2014). The genome of HHV 8 consists of latency and episomal genes, lytic reactivation genes, late kinetic genes and immune eversion (Kong, 2008). Kaposi's sarcoma herpes virus undergoes latent and lytic replication in its replication cycle. Characteristically, KSHV strictly prefers infecting B cell during latent cycle whereas lytic cycle plays a role of infecting new cells diving virus-associated pathogenesis (Jha *et al.,* 2014).

The following genes of KSHV are known to play a role in the pathogenesis of KS; K1, K2, vMIPS, K4, K4.1, K5, K9, K12, ORF-6, ORF-71, ORF-73, ORF-74, and K15 (Neipel and Fleckenstein, 1999). The genome of KSHV includes 'ORF' which is believed to have gotten its name from its genome position (Samols *et al.,* 2005). The ORF K1 gene undergoes mutation just like other human pathogen for instance the HIV-1 env; however, this mechanism causing high variability is not well understood (Uldrick and Whitby, 2011). The ORF75 is a tegument protein in KSHV that plays a role in viral lytic replication and a critical role in antagonizing nuclear domain 10. The function of nuclear domain 10 is to inhibit the replication of herpes virus. When herpesvirus is restricted from replicating by nuclear domain 10 the outcome by default is latency (Full *et al.,* 2014). The ORF75 gene has been proven to assist in lytic replication and enhancement of virus pathogenesis in the host cells (Van Skike *et al.,* 2018). The K1 genes of the HHV 8 genome has been sub-typed as A, B, C and D later followed by E and N (Zong *et al.,* 1999). The K15 gene has two diverged types that are K15 (P) and K15 (M) that reveals evidence

of recombination (Poole *et al.*, 1999). The K15 (P) has been reported as the predominant allele and is commonly found to be associated with subtype A, B, C and D of K1, whereas the K15 (M) is the minor and has not been pronounced in the subtype D (Hayward, 1999).



Latency and episomal genes lytic reactivation genes late kinetic genes immune eversion

Figure 2.1: A whole HHV 8 genome (Coscoy, 2007; Kong, 2008).

According to Jha *et al.*, (2014), on human peripheral blood mononuclear cells (PBMCs), KSHV genome undergo changes at the binding site encoded as the lytic and epigenetic alterations can also be seen as encoded by latent proteins (figure 2.2). Additional investigation demonstrated that KSHV is able to express the lytic and the latent genes at the same time and this is associated with modification of histone at specific region of the viral genome (Jha *et al.*, 2014). Epigenetic alterations show a relationship with viral gene and are regulated temporally within seven days of PBMC infection, this basis is essential for effective infection by KSHV of human PBMCs (Jha *et al.*, 2014). Studies (Lu e*t al.*, 2011; Lu *et al.*, 2012) have reported that KSHV can infect human PBMCs successfully within seven days.

Studies (Skinner *et al.*, 2010; Sharma *et al.*, 2010) have also demonstrated that the effect of epigenetic changes on the expression of genes without changing the sequences of HHV 8 DNA can have a worldwide effect on KSHV genome. Lytic and latent replication of Kaposi's sarcoma herpes virus has demonstrated an exceptional epigenetic adjustment across the whole genome in the epithelial cells during the initial infection (Toth *et al.*, 2010; Günther and Grundhoff, 2010). The modification and programming of epigenetic on KSHV genome helps in transcriptional regulation of the latent and lytic genes in the replication cycle of the virus (Toth *et al.*, 2010).

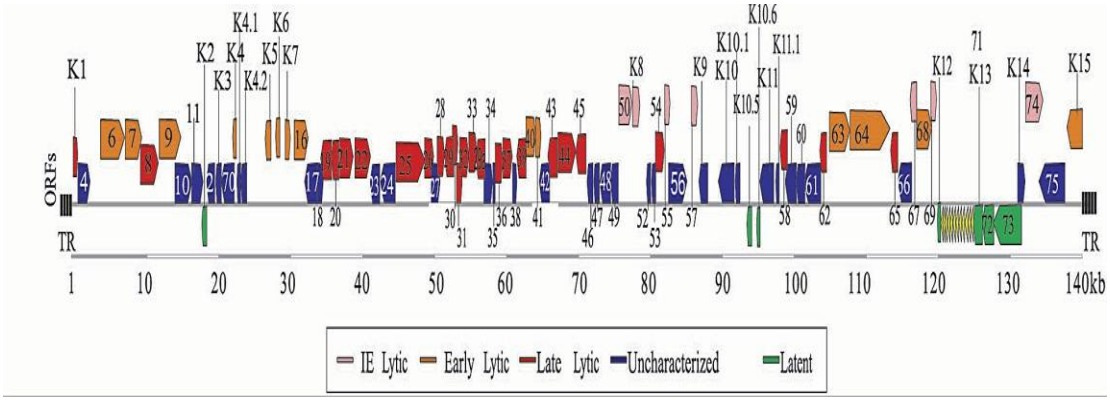


Figure 2.2: KSHV genome during infection of human PBMCs (Jha *et al.*, 2014)

Molecular biology of Kaposi's sarcoma herpes virus has been lately reviewed, the virus is enveloped and it uses ORF9 for genome replication during lytic phase (Dittmer, and Damania, 2016).

Table 2.1: Selected genes of HHV8, protein encoded and functions (Adapted from Edelman, 2005; Wen and Damania, 2010)

Gene	Protein encoded	Functions
K1	ITAM-containing signal transducing membrane glycoprotein	Involved in oncogenesis. Transformation; inhibition of apoptosis; B cell activation; downregulation of surface B-cell receptor (BCR); immunomodulation; activation of PI3K/Akt/mTOR kinases; signaling
K15	Latent antigen membrane protein (LAMP)	Immunomodulation
ORF75	FGARAT	Involve in viral lytic replication and a critical role in antagonizing Nuclear Domain 10

2.2 Association between Kaposi's sarcoma herpes virus and HIV/AIDS

Kaposi's sarcoma herpes virus is named after Moritz Kaposi, a Hungarian dermatologist, who described the 'classical KS first in 1972. The distinct feature of HIV-associated KS is that it might affect the entire skin and mucous membrane. In addition, KS might affect the lymph nodes, gastrointestinal tract, lungs and or the liver (Hoffmann *et al.,* 2017). Kaposi's sarcoma is listed among HIV/AIDS defining malignancies (Parkin *et al.,* 2014). Kaposi's sarcoma herpes virus is a lifelong infection that is established in human host and it has been linked to a number of malignancies (Neneh *et al.,* 2018). The co-infection between HIV and other viruses enhances disease progression as a result of immune suppression (Blackard and Sherman, 2008; Chakraborty *et al.,* 2010).

Kaposi's sarcoma remains one of the comments malignancies in HIV and AIDS patients (Hoffmann *et al.,* 2017). The most common cancer of people living with HIV currently is Kaposi's sarcoma (Johann and Dirk, 2017). Persons co-infected with Human Immunodeficiency Virus and Kaposi's sarcoma herpes virus are at increased risk of developing Kaposi's sarcoma (Stolka *et al.,* 2014). The commonest AIDS defining tumor in HIV infected people in Africa is Kaposi's sarcoma (Maskew *et al.,* 2011). Maskew *et al.,* (2011), further noted that Kaposi's sarcoma herpes virus come first before the development of KS which tend to worsen when a person co-infected with HIV is not on antiretroviral therapy. Kaposi's sarcoma is more dominant among HIV positive individuals (Satein *et al.,* 2008; Parkin *et al.,* 2008). Kaposi's sarcoma is common among those infected with HIV/AIDS (Schwartz *et al.,* 2008; Hoffmann *et al.,* 2017; Ferri, Fred F. (2017) and it became a hallmark of AIDS-defining illness in the 1980s (National Cancer Institute, 2015).

Klaskala *et al.,* (2007) stated that the risk of developing AIDS-KS in Africa among HIV infected individual is 30% to 50%, this is due to high prevalence of HIV-1/KSHV co-infection. Campbell *et al.,* (2009), reported that 30% of HIV-1 infected individuals in Zimbabwe are also co-infected with Kaposi's sarcoma herpes virus. The commonest cancer in Malawi and United States today is Kaposi's sarcoma caused by Kaposi's sarcoma herpes virus (Mina *et al.,* 2014). Kaposi's sarcoma is the most common cancer among HIV positive individuals (Jemal *et al.,* 2011) and the leading cancer in countries where KSHV is endemic and HIV is epidemic (Jemal *et al.,* 2011; Semeere *et al.,* 2012). Significantly, high rate of KS in the most recent years in the United States are attributed to HIV epidemic among the black communities (Matthews *et al.,* 2016). The agent that motivates proliferative growth is KSHV which is also known as Human herpes virus 8

(International Agency for Research on Cancer, 2012). Immunosuppression due to HIV sets foundation for herpes virus 8 resulting to Kaposi's sarcoma (Rohner *et al.,* 2016). The Human herpes virus 8 (HHV 8) has been documented as the causative agent of KS, which is a multifocal proliferative infection that infects the blood and lymphatic vessel (Chang *et al.,* 1994).

In another study by Gramolelli and Schulz (2015), HHV 8 was stated as the causative agent of KS. The DNA of HHV 8 was detected considerable more frequent in HIV sero-positive individuals compared to HIV uninfected group; however, these findings were statistically insignificant (Rohner *et al.,* 2016). Documentation by (Zhang *et al.,* 2012; Rohner *et al.,* 2016; Liu *et al.,* 2017) indicates that HIV is a vital factor linked with KSHV. Kaposi's sarcoma is also known as Kaposi's sarcoma-associated herpes virus and it causes a tumor by angioproliferations energized by human herpes virus 8. In addition, KS is a hallmark of an acquired immunodeficiency syndrome that might be fatal (Heyrman *et al.,* 2016). Minhas *et al.,* (2008), noted that more than 10% of Zambian children are infected with Kaposi's sarcoma herpes virus by 12 months of age, the precise risk factor that predisposed these Zambian children was not well understood but it was hypothesized that immunosuppression due to HIV-1 infection might have contributed.

The commonest malignancy that signifies AIDS in HIV-1 infected individuals is Kaposi's sarcoma (Sunil *et al.,* 2010). Higher prevalence of Kaposi's sarcoma herpes virus is witnessed among individual who are immunodeficient due to HIV/AIDS. Globally, the distribution of HHV 8 indicates great variations; however, Africa has been reported to be the region with the highest prevalence (Altuğlu *et al.,* 2016). When AIDS was first reported in 1981, the main manifestation

that befell 30%-40% of the AIDS patients was Kaposi's sarcoma (Szajerka and Jablecki, 2007). Kaposi's sarcoma associated with AIDS is chiefly found in bisexuals, homosexuals and intravenous drug users (Szajerka and Jablecki, 2007). According to Mwakigonja, *et al,* (2008), HHV 8 sero-positivity is three times more rampant in HIV/AIDS patients than in HIV sero-negative individuals. The findings by Mwakigonja *et al.,* (2008) is consistent with other reports by (Pyakurel *et al.,* 2007; Duker and Rezza, 2003; Wamburu *et al.,* 2006) which also observed an increased KS development among HIV infected patients.

According to Pyakurel *et al.,* (2007), Duker and Rezza, (2003), Wamburu *et al.,* (2006), recorded that HIV and KSHV cross-talk, and this is somewhat realized it through the transcription of HHV 8 by the HIV-Tat as documented by (Pyakurel *et al.,* 2007; Feller and Lemmer, 2008). According to studies done by Miller *et al.,* (2006); de Franca *et al.,* (2011), there is evidence that HIV infection is likely to increase HHV 8 shedding in various body fluids saliva included. Rohner *et al.,* (2016) noted that HIV infection was associated with elevated HHV 8 sero-prevalence across the globe. In addition, Rohner *et al.,* (2016) stated that it was not clear whether HIV positive individuals are at risk of being co-infected with the infectious KSHV despite the fact that HIV infection is a risk factor for KS progression. Haq *et al.,* (2016) observed that out the 335 patients that had HIV-associated KS, 39% (130) were viremic with a moderate HHV 8 viral load.

According to Fang *et al.,* (2017) an important co-infected agent with HIV is Kaposi's sarcoma associated herpes virus. The commonest cancer in several countries during the HIV and AIDS

epidemic is Kaposi's sarcoma (Parkin *et al.,* 2008). In Germany a multivariate analysis study by Feiterna-Sperling *et al.,* (2016) documented that HHV 8 infection which is the primary risk factor for KS and HHV 8- associated malignancies tend to occur early in life and as a result, management of HIV infected individuals should include testing for HHV 8 co-infection.

The fundamental cause of Kaposi's sarcoma infection is KSHV, nevertheless, it may not be adequate (Bouvard *et al*., 2009), and immunosuppression due to HIV plays a significant risk for the disease development with elevated viral load and viral shedding (Bouvard *et al*., 2009; Bagni and Whitby, 2009). The risk of developing Kaposi's sarcoma is linked to KSHV viremia (Nsubuga *et al.,* 2008) severe immunosuppression, expression of HIV protein and environmental factors. In developed countries, KS remains the second most common cancer that is HIV associated and the leading cause of tumors in regions where access to HAART is a great challenge (Sullivan and Pantanowitz, 2010; Lima *et al.,* 2017; Mosam *et al.,* 2012). Prolonged infection of HIV in the absence of antiretroviral treatment (ART) results in AIDS, later Kaposi's sarcoma (KS) a tumor known to be an opportunistic might set in (Rabkin and Yellin, 1994).

Continued replication of HIV in a host, results in the increase of viral load and depletion of the host's CD_4 cells allowing progression of KS (Dezube, 2000). Philips *et al.,* (2008) reported that correct usage of ART against HIV significantly reduced the chances of developing KS. Clinical manifestation of KS in HIV patients is a pointer of immunosuppression, Chokunonga *et al.,* 2000further noted an upsurge of morbidity and mortality associated with AIDS-KS in sub-Saharan African. According to Mbulaiteye *et al.,* (2006) HHV 8 is necessary but not sufficient

for the development of KS because other factors like immunosuppression play an essential role. Since 1996 onwards, combined antiretroviral therapy (cART) has been used to reverse AIDS associated KS in developed countries (Gallafent *et al.,* 2005).

Orem *et al.,* (2004) reported that AIDS associated KS remains the main public health burden in countries with limited access to cART and financial resources. Mortality and disease burden in HIV and AIDS patients is significantly linked to Kaposi's sarcoma (Boshoff and Weiss, 2002). Deaths linked to HIV and AIDS has been reported by Kirova *et al.,* 1998 to be on the decline by 81% when combine ART is used. The ever-increasing case of AIDS associated KS between three and twenty times in sub-Saharan Africa has been attributed to the increase of HIV infection (Parkin *et al*., 1999). Morris, in (2003) described KS as a hallmark of HIV and AIDS, in addition he observed that KS was the most recorded cancer in sub-Saharan countries.

High risk individuals such sexual workers, intravenous drug users, HIV positive people, and homosexuals are prone to develop Kaposi's sarcoma whose underlying cause is KSHV (Kumar *et al*., 2007; Del Mistro *et al.,* 2012). Higher sero-prevalence of HHV 8 observed in HIV infected individuals was attributed to sexual transmission route among adults, however, non-sexual routes might be operative among young sexually naïve adults in Nigeria (Ogoina *et al.,* 2011). In addition, Ogoina *et al.,* (2011), reported that HHV 8 associated with advanced HIV/AIDS was endemic in Northern Nigeria especially among adults. The epidemic form of KS also known as AIDS associated KS, is the highest prevalent neoplasm among homosexuals and bisexual's men infected with AIDS before the introduction of HAART (Highly Active Antiretroviral Therapy)

(Masom *et al.,* 2009). The HHV 8 has recently been considered as the causative agent of all clinical and epidemiological forms of KS tumor type (Buonaguro *et al.,* 2003).

Contrary to other findings, Malope *et al.,* (2008) stated that there was no association between HHV 8 sero-positivity, STDs, HIV, and the number of sexual partners in South Africa, in addition it worth mentioning that Malope *et al.,* (2008) conducted the study among the high-risk population. Kaposi's sarcoma infection is a Human herpes virus 8 – associated malignancy that has been a co-morbidity affecting HIV patients (Casper, 2011). The KS tumor which is a common HIV linked cancer in sub-Saharan Africa and the second commonest cancer in HIV patients globally (Gantt, 2010). According to Gbabe *et al.,* (2014) KS still remains the commonest cancer in HIV infected patients in sub-Saharan Africa and globally among AIDS patients.

One of the main causes of morbidity and mortality in AIDS patients during the AIDS pandemic has been documented to be Kaposi's sarcoma- associated herpes virus (Jha *et al.,* 2014). Uldrick *et al.,* (2010), reported that, persons that are co-infected with HIV and Kaposi's sarcoma herpes virus have been linked to KSHV inflammatory cytokine syndrome (KICS). According to Polizzotto *et al.,* (2012), KSHV has been linked with most cases of Multicentric Castleman's Disease (MCD) in HIV patients. Kaposi's sarcoma associated MCD is a rare B cell lymphoproliferative disease that affects the lymph nodes and lymphoid tissues, is aggressive and fast developing, which could result to death (Schulte and Talat, 2010). The mechanism that induces KSHV-mediated lymphoproliferative disorder is not well known, however, the infection

by KSHV in the endothelial cell is well clarified (Chakraborty *et al.*, 2012; Chandra, 2010). The B cells and T cells in the human tonsil can be predominantly infected by KSHV, with B cells yielding a sizeable quantity of transmittable virus progeny (Myoung and Ganem, 2011; Myoung and Ganem, 2011).

Since KS can be present in HIV-negative transplant and AIDS patients, it is there true to say that HIV in itself is not required for KS to develop (Insight Start Study Group, 2015). Unemori *et al.*, (2013) stated that the subject that is ongoing in the scientific inquiries is whether there exist tumor triggers that function entirely independently of immune surveillance, this is because it has been observed that a person can develop KS in the absence of massive CD_4 cell deficiency, undergo subtle impairments such as immune senescence and loss of T cell repertoire.

2.2.1 Staging and classification of Kaposi's sarcoma

There need to consider staging all cases of KS clinically. The staging of KS comprises of a complete inspection including oral and genital mucous membranes, an abdominal ultrasound, and a chest radiography. Colonoscopy and gastroduodenoscopy should also be considered besides measuring plasma level of HHV 8 because biomarker in KS have been shown to have very little value in diagnosis or prognostication (Haq *et al.*, 2016). Bower *et al.*, (2013) noted that there no widely accepted staging system for KS. There is no widely accepted staging system. In addition, Bower *et al.*, (2013) stated that the AIDS Clinical Trial Group (ACTG) staging system that was used for AIDS-related Kaposi's sarcoma developed in the pre-cART is no longer a valid.

The staging of Kaposi's sarcoma is based on the patient's immune status (CD_4 T cell count) and the systemic involvement of the disease (Johnston *et al.,* 2009). It is however not known whether staging of KS as stated by Johnston *et al.,* (2009), were developed during the first phase of HIV-AIDS epidemic where KSHV was witnessed late in life and predominantly acquired through sexual route in United State and Europe (Butler *et al.,* 2009).

A new classification of KS adopted by Cottoni in 1996 grouped KS in four stages; localized, generalized, aggressive and non-cutaneous depending on the body surface are, the number of lesions counts and or visceral involvement (Table 2.2).

Table 2.2: Classification of AIDS associated Kaposi's sarcoma. Adopted from Cottoni, 1996

	Krigel *et al.*, 1983	Mitsuyasu, Groopman, 1984	Schwartz, 1984	Alessi *et al.*, 1988	Cottoni, 1996
Stage	**Tumor extension**	**Characteristics of KS**	**Characteristics of KS**	**Characteristics of KS**	**Characteristics of KS**
1	Cutaneous, Locally indolent	Limited cutaneous (<10 lesions in one anatomic area)	Localized nodular (>15 lesions or restricted to one bilateral anatomic site & few, if any gut nodules)	Cutaneous (<5 lesions, or one anatomic area, +/-lymph nodes	Localized (<10 lesions, or body surface area <9%)
2	Cutaneous (locally aggressive +/- regional lymph node involvement	Disseminated cutaneous (>10 lesions or more than one anatomic area)	Locally aggressive (exophytic destructive lesions & locally infiltrative cutaneous lesions)	Cutaneous (<5 lesions >1 anatomic area +/- mucosa +/- gastrointestinal	Generalized (>10 lesions, or body surface area >9%
3	Generalized mucocutaneous +/- lymph node involvement	Visceral only (gastrointestinal; lympnodes)	Generalized lymphadenopathic (Widespread lymph node involvement, with/without skin lesions and no visceral involvement)	Mucocutaneous +/- lymph node and visceral or solely visceral	Aggressive (Presence of one exophytic lesion with infiltrative or invasive pattern)
4	Visceral	Cutaneous visceral	Disseminated visceral KS widespread KS, with involvement of multiple visceral organs		No-cutaneous absence of skin lesions
A	Asymptomatic	No systemic signs and symptoms		Opportunistic infections absent and CD4 <500	
B	Weight loss (10%) fever > 380C lasting 2 weeks- not due to identifiable infection	Weight loss >10% body weight Fever >37.8OC FOR >2 weeks not due to identifiable infection		Opportunistic infections present	

2.3 Transmission of Human herpes virus 8 aspect of infection

All viruses that belong to herpesvidridae family are ubiquitous except HHV 8, which displays a rare distribution pattern around the world. As a result, several questions have been asked regarding the origin of KSHV and the mode of its transmission (Tornesello *et al.,* 2010). The precise route by which HHV 8 is transmitted remains unknown, however, sexual and non-sexual modes have been reported besides that their relative importance vary with the endemicity of the infection (Mbulaiteye *et al.,* 2005). The mode of transmission of KSHV is yet to be clarified, however, childhood infection suggests a nonsexual route (Malope *et al.*, 2008). Regarding HHV 8 transmission, a study by Campbell *et al.,* (2009), suggests that there is lack of proof on the association between HHV 8 and HIV infection through sexual behavior. Butler *et al.,* (2009), reported that transmission mode of KSHV vary depending on subpopulation and geographical region, in addition, non-sexual and sexual transmission have also been stated. A meta-analysis done in China indicated that the distribution of HHV 8 varies, besides that; limited information was available to understand the risk factors linked with transmission and prevalence of KSHV (Tiejun *et al.,* 2012).

The general consensus regarding the main route of transmission of Kaposi's sarcoma herpes virus among children is via saliva (Minhas and Wood, 2014). Furthermore, the lack of gold standard for serological analysis and animal models to research on the modes of transmission has hindered the efforts to additionally describe the transmission of KSHV so as to come up with effective prevention strategies (Minhas and Wood, 2014). Horizontal transmission of HHV 8 has been reported in endemic African countries between parents and children and between siblings (Minhas *et al.,* 2008; Butler *et al.,* 2009; Butler *et al.,* 2011). It has been recorded by

Plancoulaine *et al.,* (2004) that the primary transmission of HHV 8 is during childhood from mother to child and also between siblings. Contact with infected saliva is the main transmission route of KSHV; however, sexual transmission has been postulated due to significant cases of KS witnessed among homosexuals (Martinand Osmond 2000).

Despite the fact that saliva is the main way by which KSHV is being transmitted, HHV 8 has been isolated from other body fluids and cells like semen, peripheral blood mononuclear cells (PBMCs), cervico-vaginal secretions and prostate glands (Minhas and Wood, 2014; Ganem, 2010; Sathish and Yuan, 2011). According to Hladik *et al.,* (2006), KSHV can be transmitted through blood transfusion and organ transplantation. The transmission of KSHV is mainly through horizontal via saliva; however, an extensive repeated contact is required to form transmission such as mother to child or sexual interaction (Butler *et al.,* 2009; Ignacio *et al.,* 2016). Alkharsah *et al.,* (2007), reported that maternal saliva might be linked to KSHV infection among children. Olp *et al*., (2012), stated that children from several families who had Kaposi's sarcoma herpes virus infection had KSHV genotype that differed from those of their mother and family members, this thereby led to suggestions that these children could also have acquired the KSHV infection from outside the household sources.

In Uganda where HHV 8 is considered endemic, the occurrence of KSHV via sexual transmission among heterosexual has been considered as weak (Shebl *et al.,* 2011). The transmission of HHV 8 through sexual route among heterosexual in sub-Saharan Africa remains unclear (Mbulaiteye and Goedert, 2008), however these findings are not consistent with HHV 8 sero-positivity resulting from sexual exposure. Butler *et al.,* (2009) recorded that the

transmission of KSHV occurs horizontally during childhood and commonly in a family set up. Lisa *et al.,* (2011), reported that Kaposi's sarcoma herpes virus is transmitted through horizontal route in childhood resulting from intra-family contacts and this transmission continues through into the adulthood via non-sexual route. Malope *et al.,* (2008), documented that there is a sexual risk factor for transmission of Kaposi's sarcoma herpes virus among men who have sex with men, nevertheless, conflicting evidence on heterosexual transmission has also been recorded. With regards to Kaposi's sarcoma herpes virus, sexual route of transmission is not the main source of infection in the general population (de Sanjose *et al.,* 2009). Malope *et al.,* (2008), further stated that sexual route does not play a significant role in the transmission of KSHV in the South African population.

The out-break of Kaposi's sarcoma in 1981 among the homosexual young men in the West foreshowed the onset of HIV epidemic, this remained one of the major challenges to the global health and science because KS increased more than 10,000 times with HIV being the underlying cause (Mbulaiteye *et al.,* 2011). Engels *et al.,* (2007); Malope *et al.,* (2008), stated that the sero-prevalence of KSHV among women was significantly correlated to sexual activity or co-infection with other sexually transmitted infection, however, other routes like saliva were suggested (deSouza *et al.,* 2007).

Several studies (Edelman, 2005; De Paoli, 2004) indicate that HHV 8 transmission may differ between endemic and non-endemic countries. According to Plancoulaine *et al.,* (2004) vertical or horizontal are the modes of HHV 8 transmission. According to Shebi *et al.,* (2011); de Sanjose *et*

al., (2009) transmission of HHV 8 in heterosexual adults in both endemic and non-endemic areas is through sexual relations. Kaposi's sarcoma is transmitted through blood and blood product (Cannon *et al.,* 2001). In addition, KS can be transmitted sexually; saliva can also be a carrier for the herpes virus (Campbell *et al.,* 2009).

The main route of transmission of HHV 8 is the saliva, however, semen, blood, urine and stool have also been attributed as the vehicle through which the virus can be transmitted in a population that is considered at risk of acquiring the infection like the homosexuals and human immunodeficiency virus AIDS patients (Cannon *et al.,* 2003; Casper *et al.,* 2004). Studies (Cannon *et al.,* 2003; Taylor *et al.,* 2004) have shown that HHV 8 DNA has been detected in saliva. The replication of HHV 8 in the oropharynx might be involved in the transmission of KSHV in some beings (Phipps *et al.,* 2014). Sexual transmission plays a vital role in the transmission of KSHV among the risk group population, however, high sero-prevalence of HHV 8 among children and adolescent in endemic regions of Africa is attributed to non-sexual transmission (Sarmati, 2004). The transmission mode of KSHV infection is suggestive of non-uniform specificities and can be unevenly distributed between regions (Dollard *et al,* 2010; Butler *et al.* 2009). Studies have shown that KSHV may well be transmitted through saliva, blood or blood products, sexual contact and organ transplant (Bagni and Whitby, 2009; Pica and Volpi, 2007).

According to Bhutani *et al.,* (2015) the transmission route for HHV 8 remains unclear however, both sexual and horizontal transmission has been documented. Kaposi's sarcoma herpes virus is

predominantly transmitted through saliva but sexual, vertical and blood products have also been reported (Minhas and Wood, 2014). Studies done by Butler *et al.,* (2009); Malope *et al.,* (2008) did not seem to find any evidence linking sexual route as transmission mode for Kaposi's sarcoma herpes virus infection. Among the Chinese women, Zhang *et al.,* (2014), reported that heterosexual route of transmission of KSHV might not be dominant in their population because, both salivary exchange and Kissing occurs during homosexual and heterosexual contact and it might be a challenge to delineate whether the transmission was as a result of sexual or via saliva. According to Phipps *et al.,* (2014), mothers who did not shade Kaposi's sarcoma herpes virus in breast milk were able to shed KSHV in saliva, as a result, Phipps *et al.,* (2014), concluded that horizontal transmission predominantly through saliva was the main transmission route in endemic countries.

Cofactors are essential in facilitating transmission and development of KS infection, nonetheless, whether cofactors impact directly through immune system remains unknown (Ascoli *et al.*,2009; Dollard *et al.,* 2010). Other studies (Lin *et al.,* 2008; Maizels *et al*., 2009; Maizels, 2009; Wakeham *et al.,* 2011) suggests that parasitic infections might impact the immune function and might modulate the host immune response to KSHV. The transmission of HHV 8 might be influenced by a co-infection with a parasitic infection (Hübner *et al.,* 2013) in addition to viral control and shedding amongst sero-positive persons (Pfeiffer *et al.,* 2010). Conflicting results regarding the sero-epidemiology of HIV and HHV 8 are attributed to the wide range of populations with varied transmission patterns that have been studied (Rohner *et al.,* 2016). Mbulaiteye *et al.,* (2008), stated that there was possibility of salivary and nosocomial

transmission of HHV 8 in the rural Egypt, as this might have led to the explanation of geographical difference in the sero-prevalence of KSHV.

The modes of transmission of KSHV are poorly understood, however, in areas with endemic infection, it is thought that primary infection occurs during childhood possible through saliva transmission (Mbulaiteye *et al.,* 2006). In Zhejiang, China, blood borne is the main route of transmission for Kaposi's sarcoma herpes virus (Ju *et al.,* 2012). In the general population, the transmission of Kaposi's sarcoma herpes virus is not well understood, however, sexual transmission is common among homosexuals and the transmission route for heterosexual is not well documented (Whitby *et al.,* 2009). The transmission of KSHV through saliva is backed by high occurrence and the load at which the virus is shed in the saliva of children in Uganda and among homosexual men in the United State of America (Mbulaiteye *et al.,* 2006). After analyzing the DNA sequence of HHV 8, Mbulaiteye *et al.,* (2006) noted that there is proof that some children acquired KSHV infection from their mothers and other children got the infection from someone else in the family or from the community.

Another interesting finding by Hladik e*t al.,* (2012), was that an additional risk of 4.2% was observed among individuals who received blood stored for less than four days as compared to their counterparts who received blood that was stored for more than four days. This finding by Hladik e*t al.,* (2012), indicated that transfusion of blood stored for a short period was associated with increased risk of KSHV positivity and death.

2.4 Clinical manifestation of Kaposi's sarcoma herpes virus infection

Kaposi's sarcoma is a unique vascular tumor that manifest in different clinic-epidemiological forms (Schwartz *et al.,* 2008). Clinical manifestation of Kaposi's sarcoma is a hallmark of advance HIV disease and is grouped as stage 4 by WHO, nevertheless, most clinical and laboratory use T –lymphocytes subpopulations, CD4 and CD8 to associate advanced stage of HIV infection (Obirikorang and Yeboah, 2009). Recent proof point out that pediatric KS, irrespective of the epidemiologic variant, is dissimilar to the adult KS with an increased risk for spreading and progressive disease (Jackson *et al.,* 2016). The broad range of the clinicopathological spectrum of KS proposes that KS does not signify a single disease, yet they are treated the same (Cianfrocca *et al.,* 2010). Kaposi's sarcoma has an enormously inconsistent clinical presentation (La Ferla *et al.,* 2013). Kaposi's sarcoma associated with HIV has been shown not to have a distinct pattern of localization as the disease can start on any region of the skin, but might also appear on the oral, genital or ocular mucous membrane (Hoffmann *et al.,* 2017).

The clinical manifestation might have an indolent slowly progressive component that is restricted to the skin or it could take an aggressive and a fast progressive disease (Martellotta *et al.,* 2009). The KS lesion have been shown to have dissimilar localizations involving skin, lymph nodes, oral mucosa, and a number of internal organs such as lungs and gastrointestinal tract (La Ferla *et al.,* 2013). A classic KS lesion commonly have pink, red, or purple color varying in size from millimeters to a large are of a number of centimeters. These lesions manifest in different features ranging from macular, popular, nodular or plaque-like and in most cases painless (La Ferla *et al.,* 2013; Martellotta *et al.,* 2009). According to Curtiss *et al.,* (2016); El-mallawany *et al.,* (2016);

Stefan, (2015), Kaposi's sarcoma presents with a wide-range of clinicopathological variation depending on the clinical stage (patchy, plaque, nodular) of the disease, location of KS lesions (lymph node, internal, cutaneous) and the epidemiological classification (endemic, iatrogenic, epidemic). The KS lesions will first appear on the face, trunk and limbs, and the major manifestations are oral mucosal which tend to be purple patchyes with mild pain followed by plaque and nodules (Szajerka and Jablecki, 2007).

Early presentation of Kaposi's sarcoma lesions are violet or reddish brown macules and formation of papules in the hand and foot, which may later spread to the arms and legs years later (Trujillo *et al.,* 2015). Visceral lesions tend to be common especially in the GIT and the lung (Curtiss *et al.,* 2016). In addition, Lewis *et al.,* (208), stated that majority of the patients presents with cutaneous disease, visceral disease might infrequently come before cutaneous manifestation and the brain is spared. About 10% of patients with Kaposi's sarcoma herpes virus infection have visceral and mucosal involvement (Sampaio and Rivitti, 2008).Kaposi's sarcoma associated with AIDS has been shown to be clinically more aggressive and has the ability to yield lesions in all organs except the central nervous system (Fatahzadeh, 2012).The cutaneous lesions of AIDS associated Kaposi's sarcoma have varied pigmentation commencing with one or many red/red purplish macules which progresses to papules, nodules and plaques. The KS lesions are distributed anywhere on the body, however they have been observed to be predominantly around the head, trunk, back, neck, mucous membranes and in advanced stages they might colonize stomach, lymph nodes, intestine and lungs (James *et al.,* 2005).

According to Cottoni, (1996) AIDS associated KS has been classified in four stages where in stage 1; is localized and has <10 lesions on the body surface, stage 2; has > 10 lesions on the body surface, stage 3; aggressive and existence of one exophytic lesion with infiltrative invasive pattern, and stage 4; presents with non-cutaneous absence of skin lesions. Manifestation of Kaposi's sarcoma is not only linked with herpes virus infection but can also be due to other factors for instance, HIV tat gene and inflammatory cytokines (Schwartz *et al.,* 2008). Nevertheless, the contentious issue is whether Kaposi's sarcoma is a true malignant tumor or it is just a reactive hyperplasia (Goh and Calonje, 2008). The degree of immunosuppression and the magnitudes of the disease define the course of KS. Visceral KS is characterized by its ability to spread and bleeding tendency with fatal effect. The skin lesions of KS are often aesthetic problem that lowers the self-esteem of HIV infected patients (Akasbi *et al.,* 2012).

The lesions of Kaposi's sarcoma are made of distinctive spindle cells lining the lymph node or blood vessels and a variable infiltrating inflammation, symptomatic of a reactive hyper-proliferation caused by chronic inflammation and not a true neoplasm (Martellota, 2009). Characteristically, the manifestation of KS is limited asymptomatic purple macules or nodules (Hoffmann *et al.,* 2017). The lesions of Kaposi's sarcoma could encompass a large region of the body surface, particularly oral cavity, the face and lower extremities presenting an edema resulting from lymphatic blockage (Ganem, 2010). The KS tumors might remain an altered for long period ranging from months to years, or they might develop rapidly with few weeks (Hoffmann *et al.,* 2017). In the prodromal phase, the KS lesion appears red and flat on the skin, this is known as "patchy lesion". The patchy lesions are infiltrated with T cells, B cells, monocytes and numerous neovascularity (Ganem, 2010). Rapid growth of KS lesions can be

localized accompanied by pain, yellow green coloration resulting from hemorrhage (Hoffmann *et al.,* 2017).

Advance progression of KS tumor can lead to the development of central necrosis and ulceration. This tumor might even bleed easily (Hoffmann *et al.,* 2017). Plaque-like and the nodular KS lesions every so often become confluent and might be complemented by a substantial lymphedema (Hoffmann *et al.,* 2017). The KS lesion develops a purplish erythema and progresses to form plaque and nodules that tend to ulcerate easily (Hoffmann *et al.,* 2017). **A**n advanced phase known as "plaque lesion", subsequently develops, appearing indurated, often edematous and additionally red or violaceous. Lastly, "nodular phase" are formed; they constitute a proliferation of spindle cells more often linked with ulceration involving the deepest skin layer (Ganem, 2010). During the treatment of KS, regression does not only indicate reduction in the size of lesion but also there is a color changes from dark to bright red. It has also been observed that certain KS lesions might persist for life (Hoffmann *et al.,* 2017). Hemosiderin and or melanin deposit a rise exhibiting dirty-grey-brown hyperpigmentation which are caused by inflammation arising from melanocytes stimulation. Lymphedema might also persist for several years leading to chronic complications (Hoffmann *et al.,* 2017).

Localization of KS lesion in the gastrointestinal tract might be asymptomatic or cause symptoms ranging from abdominal pain, malabsorption, diarrhea, obstruction, weight loss, vomiting and or bleeding (Martellotta *et al.,* 2009). Additional important KS that involve visceral localization could be in the lungs; this can pose a severe life threat. The involvement of KS in the lungs could

be asymptomatic or may present with dyspnea, cough or coughing of blood (Martellotta *et al.,* 2009). When a chest X-ray is done, the results tend to vary from nodular interstitial or alveolar infiltrates to an extent where an isolated pulmonary nodule or pleural effusion is witnessed. The involvement of the visceral particularly pulmonary is prevalently extensive with cutaneous included and an advanced immunodepression grade (Martellotta *et al.,* 2009).

Wide spread of KS lesions particularly involving oral cavity or visceral disease and lymphedema, are consistent with CD_4 cell count of less than 200cell/µl and existence of other opportunistic infection (Gbabe *et al.,* 2014). Recovery of the immune response is dictated by the increase in CD_4 cell count above $200/mm^3$ and has been linked with steady clearing of KS in HIV infected patients (Gazzola *et al.,* 2009).

2.5 Etio-pathogenesis of Kaposi's sarcoma herpes virus infection

Stanescu *et al.,* (2007) reported that in 1872, Moritz Kaposi described uncommon angiosarcoma that was expressed primarily as skin lesions in elderly men. All forms of Kaposi's sarcoma are caused by KSHV/HHV 8 (Uldrick and Whitby, 2011; Betsem *et al.,* 2014; Gjyshi *et al.,* 2014; Coen *et al.,* 2014). The etiologic agent for Kaposi's sarcoma is Kaposi's sarcoma herpes virus ((Xinxing *et al.,* 2014; Olp *et al.,* 2015). According to Etta *et al.,* (2018), the causative agent of Kaposi's sarcoma is Human herpes virus 8. According to Tornesello *et al.,* (2010); Tania *et al.,* (2016), Tozetto-Mendoza *et al.,* (2016) human herpes virus 8 is the etiologic agent for AIDS-associated Kaposi's sarcoma, furthermore, it is the most resistant and severe form of KS tumor. Grundhoff and Ganem, (2004) has also linked the development of Kaposi's sarcoma tumor to

Kaposi's sarcoma herpes virus. Kaposi's sarcoma is a multicentric angioproliferative tumor of endothelial cells whose etiologic agent is Human herpes virus 8, presenting with clinical heterogeneity subject to the host immune conditions (Facciola *et al.*, 2017; Uldrick and Whitby, 2011). Kaposi's sarcoma virus contains an encoded gene that can prompt proliferations of cells, hinder apoptosis resulting to transformation of endothelial cells (Riva *et al.*, 2012).

Kaposi's sarcoma can be described as a cancer that forms masses in or on the lymph node, skin, or other organs (National Cancer Institute, 2017). The lesions produced by KS can occur singularly, restricted in an area or widely spread (National Cancer Institute, 2015). The KS manifestation may worsen gradually or rapidly, appearing as a flat form or raise (Schwartz *et al.*, 2008). The cancer that is caused by Human herpes virus 8 is called Kaposi's sarcoma-associated Herpes virus (KSHV) (De Paoli *et al.*, 2016).Kaposi's sarcoma herpes virus have its place among the gamma-herpes family and similar to other herpes virus, HHV 8 has the ability to create a persistent infection particularly in the lymphoid cells (La Ferla *et al.*, 2013; Cesarman, 2014). Human herpes virus 8, belongs to gamma herpes virus family and it is the etiological agent that is linked to all the forms of Kaposi's sarcoma infection (Razonable, 2011; Zhang and Wang, 2017). The etiological agent of Kaposi's sarcoma which predominantly affects HIV infected adults is HHV 8 (Feiterna-Sperling *et al.*, 2016). The causative agent of Kaposi's sarcoma virus infection is the human herpes virus, in addition, it causes other diseases like primary effusion lymphoma and multi-center Castleman's disease (Ju *et al.*, 2012).

Kaposi's sarcoma herpes virus like other types of herpes virus encodes several genes that will help it escape immune destruction through the primary infection. These immune eversion relates to inhibition of interferon response, autophagy inhibitor, anti-apoptosis factors and clashing with Natural Killer (NK) cell-mediated control of KSHV infection (Jung and Münz, 2015).According to O'Hara *et al.,* (2009); Hosseinipour *et al.,* (2014), Kaposi's sarcoma herpes virus creates molecular latency, KS tumor cells included and at any given time, only a low percentage of KS tumor cells replicate while the majority of the cells expresses the viral latent miRNAs and viral latent gene.

In sub-Saharan part of Africa, Kaposi's sarcoma has been documented to be the commonest AIDS-associated malignancy caused by Kaposi's sarcoma herpes virus (Katz and Abera, 2015). According to Caterino-de-Araujo *et al.,* (2010) human herpes virus causes KS and HIV-1 plays a vital role in its development, however, the use of antiretroviral therapy has shown to diminish the KS lesions. Wood and Feller, (2008) observed that the oncogenesis that is linked to HHV 8 are usually as a result of HIV and AIDS.

Kaposi's sarcoma herpes virus is all the times detected in cancer tissue. The precise function of HHV 8 in the pathogenesis of KS is not completely understood and HHV8 infection does not certainly lead to KS (Minhas and Wood, 2014). Resulting from manipulation of endothelial cell, HHV 8 infection might result to viral persistence and beginning of KS (Minhas and Wood, 2014). Kaposi's sarcoma is a multicentric angioproliferative tumor of endothelial cells whose etiologic agent is Human herpes virus 8, presenting with clinical heterogeneity subject to the host

immune conditions (Facciola *et al.,* 2017; Uldrick and Whitby, 2011). While in the endothelial cells (ECs), KSHV has the ability to undergo two distinct replication cycles; lytic and latent cycle. The initial step of KSHV infection is attachment, where the virus's glycoproteins adheres to the host's cells receptor site on the ECs (Dittmer and Damania, 2013). Arising from the first step, the virus can undergo any of the following three special effects; active viral replication, viral clearance and or viral persistence in a transmuted cell (Dittmer and Damania, 2013).

In the event that KSHV starts a persistent infection, the viral DNA is moved to the host cell's nucleus where it remains as multicopy circular episomal DNA, initiating the latency phase (Mesri *et al.,* 2010; Unemori *et al.,* 2013) During this phase, very little or no virus genes are expressed with no creation of new complete virus particle (Mesri *et al.,* 2010; Unemori *et al.,* 2013). In the lytic phase, the recently produced virus particle spreads to the ECs, multiplies the number of the transformed cells (Mesri *et al.,* 2010; Unemori *et al.,* 2013).

A significant contributor of KS development is the dysregulation of autocrine and paracrine angioproliferative signaling (Sun and Cesarman, 2010). Mainly during both the lytic and latent cycle, a number of KSHV oncogenes are encoded and anti-apoptotic gene that is known to induce the proliferation of the infected ECs, transformation, production of cytokine, evasion of immune response, anti-apoptosis and angiogenesis (Sun and Cesarman, 2010). The pathogenesis of KS is presented by an abnormal neoangiogenesis, proliferation of cancer cells and inflammation of endothelial cell (Cancian *et al.,* 2013). Elevated production of proinflammatory

and proangiogenic cytokines and growth factors by the cells that are infected is the cardinal sign of the pathogenesis of angio-proliferation (Sun and Cesarman, 2010).

Mainly, AIDS-KS cell lines, stromal vessels, and spindle cells express increased levels of Vascular Endothelial Growth Factor (VEGF) and VEGF receptor-1, VEGF-2 and VEGF-379-83 (Uldrick and Whitby, 2011). The regulation of VEGF upwards is supported by a number of KSHV genes like viral interleukin-6, K1, Latency-Associated Nuclear Antigen (LANA) and viral G Protein-Coupled Receptor (vGPCR) (Uldrick and Whitby, 2011).

Predominantly, the K1 gene has been shown to encode for transmembrane glycoprotein that is related to the antibody receptor family that has been linked in mouse, with the expansion of plasmacytoid and sarcomatoid cancer and increased levels of Vascular Endothelial Growth Factor-A (VEGF-A) in the lymph node region (Pinzone *et al.,* 2015). Furthermore, the function of LANA is to promote replication of KSHV genes during latency and also change the role of p53 (tumor protein) and RB (retinoblastoma) enhancing transformation of the virally infected cells by supporting the survival of the cell (Saha *et al.,* 2010; Nunnari and Pomerantz, 2005). Uldrick and Whitby, (2011) reported that vGPCR enhances angiogenesis and transformation of cells via the mammalian Target of Rapamycin (m-TOR) pathway. The functions of cytokines in the pathogenesis of KS are to promote angiogenesis and infiltration of inflammation (Kaplan, 2013).

Oncostatin M is a cytokine that is produced by macrophages and activated T cell, it is believed to be mitogenic for HIV-KS derived spindle cells (Flepisi *et al.,* 2014). In conclusion, in recent times, the secretion and expression of Matrix metalloproteinases (MMPS) like MMP-1, MMP-2, MMP-7, and MMP-9 have been demonstrated. These molecules have shown to permit vessel destabilization and migration of infected cells (Dai *et al.,* 2013). Their expression is enhanced by Extracellular Matrix MetalloPRoteinase Inducer (EMMPRIN) that is a heavily glycosylated transmembrane protein that is a member of antibody superfamily induced by LANA as a result of direct interaction with the promoter gene with other transcription factors including zinc finger transcription factor Sp1 (Dai *et al.,* 2013).

The genesis and development of Kaposi's sarcoma arises following activation of interleukin (IL) and viral G protein-coupled receptor (Wood and Feller, 2008). The viral G protein-coupled receptor (vGPCR) plays an important role in the initiation and preservation of KS (Katz and Abera, 2015). Among the first lesion produced by the patients, production of fibroblast growth factor, tumor necrosis factor, IL-6, and vascular endothelial growth factors are essential for the commencement of KS lesion (Di Lorenzo *et al.,* 2007). Small, painless lesions are initially produced but over time they can be ulcerative and painful (Dezube, 1996).

Depending on the disease progression, the KS lesions exist in various forms however; the lesions can be plaque, patchy, nodular, and aggressive and or lymphanginoma like (Cattelen *et al.,* 2005). The CD_4cell count usually needs to be less than 200/mm^3 for Kaposi's sarcoma infection to manifest and this is a hallmark of AIDS (Dezube, 1996). The chances of developing AIDS

related KS is increases by the decline in the immune status of a HIV positive person (Ensoli *et al.,* 1990). Pelser *et al.,* (2009) reported that the replication of KSHV might be influenced by environmental factors such as soil and plants, however, this link is yet to be established.

2.6 Laboratory diagnosis of Kaposi's sarcoma herpes virus

Timely detection of KSHV infection is of great importance as this will enable prevention and treatment measures against KSHV-associated lymphoproliferative disease (Minhas *et al.,* 2008). In addition, early detection for KSHV helps in disease monitoring and its transmission (Olp *et al.,* 2013). Even with undetectable viral load for HIV and regaining of CD4 cells after uptake of cART, a significant proportion of Kaposi's sarcoma has been detected in AIDS patients (Maurer *et al.,* 2007; Krown *et al.,* 2008; Stebbing *et al.,* 2008). Cutaneous diagnosis of KS is based on clinical findings; however, questionable cases should be subjected to histological diagnosis (Hoffmann *et al.,* 2017). Early studies of individuals infected with HIV and AIDS associated KS used archival samples, and in the United States these subjects were heavily pretreated with ART, chemotherapy or both (Dittmer, 2011).

Kaposi's sarcoma can be diagnosed by obtaining a tissue biopsy from an infected patient, while the extent of the disease can be diagnosed through medical imaging (Schwartz *et al.,* 2008; (National Cancer Institute, 2017). According to de Souza *et al.,* (2007); Zong *et al.,* (2007), saliva showed to be a suitable sample for the detection of asymptomatic KSHV infection despite the recurrent shedding of the virus through saliva and in cases of low HHV 8 load especially in the absence of KS tumor.

The sensitivity of detecting KSHV depends on the sample selected for analysis, obtaining biopsy from AIDS-KS individual for instance was found to yield better results compared to the using of PBMC from the same patient (Oetvoes, *et al.,* 2014). Even though studies (Mancuso *et al.,* 2008; Marshall *et al.,* 2010; Isaacs *et al.,* 2015; Cordiali-Fei *et al.,* 2015) have demonstrated association that exist between KSHV variability and pathogenic potential especially on classic, endemic and epidemic form of KS, other studies (Tornesello *et al.,* 2010; Kouri *et al.,* 2012) have been unable to do so, this inconsistencies might be attributed to lack of standard protocol for genotyping of HHV 8 especially on the choice of target KSHV genome and also due to the coverage of HHV 8 variants.

2.6.1 Serological diagnosis of Kaposi's sarcoma herpes virus

In the year 2003, Dukers and Rezza used serological assays (Immunofluorescence, enzyme immunoassays and Western blot) for detecting HHV 8 because the virus could not be cultured. Lorin *et al.,* (2016) have reported using enzyme immunoassay (EIA), immunofluorescent assay (IFA) and antigen detection technique. Upon exposure to human herpes virus, the infected host produces antibodies against the virus which can be determined using immunofluorescence assays (Caterino-de-Araujo *et al.,* 2010). Enzyme Linked Immunoassay (ELISA) can be used in the laboratory to test for Kaposi's sarcoma herpes virus (Mbisa *et al.,* 2010). Antibodies to HHV 8 can be demonstrated by use of immunosorbent assay (ELISA), immunofluorescent assay (IFA), Western blot and immunohistochemistry (Edelman, 2005; Crabtree, 2013). The use of ELISA technique will depend on the HHV 8 antigens used and also whether the antigens are recombinant, synthetic peptides or viral lysates (Edelmen, 2005).

Dollard *et al.,* (2011), tested blood samples for the presence of KSHV antibodies using enzyme immunoassay (EIAs) and indirect immunofluorescence assay (IFA). In a study conducted by de Sanjose *et al.,* (2009), plasma samples were tested for antibodies against Kaposi's sarcoma herpes virus using ELISA and latency-associated nuclear antigen (LANA). There are two general platforms of Kaposi's sarcoma herpes virus serologic assay, the immunofluorescence-based assay and ELISA based assy (Labo *et al.,* (2014). This bead-based multiplex assay developed to detect antibodies for KSHV has been useful for sero-diagnostic and analysis of very large sample size (Labo *et al.,* 2014).

A study conducted in Turkey by (Altuğlu *et al.,* 2016), used ELISA method to detect IgG antibodies structured against HHV 8 lytic antigens. The findings by (Altuğlu *et al.,* 2016), revealed that HIV positive individuals were HHV 8 sero-positive. Antibodies detection of Kaposi's sarcoma herpes virus is quite controversial because KSHV expresses several antigen and host responses are quite highly variable as a result a recombinant ELISA for LANA improves the testing (Mbisa *et al.,* 2010). Kaposi's sarcoma herpes virus is diagnosed by detection of the virus protein latency-associated nuclear antigen (LANA) from a biopsy (Johann and Dirk, 2017). Immunofluorescent assay entails integration of virally infected cell lines that are latently infected with expression of LANA-1 or cells that have an expression of lytic antigens as a result of chemical induction (Edelmen, 2005; Crabtree, 2013). The western blot technique uses an electrophoretically separated virally infected cell lysates or it can use a whole viral lysate, which are then moved to nitrocellulose followed by reactive antigen detection (Hudnall, 2004). The serological methods have low specificity and sensitivity (Edelman, 2005).

According to Hosseinipour *et al.,* (2014), all Kaposi' sarcoma herpes virus infected cells tend to transcribe the so-called latent messenger RNAs and a negligible set of viral proteins. Pereira *et al.,* (2013) stated that Kaposi's sarcoma latency associated nuclear antigen (LANA) has become the determining diagnostic marker for KS. Pereira *et al.,* (2013) further stated that LANA is a robust specific monoclonal antibody test that is commercially available and is an automated immunohistochemical staining technique.

A positive result by LANA test unequivocally confirms the diagnosis of KS in a fitting clinicopathological context (Pereira *et al.,* 2013). According to Cesarman, (2011); Broccolo *et al.,* (2016), expression of LANA is not confined to KS lesions, Broccolo *et al.,* (2016) further states that the DNA of KSHV is frequently present in HIV positive patients with KS even at the time of ART introduction or with the progression of KS, but might be undetectable in nearly all patients on ART and those experiencing KS regression. According to van Bogaert (2012), immunohistochemical biopsy has be demonstrated to be the 'gold standard' for the diagnosis of Kaposi's sarcoma herpes virus.

Krown *et al.,* (2012); Mohanlal and Pather, (2015) stated that LANA staining is variable, as it depends to some degree on the clinical progression stage of the disease, particularly those involving the skin lesions. Mohanlal and Pather, (2015) observed that variable LANA staining pattern in cases of KS does not relate in any way to the patients age, gender, the clinical subtype of KS, the distribution, or even the CD_4 cell count. In addition, Mohanlal and Pather, (2015) stated that factors that influences the expression levels of LANA staining remains unknown

despite the fact that stages of KS and the immunohistochemical technique have been shown to influence variability in staining.

Undetectable LANA expression when an immunohistochemistry is done can be attributed to very low viral copies of KS cells, besides, failure to demonstrate LANA does not automatically rule out the diagnosis of KS especially in an appropriate clinicopathological setting (Mohanlal and Pather, 2015). According to Snodgrass *et al.,* (2016) Polymerase Chain Reaction (PCR) technique has shown to be reliable when it comes to detection of HHV 8 in KS lesions even in the absence of LANA expression. Latest study by Speicher *et al.,* (2015), stressed the potential of contamination of KS biopsies followed up by false positive results when PCR method is used in the detection of HHV 8, therefore, a negative LANA expression should in most times prompt a careful reconsideration of all the clinico-pathological features and the need to factor in a differential diagnosis be considered. Hosseinipour *et al.,* (2014) latency locus (LANA, vCyc, vFLIP, kaposin, and microRNAs) for the virus was transcribed. The findings by Hosseinipour, *et al.,* (2014), revealed that multiple subtypes of KS existed in HIV and in KS patients who were treatment naïve.

Among HIV-1 cohort in Zambia, Kumar *et al.,* (2013), studied the prevalence of Kaposi's sarcoma herpes virus and its antibodies titers; the findings revealed that HIV-1 associated KS individuals had significantly higher prevalence of neutralizing antibodies in comparison to non-KS but KSHV infected patients. These findings by Kumar *et al.,* (2013), indicated that lytic replication by KSHV occurs during KS development might have contributed to the production of

neutralizing responses. In one of the study by Lepone *et al.,* (2010), it was stated that identification of Human Leucocyte Antigen (HLA) had restricted CD_8 T cell epitope that was present in LANA-1, K12 and K8.1 protein, these epitopes were considered important induction of immune mediators in KSHV seropositive individuals. Furthermore, Robey *et al.,* (2010), made available a list of epitopes that are known to KSHV immunogenic open reading frames (ORF). Robey *et al.,* (2010) stated that these were grave steps that were made in understanding the function played by T cell in the pathogenesis of KSHV infection.

2.6.2 Histo-pathological diagnosis of Kaposi's sarcoma herpes virus

The diagnosis of KS can be realized through Haematoxyline and Eosin (H & E) staining technique (Mwakigonja *et al.,* 2008; Tumwine *et al.,* 2010). The histopathological diagnosis of KS is not of little value (Johann and Dirk, 2017). Microscopic examination of the H & E stained tissue biospsy entails looking for proliferation of spindle cells and oedema (Kumar *et al.,* 2013). While Kaposi's sarcoma can be pointedly suspected in clinical setting, a study by Amerson *et al.,*(2016) confirmed that clinical diagnosis of KS has limited predictive value. In 1979, Ackerman, stated that the gold standard for diagnosis of KS was by histopathological confirmation, however, it was observed that the diagnosis was not always straight forward especially when the pathologist was not familiar with the spectrum of histopathological features of KS. A well trained histopathologist can accurately diagnose KS however typically they are, provided the clinical lesions are well established (Luzar *et al.,* 2007; Grayson and Pantanowitz, 2008; O'donell *et al,* 2010; Bunn *et al.,* 2013).

Kaposi's sarcoma has a wide morphological spectrum that might mimic several unrelated non-neoplastic and neoplastic conditions, resulting to diagnostic challenges to the pathologist. As a result, a pathologist should be conversant with variants of KS including telangiectatic, keloidal, anaplastic, cavernous hemangioma-like, KS with myoid nodules, solid, desmoplastic, bullous, pyogenic granuloma-like, ecchymotic, pigmented KS, intravascular, and KS with sarcoid-like granuloma (Luzar *et al.,* 2007; Grayson and Pantanowitz, 2008; O'donell *et al,* 2010; Bunn *et al.,* 2013; Sutton *et al.,* 2014).

The spindle-shaped cells have been shown to be present in all forms of KS, they signify a unifying feature, forms the basis of diagnosis and constitutes the huge part of the proliferating cell fraction (Grayson and Pantanowitz, 2008; O'donell *et al,* 2010; Amerson *et al.,* 2016). The spindle- shaped cells that are seen in KS lesions are thought to be of endothelial lineage, though they also have features of smooth muscles cells and pericytes. There is even proof that KS is a mixture of cancer cells (Liu *et al.,* 2010; Ojala and Schulz, 2014). The tumor stems cells that are present in KS could preserve the cancer and spawn the highly proliferative spindle cells. The endothelial stem cells would be de-differentiated by loss of differentiation markers (Cheng *et al.,* 2011; Ojala and Schulz, 2014).

In the prodromal phase, Kaposi's sarcoma presents like a flat red patchy containing inflammatory infiltrate, scarce spindle cells with extravasation of erythrocytes (Speicher *et al.,* 2015). Patchyes progress into plaques and the spindle cells predominates. At an advanced stage, there is development of macroscopic visible nodules known as the nodular stage. Spindle cells at

nodular stage are arranged either around vascular space containing erythrocytes or they form fascicles flowing in different direction (Orenstein, 2008; Gramolelli and Schulz, 2015). According to Tumwine *et al.,* (2010); and Speicher *et al.,* (2015), histological diagnosis does not detect the presence of causative agent and the pathologist sometimes find it difficult to differentiate KS from other cancers, as certain conditions like bacillary angiomatosis, spindle cell haemangioendotheliomas, granuloma annulare, spindle cell melanoma, and fibrohistiocytic tumors. Diagnosis of KS by histology includes presence of spindle-shaped cells that have vascular channels lined up by an abnormal endothelial cell assembled like catch of fish. In addition, extravasated erythrocytes, hemosiderin and fibrosis might also be seen (Hoffmann *et al.,* 2017).

2.6.3 Molecular detection and characterization of Kaposi's sarcoma herpes virus

Kaposi's sarcoma herpes virus can be diagnosed by the use of Polymerase chain reaction (PCR) in tissues and it has shown to give utmost specificity compared to all other test being used (Crabtree, 2013). Identification of Kaposi's sarcoma herpes virus can be done by use of Polymerase Chain Reaction (PCR) in tissues (Biggar *et al.,* 2000). The use of PCR technique in the detection of KSHV has been shown to give utmost specificity compared to the use of test that determines exposure to infection (Crabtree, 2013). Crabtree in (2013) stated that the use of PCR technique in diagnosis of KS can detect approximately 95% of all the cases. However, the cost associated to the use of PCR is quite expensive as a result limiting the clinical application of HHV 8 DNA detection in resource limited facilities (Biggar *et al.,* 2000; Edelman, 2005; and Crabtree, 2013).

Tornesello *et al.,* (2010), stated that DNA samples obtained from patients living in Cameroon, Kenya and Uganda were subjected to PCR amplification of HHV 8 and later followed by direct nucleotide sequencing and phylogenetic analysis. Hosseinipour, *et al.,* (2014), detected Kaposi's sarcoma herpes virus viral load from plasma samples, in addition to that, real-time quantitative PCR was used to transcription of KSHV. Jalivand *et al.,* (2012), reported to have successfully extracted DNA from PBMC or plasma sample and paraffin embedded blocks (sections about 10 mm) and thereafter performed DNA amplification on them using nested PCR technique. Kakavand-Ghalehnoei *et al.,* (2016), also reported to have used PCR technique to amplify DNA of HHV 8. Polymerase chain reaction has been used by Tania *et al.,* (2016) to detect ORF K1 fragment of HHV 8, to add-on, the study used saliva from patients with HIV and AIDS without previous manifestation of KS. Later on, Tania *et al.,* (2016), stated that phylogenetic analysis of HHV 8 was carried out to look at the distribution of HHV 8 genotypes.

According to stebbing e*t al.,* (2008); Tania *et al.,* (2016), PCR primers have been used to amplify HHV 8 DNA and thereafter genotyping done to determine the distribution of KSHV strains. Exploration of biomarkers might hypothetically be useful for the prognosis of Kaposi's sarcoma based on different loci of KSHV genome (Marshall *et al.,* 2010; Mancuso *et al.,* 2011; Borges *et al.,* 2012; Cordiali-Fei *et al.,* 2015) or based on a whole KSHV genome (Olp *et al.,* 2015). In addition, the exploration of biomarkers has progressed parallel to the molecular characterization of HHV 8 strains infecting people from different epidemiological and clinical forms of KS (Marshall *et al.,* 2010; Mancuso *et al.,* 2011; Borges *et al.,* 2012; Cordiali-Fei *et al.,* 2015) for instance short targets of KSHV were carefully chosen from AIDS-KS to identify

characteristic molecular pattern but had to be with restricted coverage of the repertoire of KSHV genotype (Ishak *et al.,* 2007; Kasolo *et al.,* 2007; da Silva *et al.,* 2011).

In Zimbabwe among AIDS- Kaposi's sarcoma patient, the laboratory analysis by Tiffany *et al.,* (2008) entailed extraction of DNA from peripheral blood mononuclear cells (PMBCs) and/or plasma, amplifying the DNA by nested PCR, thereafter the amplified DNA were directly sequenced. Furthermore, Tiffany *et al.,* (2008), had to molecularly clone DNA extract from one patient and the results generated from it were analyzed. In Brazil, Tozetto-Mendoza *et al.,* (2016), detected fragments of ORF K1of KSHV from saliva and blood sample using polymerase chain reaction technique which were then genotyped for genetic variability

Diagnosis of Kaposi's sarcoma herpes virus is improved when mRNA of the KSHV is detected from the KS lesion as opposed to using blood sample (Martín-Carbonero *et al.,* 2008; Bower *et al.,* 2013). Pathogenic molecules for KSHV such as K1, K15 and some viral interferon regulatory factors have been detected as mRNAs. In addition to that, "extended" viral transcription has been detected in abortive gamma-herpes virus replication (Chandriani and Ganem, 2010; Chang and Ganem, 2013; Arias *et al.,* 2014; Canny *et al.,* 2014). Sequence analysis of KSHV genome can be done using next-generation sequencer machine. The output readings of the assembled Kaposi's sarcoma herpes virus genomes are read from IIIumina HiSeq 2500 and are filtered using trimmomatic (Bolger *et al.,* 2014). During the comparative and phylogenetic sequence analysis, the KSHV is assembled, aligned using Kalign2, multiple alignment can then be done to generate the genomes maximum-likelihood and phylogenetic tree

drawn using PhyML (Guindon *et al.,* 2010) with 1,000 bootstrap replicates and the tree visualized using MEGA6 (Tamura *et al.,* 2013). Mutations on the genome can be visualized using Vista software (Frazer *et al.,* 2004).

Kourí *et al.,* (2012), obtained biopsy from Kaposi' sarcoma patients in Cuba, extracted the DNA, amplified the K1 gene using nested PCR, sequenced the products of the PCR, assembled the sequences and edited them using the sequencher TM program. Thereafter, Kourí *et al.,* (2012), aligned the sequences using LUSTAL X version 1.81, drew a phylogenetic tree of K1 amino acid sequences using MEGA program, version 4.9. Finally, Kourí *et al.,* (2012), obtained bootstrap values was from a consensus tree based on 1000 randomly generated data set. From biopsy, the sequencing of a whole KSHV genome has been done successfully in Western region where the virus is not endemic (Olp *et al.,* 2015). Olp *et al.,* (2015) reported that they managed to sequence 16 distinctive Zambian KS-derived, Kaposi's sarcoma herpes virus genome using a targeted KSHV enriched protocol followed by IIIumina deep-sequencing. Ouyang *et al.,* (2014), was able to collected PBMCs and formalin-fixed paraffin-embedded tissue, extracted the DNA from them, amplified the KSHV ORF-K1 gene using nested PCR, cloned the purified K1 gene fragments into pGEM-T, constructed a phylogenetic tree from sequenced products using neighbor-joining (NJ) analysis by phylip (version 3.68) and MEGA (version 4.0.2). Ouyang *et al.,* (2014), evaluated the statistical reliability of NJ tree using 1000 bootstrap samples.

A whole-genome of Kaposi's sarcoma herpes virus was sequenced from saliva; this was obtained from individual in Uganda who was free of KSHV-associated disease. The purpose was to study

variability between KSHV sequences isolated from different sources (Asiki *et al,* 2013). Several studies (Butler *et al.,* 2009; Pfeiffer *et al.,* 2010) done in Uganda have provided valuable contribution towards understanding the sero-epidemiology and transmission of Kaposi's sarcoma herpes virus. Sequences obtained from Ugandan individuals were subjected to comparative sequence analysis, aligned using MAFT (v7.0) (Katoh and Standley, 2013) and viewed using Ali View software.

2.6.4 Use of animal model & non-animal model to understand pathogenesis of KSHV

Animal model and non-animal model has been developed to study Kaposi's sarcoma herpes virus infection, nevertheless, primate's models have not been able to establish persistent KSHV infection (Chang *et al.,* 2009). Therefore, Chang *et al.,* (2009) stated that, there is still need to explore other animal models such as rodents to study the pathogenesis of KSHV in both latent and lytic viral gene expression.

Brehm *et al.,* (2010), stated that a humanized mouse has been shown to be a perfect model for researching on human viral infection. Wege *et al.,* (2008), reported that, the humanized mouse can produce the human mucosal immune systems and HLA restricted antigen, specific humoral and cellular responses. Wang *et al.,* (2008) used this humanized mouse model to study KSHV infection and the findings were that latent and lytic viral transcript, as well as viral proteins were demonstrated.

2.7 Antiviral therapy and prevention of KSHV

Currently there is no vaccine against KSHV and for one to avoid KS infection; the need to minimize the chances of being infected by the virus is of great importance (Engels *et al.*, 2006). Patients who have been newly diagnosed with HIV should be put on prompt antiretroviral treatment. In addition, chemotherapy might be used for the management of KS in the early stages as this has shown to be essential in 20% of the cases (Bower *et al.,* 2009). A decrease in HIV plasma viremia implies that the infected patient's immune status becomes reconstituted and the KS lesion tend to stabilize or resolve completely without the need to use other specific form of treatment (Cattelan *et al.,* 2005).

Progress have been made as far as treatment of KS is concerned, however, the clinical course associated with KSHV ranges from indolent state to severe and might lead to morbidity and mortality (Krown, 2011; Dittmer *et al.*, 2012). There is no standard therapeutic guideline for KSHV due to its heterogeneity however; different therapeutic options are available for use. Rate at which KS tumor grows, symptoms presented by the infected patient, immune status and concurrent HIV related complications are considered when making treatment decision (Ruocco *et al.,* 2013). Due to difference in the epidemiology of HHV 8 genotype, HIV and sequence of acquisition of HIV and HHV 8, both are likely to influence the incidence of Kaposi's sarcoma and the outcome of ART (Bohlius *et al.,* 2015). Antiretroviral therapy has led to the decline in the incidence of HIV associated Kaposi's sarcoma in both endemic and non-endemic regions where HHV 8 are distributed (Bohlius *et al.*, 2015).

The need to develop a vaccine against KSHV has been imperfect due to lack of an animal model that can be used to gauge the protective effects of the virus (Wu *et al.,* 2010). The transmission and shedding of KSHV might be reduces by developing a vaccine that will control the lytic replication and decrease the viral load of HHV 8 among the infected subjects (Wu *et al.,* 2012). The availability of KSHV is likely to reduce the morbidity and mortality associated with the virus especially in endemic African continent (Wu *et al.,* 2010). Franceschi *et al.,* (2008), stated that the ongoing argument among researchers and policy makers is whether a vaccine is needed for Kaposi's sarcoma herpes virus since incidences of KS are decreasing due to effectiveness of ART in developed countries. Franceschi *et al.,* (2008) further stated that high prevalence of KSHV in Africa and endemic regions is questionable as to whether ART and other public health prevention strategies might be effective in preventing Kaposi's sarcoma herpes virus infection in developing countries. In conclusion, Franceschi *et al.,* (2008), reported that the first step towards developing a protective vaccine against KSHV is the need to understand the viral antigens and the production of protective immune response against KSHV antigens.

Mixed Kaposi's sarcoma results have been seen during clinical experience when anti-herpes virus drugs are administered to patients with KSHV (Pantanowitz *et al.,* 2008; Uldrick *et al.,* 2011). Regarding treatment of Kaposi's sarcoma herpes virus, it still remains unclear which patient benefit, whether those that are on antiretroviral therapy concurrent with anti-KSHV treatment (ganiclovir, cidofovir, valganciclovir) or those that are on the first-line therapy of ART alone since ART alone can result to resolution of KS (Nguyen *et al.,* 2008; Brook *et al.,* 2010; Mosam *et al.,* 2012).Antiretroviral therapy is very important in management of HIV-associated KS, nevertheless, recurrence of Kaposi's sarcoma has still been observed in patients who do not

respond to ART (Krown *et al.,* 2008; Nguyen *et al.,* 2008). It has been observed by Achenbach *et al.,* (2012); Cox *et al.,* (2013); Letang *et al.,* (2013), that 10%-20% of patients with Kaposi's sarcoma might develop worsening KS (KS-inflammation reconstitution syndrome-KS-IRIS) disease even after initiating ART.

The WHO ART guideline of 2013 that recommended commencement of ART in asymptomatic adults with CD4 T cell count below 500 cell/ μL was aimed at preventing the development of KS in HIV positive people (World Health Organization, 2013). Cases of KS in HIV infected patients has significantly reduced since the introduction of ART (Bohlius *et al.,* 2014). According to Davidson *et al.,* (2014) early introduction of ART decreases the risk of developing Kaposi's sarcoma to approximately 80%, this was observed in a study done in South African patients among HIV infected cohort.

In Cuba, despite wide distribution of antiretroviral treatment, Kaposi's sarcoma has not been reported to be on the decline (Vivian *et al.,* 2012). This finding by Vivian *et al.,* (2012), was similar to Maurer *et al.,* (2010) who reported an increase in AIDS associated KS among HIV infected individual with HIV viral loads that were suppressed and their immune reconstituted. In Africa, discrepancy on AIDS-KS treatment outcome has continued despite availability of ART, whereas in Europe and North America complete or partial response to ART with chemotherapy resulted to the decline in AIDS-associated KS (Cooley *et al.,* 2007; Nguyen *et al.,* 2008; Martin-Carbonero *et al.,* 2008). Borok *et al.,* (2010), made an observation that only 19% of Zimbabweans had partial or complete resolution of AIDS associated KS and 16% died despite

being on ART, to add on, these patients had HIV-1 plasma below the limit of detection and their CD4 cells had significantly increased.

The treatment of Kaposi's sarcoma is different from that of other tumor because it is a systemic disease that is associated with manifestation of vascular tumors (de la Puente *et al.,* 2015). To date, there is no standard treatment guideline for Kaposi's sarcoma; however, treatment depends on the subtypes, staging of KS and the patient's immune status (Vogt *et al.,* 2008). Present treatment of Kaposi's sarcoma comprises of topical treatment for instance, radiotherapy, surgery, local chemotherapy or topical immunotherapy (Schartz *et al*., 2008; Schwartz *et al.,* 2008; Trakatelli *et al.,* 2010; Brambilla *et al.,* 2010), and topical β-receptor blocking agent (Alcántara-Reifs *et al.,* 2016). Systemic chemotherapy or immunotherapy may be administered to Kaposi's sarcoma patients with dispersed lesions (Di Lorenzo *et al.,* 2008; Brambilla *et al.,* 2008). Following the commencement of antiretroviral therapy, Makombe *et al.,* (2008); Nelson *et al.,* (2010), stated that, AIDS patients with Kaposi's sarcoma had worse clinical outcome compared to AIDS patients without KS.

2.7.1 Kaposi's sarcoma and cART (combination antiretroviral therapy)

Patients infected with AIDS-associated KS respond to cART by 50% depending on geographical location and severity of the presentation, thereby, resulting to immune reconstitution and HIV suppression (Semeere *et al.,* 2012; Krell and Stebbing, 2014; Krown *et al.,* 2014; Chinula *et al.,* 2017). According to Achenbach *et al.,* (2011); Letang *et al.,* (2013); Kowalkowski *et al.,* (2015), to treat AIDS-KS, up-to-date cART and monitoring its efficacy are crucial. Kaposi's sarcoma

immune reconstitution occurs when a portion of AIDS-KS responds to introduction of cART with disease advancement (Achenbach *et al.,* 2011; Letang *et al.,* 2013; Kowalkowski *et al.,* 2015). Withdrawing the use of cART should not be an option, however, simultaneous chemotherapy and maybe immune modulating adjuvants may be of help in the short-term (Speicher *et al.,* 2013). The use of steroid has shown both KS disease stabilization and acceleration (Catricalà *et al.,* 2009).

A robust randomized clinical trial has established that cART provides an effective protection against KS development, even in patients with moderately preserved immune system. Decline in the risk of infection that is linked to cancer was driven by reduction in the incidence of KS (Borges *et al.,* 2016). Comparable findings were also witnessed in the SMART trial, where patients who were on CD_4 T cells-guided cART strategy confirmed that the incidence of KS lesion was higher among those who had interrupted treatment than those on constant cART (Silverberg *et al.,* 2007). A new study conducted from the larger region of European cohort indicated that, the incidence and the risk factors for developing KS changes over time from commencement of cART. In addition, it was recorded that whereas a low CD_4 cell count poses a risk of developing KS, HIV-1 viral load that is detectable became a significant risk factor in patients who started cART many years prior, independently of their immunodeficiency status (Cancer Project Working Group for the Collaboration of Observational HIV Epidemiological Research Europe (COHERE) study in EuroCoord, & Cancer Project Working Group for the Collaboration of Observational HIV Epidemiological Research Europe (COHERE) study in EuroCoord, 2016).

Bower *et al.,* (2013), in a prospective cohort study conducted in the United Kingdom, it was noted that 18% had developed KS while on up-to-date cART and among them 9% had HIV viral load of < 50 copies/ml. Decreased HIV plasma viremia and immune reconstitution tend to stabilize KS lesions or at times it resolves the KS completely without any specific therapy. Pursuant to that, Bower *et al.,* (2013), stated that there is need to initiate cART in HIV infected patient who are naïve to ART and have been newly diagnosed with KS.

The survival years was five, and at 95% among 213 cART-naïve HIV-1 patients who had clinical presentation of KS at an early stage but had cART initiated. It was further noted that progression-free survival was at 77% (Bower *et al.,* 2013). Studies by Sgadari *et al.,* (2003); Gantt *et al.,* (2011); Gantt et *al.,* (2014); Kowalkowski *et al.,* (2015), suggest that HIV protease inhibitors have shown to have direct anti-KS activity besides being an anti-retroviral agent. Bruyand *et al.,* (2015) reported that there was no clinical benefit in comparing the efficacy of non-protease inhibitor and protease inhibitor regimen. In the United State, a third of AIDS-KS develops in the framework of successful cART and the affected patients have undetectable HIV viral load with near-normal CD_4 cell count (Maurer *et al.,* 2007; Krown *et al.,* 2008).

It is a known fact that HIV disease develops following incomplete CD4 T cell receptor reconstitution (Jiang *et al.,* 2009). Nichols *et al.,* (2011) reported that regression of KS is as a result of changing cyclosporine which is an immune suppressive regimen to rapamycin (mTOR) inhibitor. Krown *et al.,* (2012) is also in agreement that even a well-established KS lesion will

respond to rapamycin independently of immune reconstitution in patients that have AIDS associated KS. A study conducted on animal models and in vitro experiment noted that protease inhibitors (PIs) had anitproliferative effect (Kowalkowski *et al.,* 2015), in addition, it was observed that the incidence KS reduced with extended use of protease inhibitor. There is also some proof that PIs might reduce oral shedding of HHV 8 (Gantt *et al.*, 2015). However, in another study (Grabar *et al.,* 2006), recommended that non-protease inhibitors based regimen are equal to PIs with regards to the clinical results of ART-naïve HIV infected patients presenting with AIDS-associated KS. In addition, Martin *et al.,* (2014) reported that PI was superior to other ARVs among patients that had been infected with KS.

HIV-associates KS lesion stabilizes when the infected patient is initiated on cART. Further to that, HIV plasma viremia decreases and immune response becomes reconstituted and in patients that have advanced disease progression, cART should be supplemented with cytotoxic chemotherapy (Hoffmann *et al.,* 2017). Combined antiretroviral therapy (cARTs), has significantly altered the clinical course of Kaposi's sarcoma since its introduction. Nonetheless, certain cases that are very aggressive can still transpire, characteristically as an immune reconstitution inflammatory syndrome (IRIS) few weeks or months following commencement of cARTs (Letang *et al.,* 2013). Kaposi's sarcoma that is linked to IRIS frequently comes with fast developing visceral lesions and it causes high mortality in individuals diagnosed with elevated HHV 8 and high HIV viremia (Letang *et al.*, 2013).

To treat HHV 8 and dissolve the KS lesions, a combination of chemotherapy, radiation, surgery and biological therapy is needed as opposed to single use of HAART drug (Schneider and Dittmer, 2017). Systemic chemotherapy is suitable for HIV and AIDS patients who do not respond to HAART (Highly Active Antiretroviral Treatment), have wide spread of KS lesions, concurrent life threatening disease involving the visceral (Krown *et al.,* 2004). Target therapy can be used on patients whose KS lesions persist to progress despite being on chemotherapy or HAART have (Vaccher *et al.,* 2005). The use of highly active antiretroviral therapy (HAART) in the control of KS is the vital part in the management of HIV-associated KS (Uldrick and Whitby, 2011; Martorelli *et al.,* 2012). In addition, study by Casper and Wald, (2007) indicated that the resolution of KS lesion is impacted by components of HAART. A combination of HAART that contains HIV protease inhibitor might be superior for treatement of KS patients, in addition ganciclovir has been reported to reduce viral load in KS sero-positive patients and as a result, target-based treatment and cytokine signaling might be a strategy to treat KS patients whose lesions progresses even with the use of chemotherapy (Gantt *et al.,* 2011; Gantt *et al.,* 2013; Ruocco *et al.,* 2013; Dittmer *et al.,* 2012). According to Semeere *et al.,* (2012) cART reduces the chances of developing epidemic KS by up to 90%. The use of combined ART is likely to influence the association between HIV/HHV 8 by triggering the immune response against KSHV thereby resulting to reduce HHV 8 viremia (Sullivan *et al.,* 2010).

Use of antiviral drug to inhibit the lytic phase of KSHV has not shown to be of great efficacy for the treatment of KS lesions; however, few cells have revealed that lytic replication plays an important role in the KS tumorigenesis (Gantt and Casper, 2011). In another pilot study conducted by Lechowicz *et al.,* (2009), it was reported that valproic acid was able to prompt

KSHV lytic replication in patients with AIDS-associated KS on HAART and as a result of that, studies by Fu *et al.,* (2008) and Reid, (2011) focused on the need to increase the treatment regimens and the lytic inducing agents in KS patients.

Undoubtedly, HAART epitomize the pillar of treatment because it hinders viral replication and improves the immune system decreasing the immunodepression that is the cardinal cause of KS (Facciola *et al.,* 2017).In developed countries the incidence of KS have drastically reduced due to accessibility and availability of Highly Active Antiretroviral Therapy (HAART), it still considered the most prevalent cancer in HIV infected patients globally (Simard *et al.,* 2011; Lucia *et al.,* 2011; Facciola *et al.,* 2017; Colafigli *et al.,* 2017).

Significant number of KS cases manifest in HIV infected individual when their CD_4 T-cell count is below 200/ml, even though it has been observed in infected individuals on long-term HAART, well controlled HIV-infection and those with CD_4 T-cell count above 200/ml (Dittmer and Damania, 2013; Uldrick and Whitby, 2011). Kaposi's sarcoma is an incurable disease; as a result, the main aim of the treatment is to attain a long lasting remission of the tumor (Semeere *et al.,* 2012). Drugs are available that can be used for local therapy or systemic treatment. Since the introduction of HAART, there has been tremendous improvement and survival of patients with KSHV (Semeere *et al.,* 2012).

Biggar et *al.,* (2005) observed that HAART is able to reduce the inflammation attributed to KS and improve the immune response of the infected patients. A novel treatment targeting the local region of the skin lesions has been suggested; the use of electrochemotherapy (ECT) that entails a combination of electroporation with an administration of two highly cytotoxic drugs, cisplatin and bleomycin (Latini *et al.,* 2012; Testori *et al.,* 2010).

Among the HIV/AIDS, Kaposi's sarcoma can be prevented and treated by use of highly active antiretroviral therapy and in certain cases chemotherapy might be required (Hoffmann *et al.,* 2017). Patients that are infected with AIDS- associated KS and are not responding to HAART, "target" based therapy should be adopted, this is founded on the use of an anti-angiogenic drugs, metalloproteinase and cytokine signaling pathway inhibitor (Cattamanchi *et al.,* 2011; Gantt and Casper, 2011; Peters *et al.,* 2007). There are four prognostic features that can be used to attain a precise prognostic index to manage the treatment choice; AIDS –defining illness, age ≥ 50 years, a CD4 T cell count and S stage (Stebbing *et al*., 2006; Martellotta *et al.,* 2009). These parameters have a prognostic score ranging from 0 to 15. In line with this prognostic index, infected patients with increased risk (Score >12) ought to be on HAART and systemic chemotherapy, patients with reduce risk (Score <5) should be treated with HAART alone and only those patients that have progressive disease ought to be treated with chemotherapy (Stebbing *et al*., 2006; Martellotta *et al.,* 2009).

Continued existence following the diagnosis of KS in the modern HAART era has been reported by Freeman (2013) as less than the highest standard in regions that have resource poor

surroundings. The introduction of HAART has led to tremendous decline in the KS cases; however, continued cases have been reported in regions that have poor access to ARVs (Gbabe *et al.,* 2014).

2.7.2 Chemotherapy and Immunotherapy

The strategy adopted to treat KS depends on location and spread of the infection, if the infection is confined on the skin excision by surgery or electrochemotherapy is the most preferred approach, due to adverse effects, local radiotherapy tends to be abandoned (Riva *et al*., 2012).

The use of cytotoxic chemotherapy constituted the standard of care for Kaposi' sarcoma cases (Molyneux *et al.,* 2013; Chagaluka *et al.,* 2014). Raimundo *et al.,* (2013) reported that paclitaxel shows clinical efficacy against Kaposi's sarcoma, in addition, it is often used as second line in situations where doxorubin is unavailable. Chemotherapy should be prescribed to patients with rapid development of KS involving visceral, lymphoma or those who the KS lesion persist despite being on antiretroviral (Di Trolio *et al.,* 2006). Management of KSHV has taken a new approach that entails the use of static agents, cytokines and agents that are angiogenesis inhibitors (Casper *et al.,* 2008).

According to Bower *et al.,* (2013) a good prognosis has been observed when cART is combined with cytotoxic chemotherapy especially in patients that have rapid progression of KS or those with visceral disease or lymphedema. A complete remission rate of about 80% has been

observed in Western countries when pegylated liposomal doxorubicin hydrochloride is used, however, the general quality of chemotherapy is moderate (Lichterfeld *et al.,* 2005; Di Trolio *et al*., 2006; Cooley *et al.,* 2007). According to Cooley *et al.*, (2007), liposomal daunorubicin which was believed to be a substitute for pegylated liposomal was found to be somewhat less effective. In agreement with the former study Cianfrocca *et al.,* (2010) paclitaxel was also reported to be slightly less effective compared to pegylated liposomal; however, it was found to be more myelotoxic and mostly led to alopecia. Martorelli *et al.,* (2012) reported that the recent first-line systemic treatment for advanced, progressive AIDS-KS comprises of liposomal anthracyclines for example daunorubicin and doxorubicin. With regards to chemotherapy, Gbabe *et al.,* (2014) noted that there was no difference between liposomal doxorubicin, liposomal daunorubicin and paclitaxel.

Krown *et al.,* (2011) stated that in order to preserve an existing KS lesion, KSHV replication is not required because the virus is mostly in a latent state hence gancilovir has been shown not to have effect on an already formed KS lesion. There is insufficient study on the use of pegylated IFN in individuals infected with HIV-associated KS, in addition the regimen is not licensed for use by KS patients as the dosage remains unknown, however, cases of promising reports have been noted among AIDS patients (Robey and Bower, 2015).

2.7.3 Local therapy for Kaposi's sarcoma

The adoption of local therapy has been shown to be well tolerated and cost friendly. The method of local therapy to be used will depend on the size and location of the KS tumor and it ranges

from cryosurgery, IFNs, soft X-ray radiation, electron beam treatment, cobalt radiation and alitretinoin gel usage (Schartz *et al.,* 2008). According to Brambilla *et al.,* (2008)**,** Can important strategy in treating KS-associated lymphedema entails the use of compressive treatment with elastic stocking.

Donato *et al.,* (2013) suggested that KS is extremely radiosensitive tumor as a result; a radiation field should be extended between 0.5-10 in order to reach the tumor cells that might be spreading along the vascular channels. Donato *et al.,* (2013) further reported that plaque-like or macular KS lesions respond quite well to day-to-day doses of soft X-ray radiation, however, large KS lesions with lymphedema manifestation should be managed with a fast electron beam radiation and a manual compression lymphatic message.

Topical treatment of skin KS has been shown to respond adequately with imiquimod published, however, no clinical trial has been conducted (Fairley *et al.,* 2012; Prinz Vavricka *et al.,* 2012; Lebari*et al.,* 2014). Other agents that have shown promising clinical outcomes include thalidomide and its derivatives which are currently undergoing clinical trials (Rubegni *et al.,* 2007; Steff *et al.,* 2013; Polizzotto *et al.,* 2016; Pourcher *et al.,* 2017). Kaposi's sarcoma patients infected with few local lesions can be treated with radiation therapy or cryosurgery (Zimmerman and Crawford, 2012). A combination of chemotherapy and antiretroviral treatment has been shown to be significantly effective than when radiation therapy or cryosurgery are used individually (Anglemyer *et al.,* 2014).

2.7.4 New therapeutic approaches

A number of novel treatments with regards to Kaposi's sarcoma pathogenesis have been proposed, such as cytokines, inhibitors of angiogenesis and antiviral agents (Hoffmann *et al.,* 2017). Casper *et al.,* (2008) reported that valganciclovir is the most promising virustatic agent that significantly reduces the replication of HHV 8. In addition, Casper *et al.,* (2008) stated that several new treatments for KS are under investigation. Krown *et al.,* (2011) questioned the efficacy of valganciclovir in the management of classical KS lesions because data on its clinical efficacy is yet to be published. Antiviral treatment without value when used includes cidofovir, foscarnet, ganciclovir or valganciclovir (Casper *et al.,*2008), this is so because neoplastic KS spindle cells contains a latent HHV 8 and its eradication is impossible (Riva *et al.,* 2010; Schulz, 2006; Ariza-Heredia and Razonable, 2011). Novel treatment that target specific regions are currently being investigated, however, one could commence antiviral therapy in the event of an aggressive disseminated KS that is accompanied by elevated HHV 8 viremia (Koon *et al.,* 2014).

For endothelial proliferation to occur, VEGF is essential and inhibition of VEGF constitute the treatment approach for KS (Uldrick *et al.,* 2012; Koon *et al.,* 2014; Ignacio *et al.,* 2016). Drugs that work as anti-VEGF have shown to work in some patients and fail to work in others; this discrepancy is perhaps attributed to insufficiency of the drug delivery to the KS lesion (Cavallin *et al.,* 2014). Several promising case report have been documented on the efficacy of lenalidomide which is an immunomudulatory drug that has an antiangiogenic effect (Martinez *et al.,* 2011; Steff *et al.,* 2013). A moderate response rate of bevacizumab an anti-VEGF-A monoclonal antibody has been seen in HIV patients that have KS progression and are on cART (Uldrick *et al.,* 2012). According to Koon *et al.,* (2011); Koon *et al.,* (2014), there is an ongoing

study that is currently conducted on the combination of liposomal doxorubicin and bevacizumab, in addition promising results has been witnessed from the phase II study on imatinib and halofuginone.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design

This was a hospital based cross-sectional descriptive study. The Formalin fixed paraffin embedded blocks were traced from archives following histological reports of the patients that were diagnosed with KS or KS-like between 2013 and 2016. Consecutive sampling technique was used to pick the archived tissue blocks from Thematic Unit of Anatomic pathology, Department of Human pathology, College of Health Sciences, University of Nairobi and Department of Laboratory Medicine, Histology section, Kenyatta National Hospital.

3.2 Study site

This study was carried out at Kenyatta National Hospital (KNH). Kenyatta National Hospital is situated in the capital City Nairobi County, Kenya and is largest teaching and referral hospital in Kenya, East and Central African region. This health care facility covers an area of 45.7 hectares and within the KNH complex are College of Health Sciences (University of Nairobi); the Kenya Medical Training College; Kenya Medical Research Institute and National Laboratory Service (Ministry of Health). Kenyatta National Hospital has 50 wards, 22 out-patient clinics, 24 theatres (16 specialized) and Accident & Emergency Department. Out of the total bed capacity of 1800, 209 beds are for the Private Wing.

3.3 Study population

The study population were cases previously diagnosed with KS or KS-like between 2013 and 2016. The bio-medical records of the studied cases was obtained from the registry, data and information collected were then harmonized with the questionnaire (Appendix IV) of this study. The data obtained from the bio-medical records had details on the demographics, HIV-1 status, CD_4 cell count, tumor location, number of tumors, and the antiretroviral treatment status.

3.4. Inclusion and exclusion criteria

3.4.1 Inclusion criteria

For this study, the inclusion criteria were: -

1. The tissue blocks retrieved were from patients of 18 years of age and above
2. Tissue blocks that were previously proven as KS or KS like.
3. A comprehensive bio-medical data linking the blocks and registry records.
4. Multiple tissue blocks of the same KS or KS-like case

3.4.2 Exclusion criteria

For this study, the exclusion criteria were: -

1. Incomplete bio-medical data linking the blocks and registry records.
2. Fragmented tissue blocks

3.5 Sample size determinations

Sample size was determined using standard statistical formula (Fishers *et al*., 1998):

$$n=Z^2pq/d^2$$

Where;

Z^2=1.96, p= 5.6% based on the prevalence HIV infection, trends, and risk factors among persons aged 15-64 years in Kenya: results from a nationally representative study (Kimanga *et al.,* 2014).

$q=0.944$, $d^{2=}0.05^2$

$$n=1.96^2 \text{ X } ((0.056) \text{ X } (1-0.056))/ (0.05)^2 = 81$$

Approximately 81tissue blocks were used in this study.

Consecutive sampling was done to pick the tissue blocks that met the inclusion criteria for the study. Consecutive sampling involved picking the tissue blocks that met inclusion criteria until such a time that the required sample size (81) was met.

3.6 Sample handling

The study retrieved all the blocks from the archives that had been previously diagnosed as KS or KS-like between 2013 and 2016. Histological report was used to retrieve respective cases from the routine block storage and archived. The criteria for inclusion as HIV positive case included information gotten from the clinician in the histological request form indicating that these were follow up at the comprehensive care center (CCC). The clinical information on the form indicating gender, age, HIV status, if patient was on ART or HAART treatment, Location of the KS lesion, number of the KS lesion, distribution of the KS lesion, CD_4 cell count and histology diagnosis was then harmonized with the questionnaire for the study.

3.7 Laboratory analysis of Kaposi's sarcoma herpes virus

3.7.1 Retrieval of tissue blocks

These tissue blocks were from the years January, 2013 to December, 2016.The picked tissue blocks were previously diagnosed as KS or KS like based of clinical appearance and Hematoxylin and Eosin (H & E) staining. Each and every tissue block was assigned a new study number for identification purposes.

3.7.2 Tissue sectioning

To begin the process of tissue sectioning, the blocks were placed on an ice-cold plate for 20 minutes before rotary microtome was used to produce serial sections. A rotary microtome was used to cut the tissue blocks to about 10 µm in thickness. The study used sterile tissue blades to section the archived blocks. Different gloves and tissue blades per FFPE block were used to prevent carry-over of DNA. After sectioning each tissue block, the surface of the rotary microtome was sterilized using DNAZap ^{TM} PCR DNA (Thermo Fisher Scientific Company Cat No. /ID: AM9890)- degradation solutions. Each tissue ribbon was fished by a forceps and transferred to a 37^0C water bath in order to float the sectioned tissue. The sectioned tissue ribbon was placed in the water bat to allow them to stretch for a few seconds. Each sectioned tissue ribbon was picked on a glass slide at an angle, slide sectioned given time to drain for few minutes before they were transferred to a hot plate to dry. The tissue sections were processed for H and E staining.

3.7.3. Hematoxylin and Eosin Staining

The procedure that was used for H and E staining entailed; Deparaffinized the tissue section by use of xylene, hydrated the tissue section by passing it through decreasing concentration of alcohol and water, stained in hematoxylin for 5 minutes, washed in running tap water until section ‘’blue’’, differentiated in 1% acid alcohol for 5 minutes, washed in running tap water until the section was blue which was achieved by dipping in an alkaline solution, stained with 1% Eosin Y for 10 minutes, washed in tap water for 5 minutes, dehydrated in increasing concentration of alcohol and cleared with xylene, mounted in a mounting media then observed under microscope X10 and X20. Reporting of the H and E stained slides was done by a qualified histo-pathologist.

The diagnosis of KS based on histo-pathological criteria included identification of spindle cell proliferation, presences erythrocytes that were filled with vascular slits and proliferation of small vessels, with some vessels presenting with extracellular haemorrhage and haemosiderin deposition. The KS lesions in this study were classified as patchy, plaque and or nodular in stage.

3.7.4 Molecular characterization of Kaposi’s sarcoma herpes virus

3.7.4.1 DNA extraction of Kaposi’s sarcoma herpes virus

The DNA of Kaposi’s sarcoma herpesvirus was extracted from the tissue blocks that were sectioned. Deoxyribonucleic acid was then extracted from the 10 µm width of the sectioned tissue blocks. Four folds of the tissue sectioned was collected and inserted in a 1.5 ml microcentrifuge tube. Deoxyribonucleic acid extraction was done using Qiagen kit GeneRead DNA FFPE (Qiagen®company Cat No./ID: 180134). The extraction kit removes paraffin and

reverses formalin cross-links from the DNA tissues before it is bound to the QIAamp MinElute column. The quantification of the extracted DNA of Kaposi's sarcoma herpes virus was done using QIAxpert machine and the quantity of the viral DNA ranged from 8 ng to 18 ng before amplification by nested PCR.

3.7.4.2 DNA analysis of Kaposi's sarcoma herpes virus

The Taq PCR Core Kit-Qiagen (Cat. No. 201223) was used for detecting HHV-8 gene. The study targeted three KS genes i. e K1, K15 (P) and ORF75 and used nested PCR technique. Nested PCR technique employs the use of two sets of primers for each targeted KS gene. The set of primers that were used for this study included; K1 gene 868 bp product size and the primer sequence was K1a-f 5'ATGTTCCTGTATGTTGTCTGC3'; K1a-r 5'AGTACCAATCCACTGGTTCG3'; K1 gene 840 bp product size, the primer sequences was K1b-f 5'GTCTGCAGTCTGGCGGTTTGC3'; K1b-r 5'CTGGTTGCGTATAGTCTTCCG3'; K15 (P) 365 bp product size and the primer sequence was K15P-f 5'TGCAGGCTTGGTCATGGGTTAC3'; K15P-r 5'GGGACCACGCTGCAATTAAATG3'; K15 (P) 285 bp product size and the primer sequence was K15-f 5'ACGCATACATGTACTGCCAC3'; K15-r 5'CTTTGATATTGCCAGTGGTG3'; and ORF75 gene 895 bp product size, the primer sequence was KS 1000-f 5'CGGTTCGGTGGCATACAGGC3'; KS 1034-r 5'CTGACTACAGAGGGTGTCCCCG3'; ORF75 gene 804 bp product size, the primer sequence was KS 2000-f 5'GGAAACAGGGTGCTGTG3'; KS 2034-r 5'CATGGCCTACGACGTCAC3'.

The PCR cycling condition of all the three (K1, K15P and ORF75) regions were similar and it consisted of 30 number of cycles which entailed; initial denaturation at 94^{o}c for 3 minutes, denaturation at 94^{o}c for 1 minute, annealing at 63^{o}c for 1 minute, extension at 72^{o}c for 1 minute and final extension at 72^{o}c for 10 minutes. The PCR products were separated using 1% agarose (SEaekem LE® agarose; FMC BioProducts, Rockland, Marine USA) gel in 1X TAE Buffer (0.04M Tris acetate, 0.001 M EDTA). Five micro-litres of Ethedium bromide 0.5 µg per ml (Promega®, Madison, Wisconsin, USA) was added to the gel to stain KSHV DNA. A 1 kb DNA molecular weight marker (Promega®, Madison, Wisconsin, USA) was used to estimate the KSHV DNA band size. The amplified products of the second nested PCR were mixed with gel loading dye before loading into the wells on the gel. The electrophoresis was executed at a constant voltage of 100 volts/cm using a Mupid®2 plus submarine electrophoresis system power supply source. The DNA bands (figure 4.6) were visualized and images captured using the syngene^{TM} Gene Genuis computer system (Synoptics LTD, Cambridge, United Kingdom) as per manufacturers protocol.

3.7.4.3 Purification of sequences and nucleotide sequencing

The amplified products of the nested PCR were purified then subjected to direct nucleotide sequencing (next-generation sequencing) using the following primers: forward (5'GGCCCTTGTGTAAACCTGTC3') and reverse (5'CCTGAATGTCAGTACCAATCCA3'). A total of 60 amplified DNA extracts that were positive for K1, K15P and ORF75 KSHV genes were subjected to sequencing.

Both the forward and reverse products of nested PCR were sequenced. The products of the DNA sequence were aligned using Multiple Alignment using Fast Fourier Transform (MAFFT) version 7. The MAFFT helped in the alignment of multiple sequences. Neighbour-joining bootstrap value 1000 of the aligned sequences was carried out in Molecular Evolutionary Genetic Analysis (MEGA) version 6 software packages. The MEGA helped in the drawing of phylogentic tree and a bootstrap value above 70% (in the phylogentic tree) was considered significant.

3.8 Data management

The data clerk from the Histology section, Kenyatta National Hospital was approached by the principal investigator for this present study to obtain the bio-data and histological reports for the years January, 2013 to December, 2016. The bio-medical and clinical reports of the archived tissue blocks obtained from the registry were harmonized with the questionnaire (Appendix IV) of this study. From the histological report files, KS or KS like were noted and assigned study number. The principal investigator moved forward to the archive section and fished out all the tissue blocks, matched the blocks and records. Out of the total, only 81 tissue blocks that met the inclusion criteria were picked and used for this study.

The bio-data, clinical reports and the results of laboratory analysis were entered in an excel sheet. This data was cleaned and then entered into the Statistical Package for Social Sciences (SPSS) version 21.0 where Logistic regression analysis, chi-square and t-test was used to determine the associations among HHV 8, histological morphology of KS, gender, age, number

of KS lesions, distribution of the KS lesions, histo-pathological confirmation and the site of KS locations. A p- value less than 0.05 were considered statistically significance.

3.9 Ethical consideration

University of Nairobi Institute of Tropical and Infectious Diseases (UNITID) cleared this proposal and allowed the principal investigator to proceed with obtaining Ethical clearance from Kenyatta National Hospital/ University of Nairobi Ethics and Research Review Committee (KNH/UON ERC).

The Ethical clearance number: P682/11/2014 (Appendix I) for this study was gotten from KNH/UoN ethics and research committee in June 2015. The information document forms (Appendix II), was read and explained to the dark clerks by the principal investigator. In addition, the information consent form (Appendix III) was signed for by the data clerks. Finally, the questionnaire (Appendix IV) was used by the principal investigator as a guide to counter check on the important bio-medical data that was essential for this study.

CHAPTER FOUR

RESULTS

4.1 Aspects of demographic characteristics

4.1.0 Demographic Data

4.1.1 Age

Mean age was 39.3 (SD=9.5) years, range 19-63 years, mode 34 years, and median 38 years. Of the 81 cases studied, 39 (48.2%) were age group 30-39 years, while 23 (28.4%) were age group 40-29 years. Six (7.4%) were age group 50-59 years and 4 (4.9%) 60-69 years.

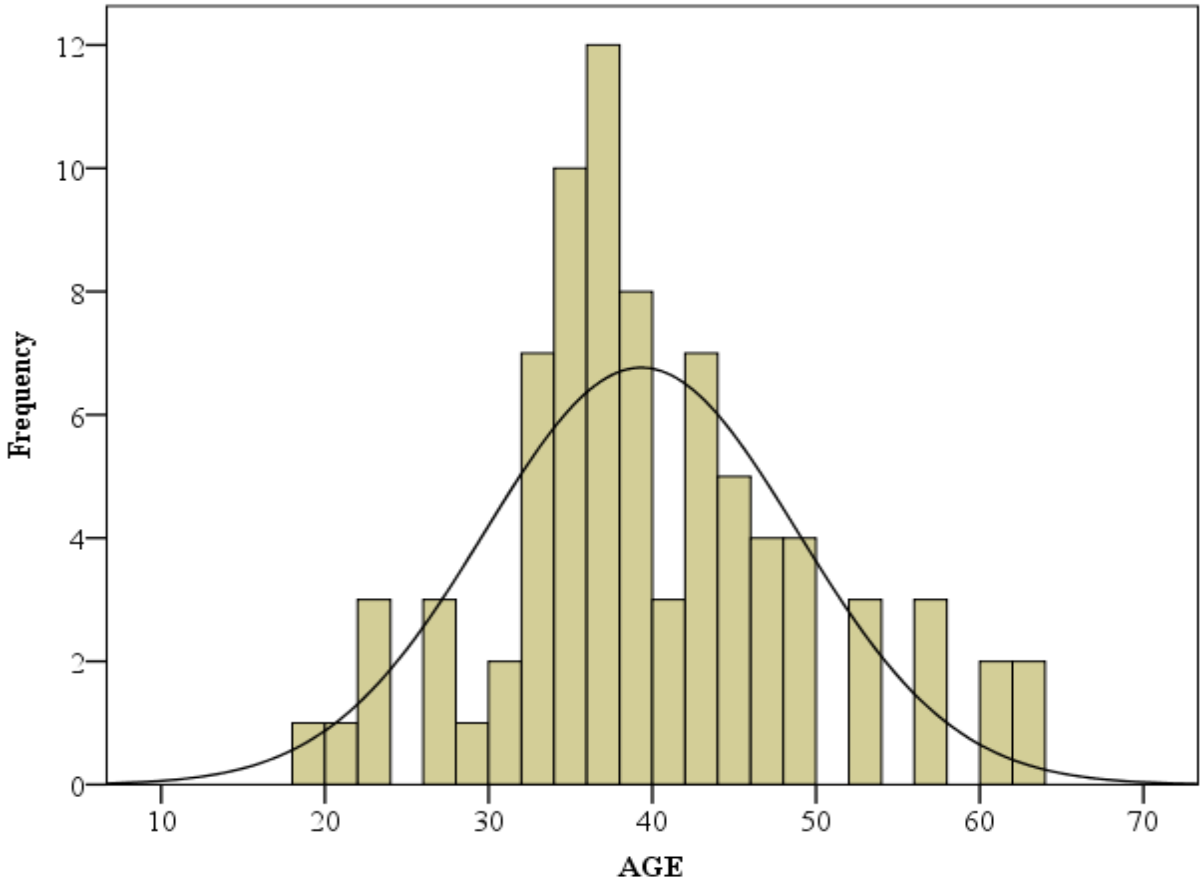


Figure 4.1: Age distribution of cases

Age distribution in figure 4.1 is seen to be skewed to the right with older cases dominating and peak age is 39 years. Majority 39 (48.2%) of the cases in the age group 30-39 years clustered around the mean age of 39.3 years.

Table 4.1: Age group of studied cases

Age Group	Frequency	Percentage
18-29 years	9	11.1
30- 39 years	39	48.2
40- 49 years	23	28.4
50- 59 years	6	7.4
60- 69 years	4	4.9

In table 4.1, majority 39 (48.2%) of the cases were age group 30-39 years with the least being 4 (4.9%) 60-69 years.

4.1.2 Gender

Males were 46 (56.8%) and females 35 (43.2%). Ratio of males to females was 1:0.8.

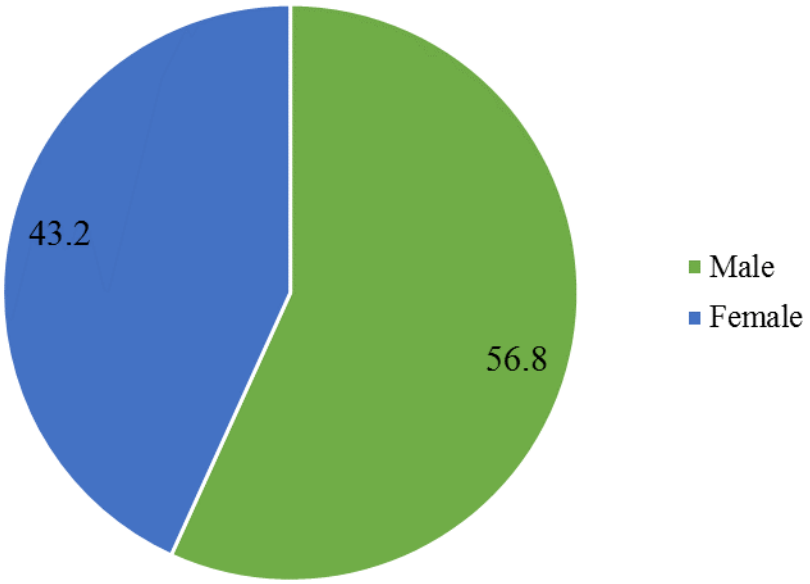


Figure 4.2: Gender profile of cases

It was observed that males were greater in number compared to females as seen in figure 4.2.

4.2.1 HIV Status

All the studied cases were HIV positive. Eighty-one (100%) cases had been diagnosed as HIV positive.

4.2.2 ARV treatment

Of the 81 studied cases, 77 (95.1%) were on antiretroviral (ARV) treatment. The treatment status of the studied cases was grouped into two as shown in figure 4.4. It was observed that 4.9% (4) were not on any antiretroviral regimen while 95.1% (77) had been put on treatment against HIV.

4.2.3 CD_4 Cell Count

The CD_4 cell count of 52 (64.2%) cases were 201-350 cell/mm^3. None of the cases had a CD_4 cell count above 350 cell/ mm^3. The distribution CD_4 cell count among the studied cases indicated that majority 64.2% (52) had counts of 201- 350 cells/$mm^3$and 35.8% (29) with 0- 200 cells/ mm^3.

4.2.4 Distribution and number of KS Lesions

Of the 81 studied, 53 (65.4%) has a generalized lesion. The distribution of KS lesions was categorized as either generalized or localized. Majority 65.4% (53) had generalized KS lesions while 34.6% (28) presented with localized lesions. The distribution and number of KS lesions is shown in table 4.2. Of the 81 studied cases, 45 (55.6%) had more than 10 KS lesions. The

number of KS lesions was recorded as more than 10 or less than 10. The observation made was that 55.6% (45) of the cases had more than 10 KS lesion and 44.4% (36) less than 10. The distribution and number of KS lesions shown in table 4.2

Table 4.2: Distribution and number of KS Lesions

Distribution of KS Lesions	n (%)
Generalized lesion	53 (65.4%)
Localized lesions	28 (34.6%)
Number of KS Lesions	
More than 10 KS lesions	45 (55.6%)
Less than 10	36 (44.4%)

4.2.6 Site of KS Lesion

Of the 81 studied cases, 33 (40.7%) had KS lesions on the lower limbs, while 25 (30.9%) and 17 (21.0%) had lesions on the trunk/chest/back, and upper limbs respectively. One (1.2%) lesion from eyelids and genitalia and 4 (4.9%) lesions from the palate/mouth (Figure 4.3).

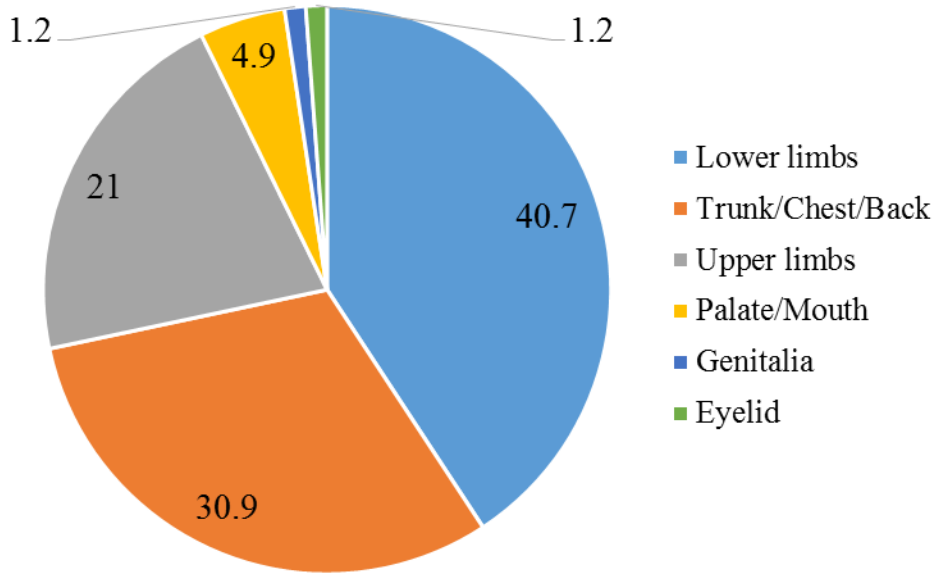
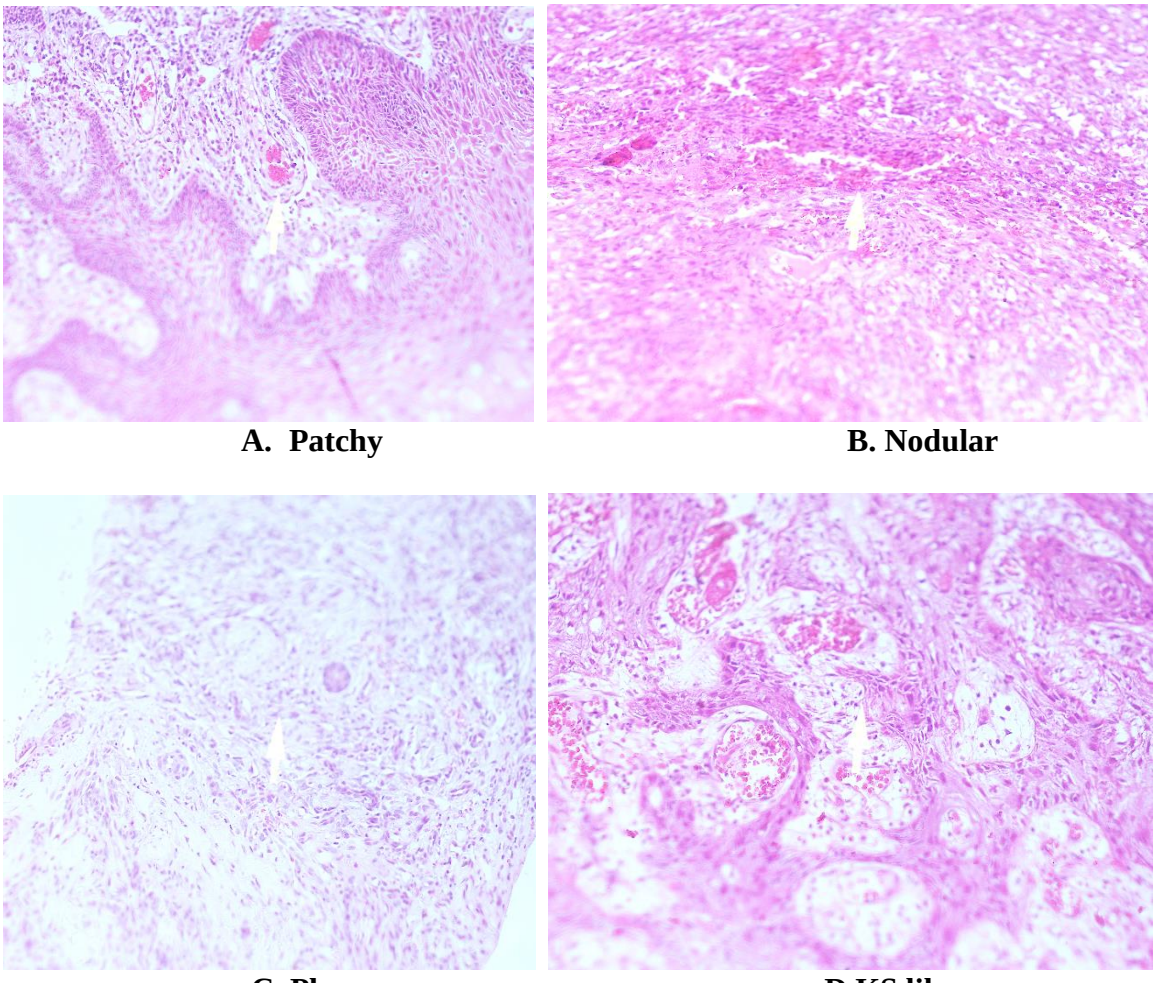


Figure 4.3: Site of KS lesions on cases

It is remarkable how the KS lesions were distributed among the studied cases. Worthy of attention was the 40.7% (33) on the lower limbs, 30.9% (25) on the trunk/chest/back and 21.0% (17) on the upper limbs.

4.2.7 Morphology of KS Lesions

The morphology of KS lesions was as follows; patchy, nodular, plaque and KS like (Figure 4.4).



A. Patchy **B. Nodular**

C. Plaque **D KS like**

Figure 4.4: Microscopic (X20) description of histological subtype and KS like

Of the 81 studied cases, 49 (60.5%) had nodular KS lesions, while 19 (23.5%) and 13 (16.0%) were plaques, and patchy lesions respectively. Results on the morphology of KS lesion indicated

that the cases of nodular type were more than plaques and patchy combined (Figure 4.5). The nodular type of KS morphology was strongly expressed among the studied cases.

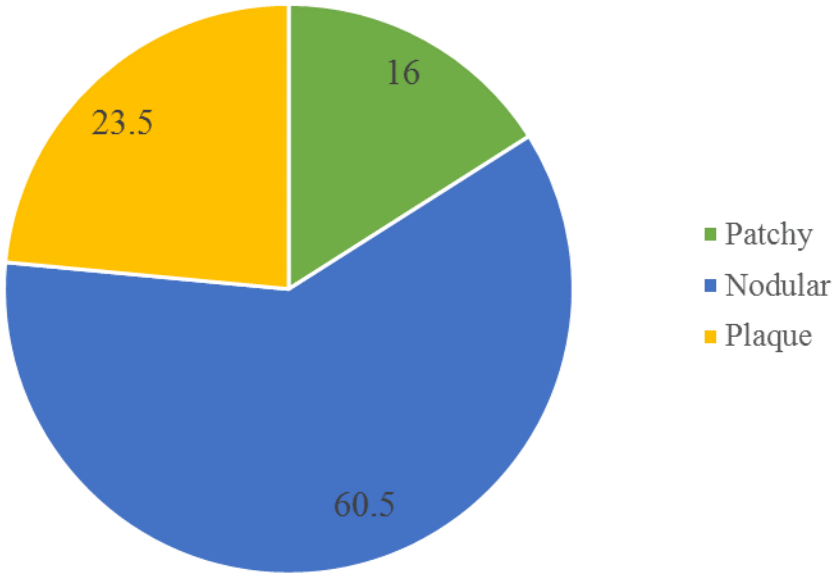


Figure 4.5: Morphology of KS lesions on cases

4.2.8 Histology of KS

Sixty-eight (84.0%) of the 81 studied cases had a KS histology (Figure 4.6).

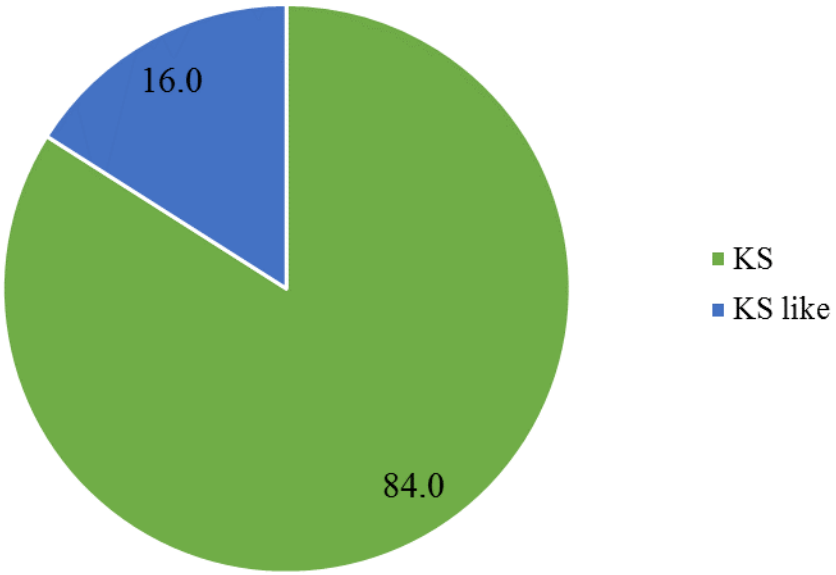


Figure 4.6: Histology of KS

On the histology description of the 81 cases studied, 84% (68) were identified as typical KS and 16% (13) having features suggestive of KS called ‘‘KS like’’.

4.2.9 K1 Gene Status

The K1 gene was present in 72 (88.9%) of the 81 studied cases (Figure 4.7).

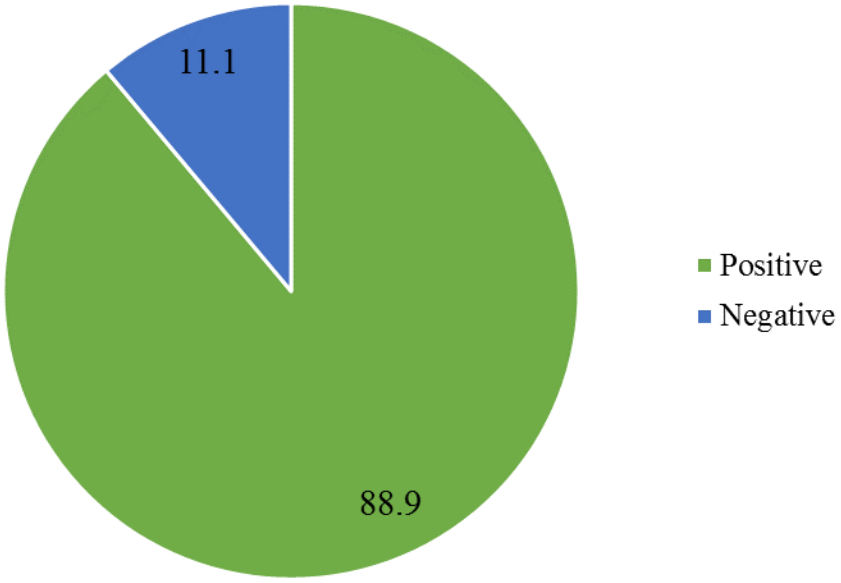


Figure 4.7: KI gene status in cases

The KI gene was highly expressed at 88.9% (72) out of the total number of 81 studied cases.

4.2.10 K15P Gene

The K15P gene was present in 72 (88.9%) of the 81 studied cases (Figure 4.8).

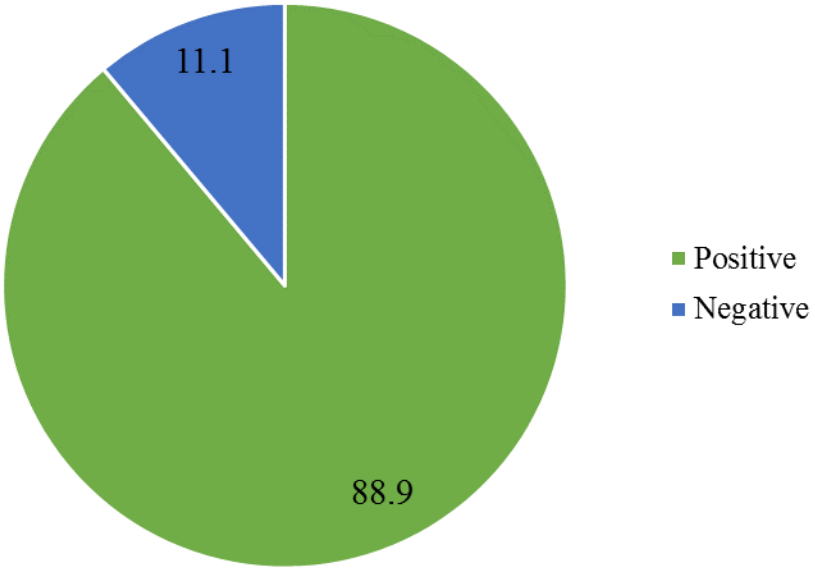


Figure 4.8: K15P gene status in cases

The K15P gene was highly expressed at 88.9% (72) out of the total number of 81 studied cases.

4.2.11 ORF75 Gene

The ORF75 gene was present in 49 (60.5%) of the studied 81 cases (Figure 4.9).

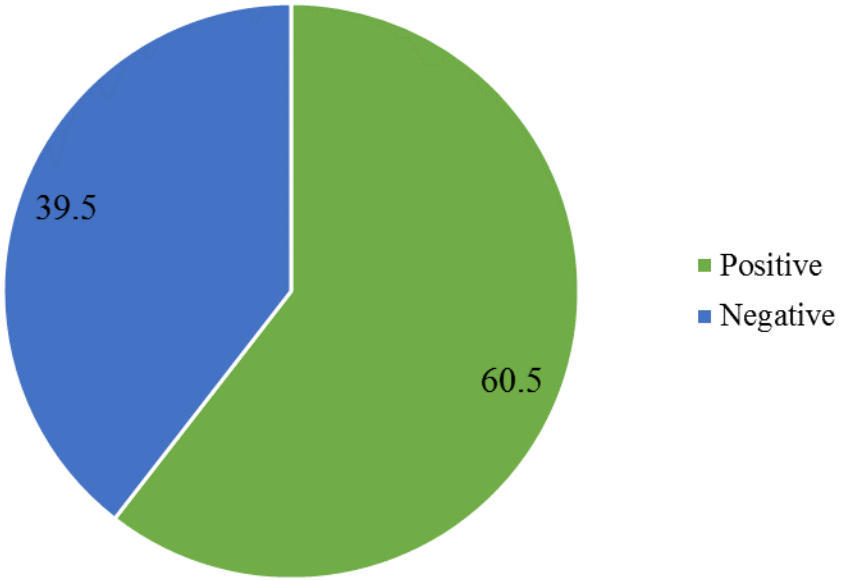


Figure 4.9: ORF75 gene status in cases

The ORF75 gene was expressed at 60.5% (49) out of the total number of 81 studied cases. The 39.5% (32) of the cases were negative implying that ORF75 gene was not detected.

4.3.1 Age and Gender

The mean age of males was 40.5 (SD=10.4) years, range 19-63 years, and median 40 years. The mean age of females was 37.6 (SD=7.9) years, range 20-61 years, and median 36 years. The mean difference in age between male and female cases was 2.9 years, t=1.3, p=0.10.

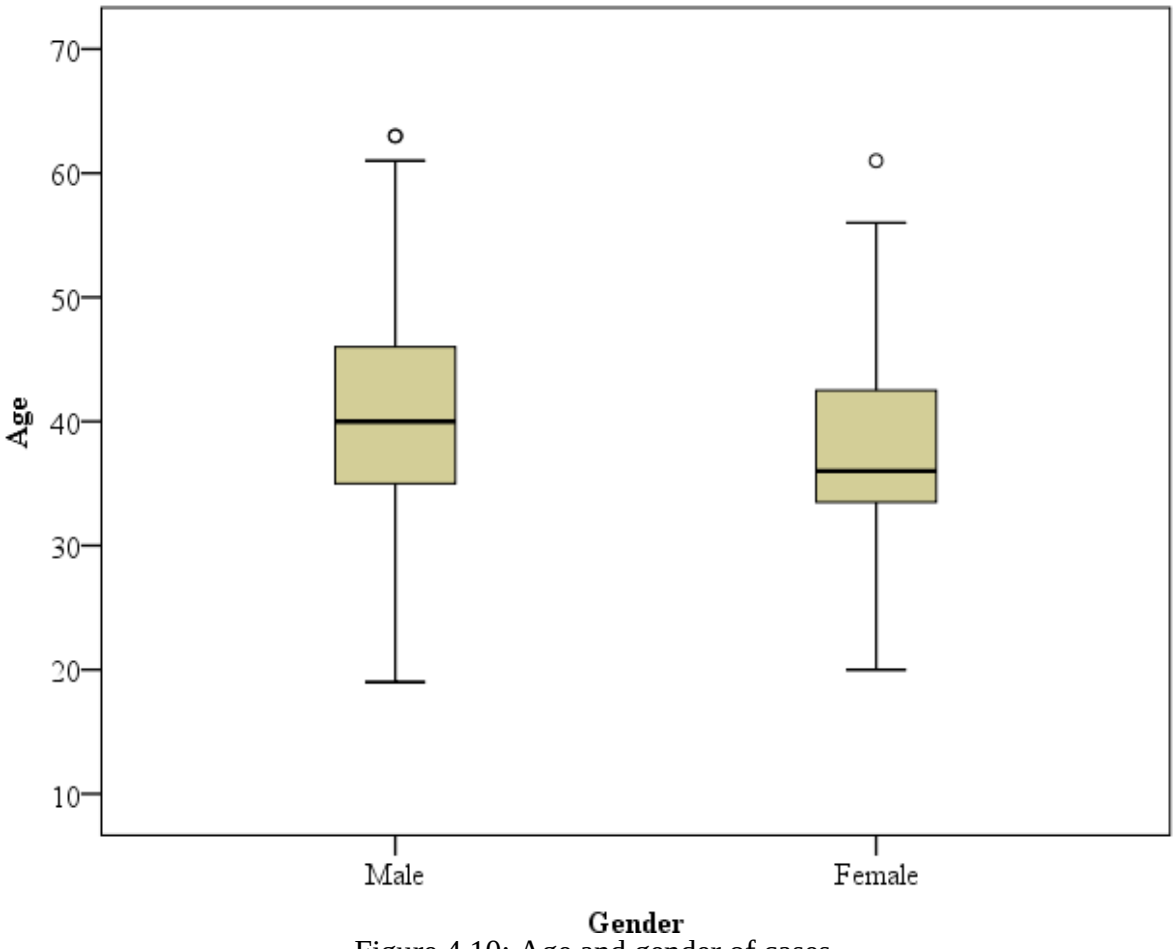


Figure 4.10: Age and gender of cases

Of the studied cases, 17 (37.0%) males and 22 (62.9%) females were age group 30-39 years, while 15 (32.6%) and 8 (22.9%) respectively were age group 40-49 years (Figure 4.10). The Pearson's chi-square statistic for age and gender was 6.0, p=0.19. There were more males 56.8% (46) than females 43.2% (35) cases in this study (Table 4.3).

Table 4.3: Age and Gender

	Gender	
	Male n (%) 46 (56.8)	Female n (%) 35 (43.2)
Age Group		
18-29 years	6 (13.0)	3 (8.6)
30- 39 years	17 (37.0)	22 (62.9)
40- 49 years	15 (32.6)	8 (22.9)
50- 59 years	5 (10.9)	1 (2.9)
60- 69 years	3 (6.5)	1 (2.9)

Male cases were greater in number in all the age groups except the ages of 30- 39 years where female preponderance was observed (Figure 4.11).

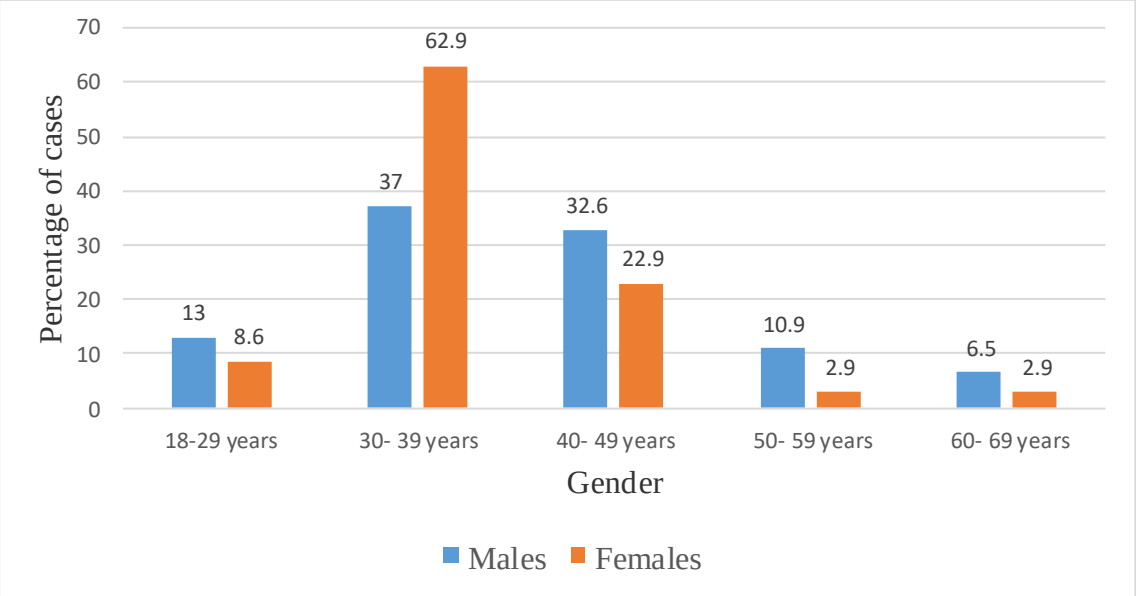


Figure 4.11: Age group and gender cases

4.3.2 Age and ARV

The mean age of cases who were on ARV was 39.5 (SD=9.6) years, range 19-63 years, and a median of 38 years. Among cases who were not on ARVs, the mean age was 34.7 (SD=7.9), range 26-42 years, and median 25.5 years. The mean difference in age between the studied cases on ARV was 4.8, t=0.981, p=0.32 (Figure 4.12).

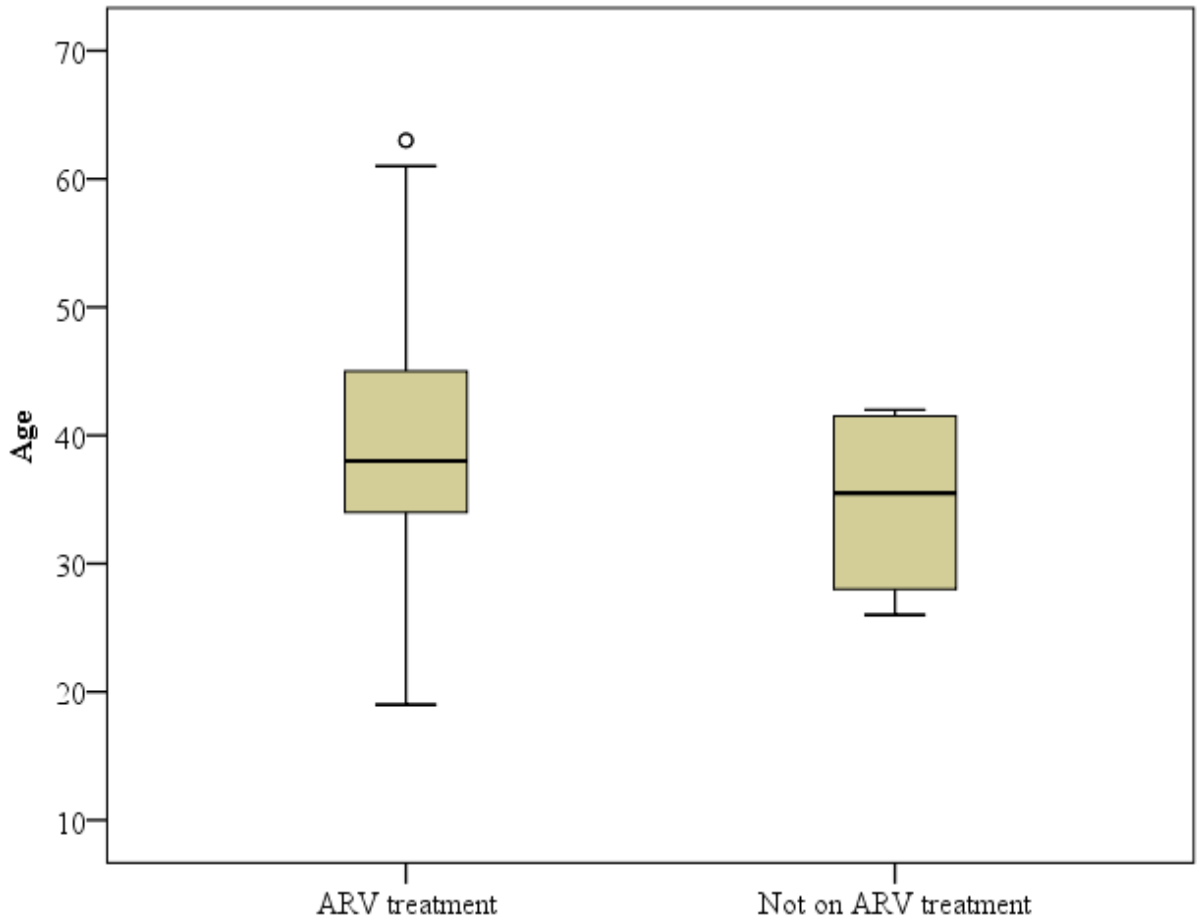


Figure 4.12: Age and ARV

It was observed that majority of cases 95.1% (77) were on ARV treatment and 4.9% (4) not on ARV treatment. Thirty-eight (49.4%) cases under ARV treatment and 1 (25.0%) not under treatment were age group 30-39 years. Twenty-one (27.3%) cases under ARV treatment and 2

(50.0%) not under treatment were age group 40-49 years (Table 4.4). Pearson's chi for age and treatment was 2.4, p=0.66.

Table 4.4: Age and ARV treatment

	Treatment n (%)	
	ARV treatment 77 (95.1)	Not on ARV treatment 4 (4.9)
Age Group		
18-29 years	8 (10.4)	1 (25.0)
30- 39 years	38 (49.4)	1 (25.0)
40- 49 years	21 (27.3)	2 (50.0)
50- 59 years	6 (7.8)	0 (0.0)
60- 69 years	4 (5.2)	0 (0.0)

There were no cases of age 50 to 69 years that failed to take ARV treatment (Figure 4.13). In all the age groups, it was observed that more cases were on ARV treatment except 40-49 years where 50% (2) were not on treatment.

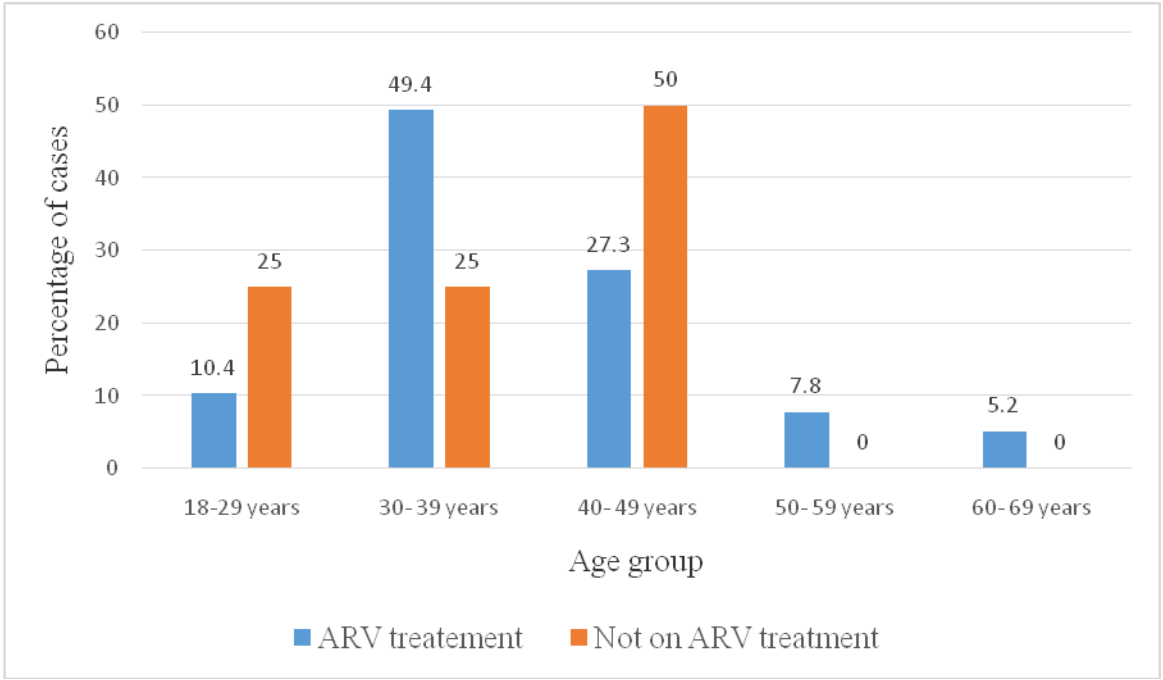


Figure 4.13: Age group and ARV treatment status

4.3.3 Age and CD4 Count

The mean age of the studied cases with a CD_4 cell count 0-200 Cells/mm3 was 38.5 (SD=10.5) years, range 19-61 years, and a median of 38 years. Among the studied cases with a CD_4 cell count 201-350 Cells/mm^3, the mean age was 39.7 (SD=9.0), range 23-63 years, and median 37 years. The mean difference in age of the studied cases with a CD_4 cell count 0-200 Cells/mm^3 and 201-350 Cells/mm^3 was -1.1, t=0.15, p=0.60 (Figure 4.14).

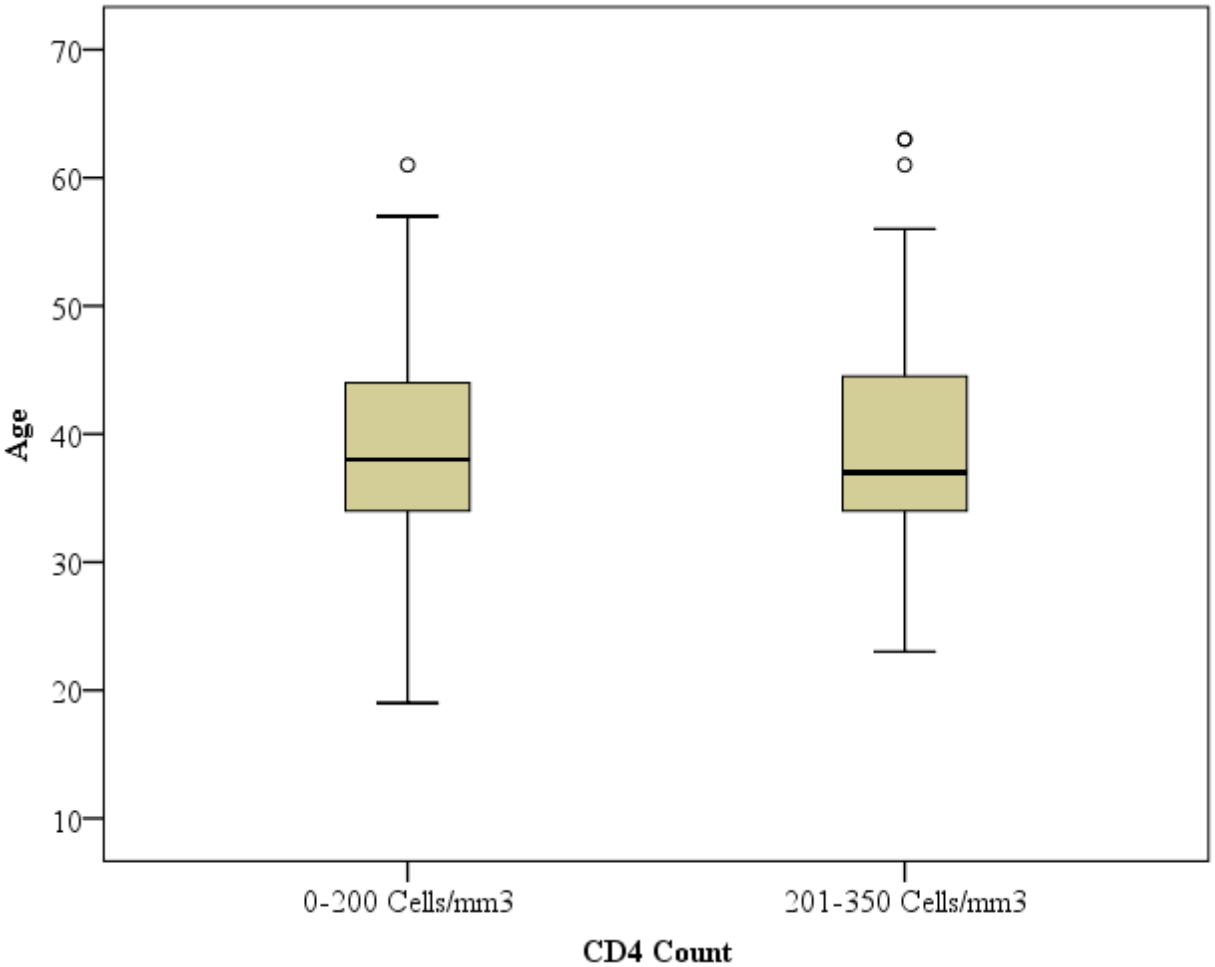


Figure 4.14: Age and CD4 count of cases

Eleven (37.9%) cases with a CD_4 level of 0-200 Cells/mm3 and 4 (7.7%) a CD_4 level of 201-350 Cells/mm3 were age group 30-39 years. Ten (34.5%)with a CD_4 level 0-200 Cells/mm3 and 13 (25.0%) a CD_4 level of 201-350 Cells/mm were age group 40-49 years. The Pearson's chi-square statistic for age and treatment was 3.3, p=0.50. Majority of the cases 64.2% (52) had CD_4Count between 201-350 cell / $mm^3$and 35.8% (29) was found to have CD_4Count ranging from 0-200 Cells/mm^3 (Table 4.5).

Table 4.5: Age and CD_4 count

	CD_4 Count data	
	0-200 Cells/mm^3n (%) 29 (35.8)	201-350 Cells/mm^3n (%) 52 (64.2)
Age Group		
18-29 years	5 (17.2)	4 (7.7)
30- 39 years	11 (37.9)	28 (53.8)
40- 49 years	10 (34.5)	13 (25.0)
50- 59 years	2 (6.9)	4 (7.7)
60- 69 years	1 (3.4)	3 (5.8)

Remarkable observation made was that, cases in the age group 18-29 years and 40-49 years had higher peaks of 0-200 cells of CD_4 cell count at 17.2% (5) and 34.5% (10) respectively compared to 201-350 cells of CD_4 cell count among their counterpart in the same age group (Figure 4.15).

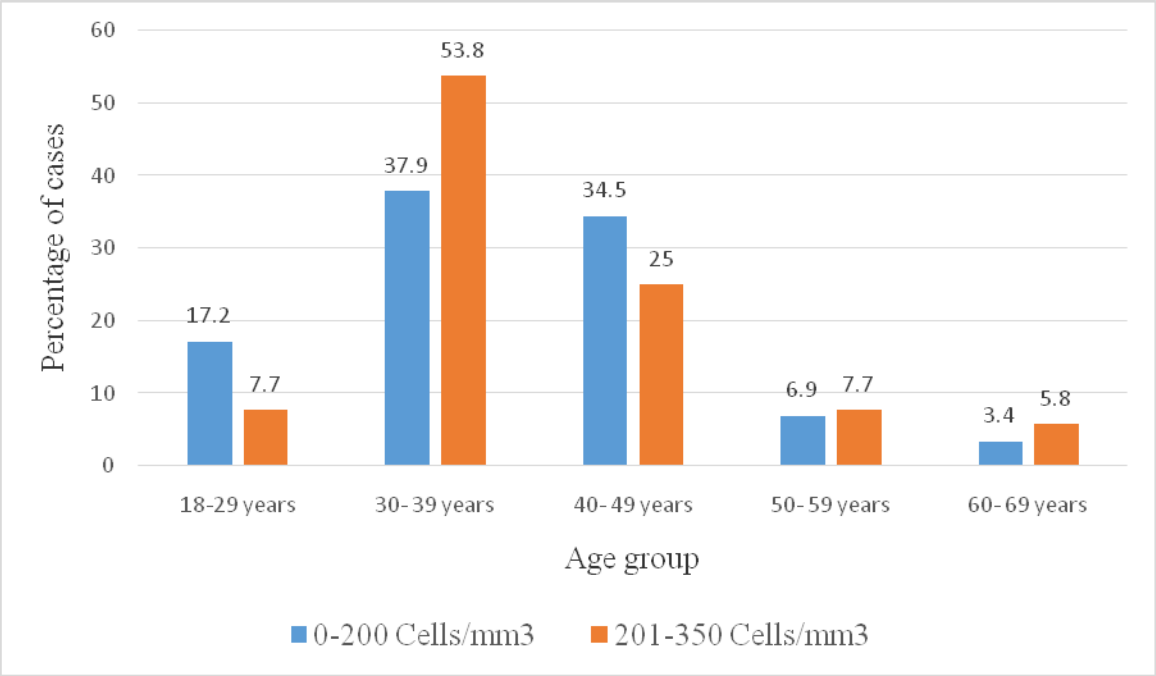


Figure 4.15: Age group and CD_4 count

4.3.4 Age and Number of Lesions

The mean age of the studied cases with >10 KS lesions was 39.6 (SD=10.4) years, range 20-63 years, and a median of 38 years. Among the studied cases with <10 KS lesions, the mean age was 38.8 (SD=8.4), range 19-63 years, and median 37.5 years. The mean difference in age of studied cases with >10 KS lesions and <10 KS lesions was 0.7, t=0.36, p=0.71 (Figure 4. 16).

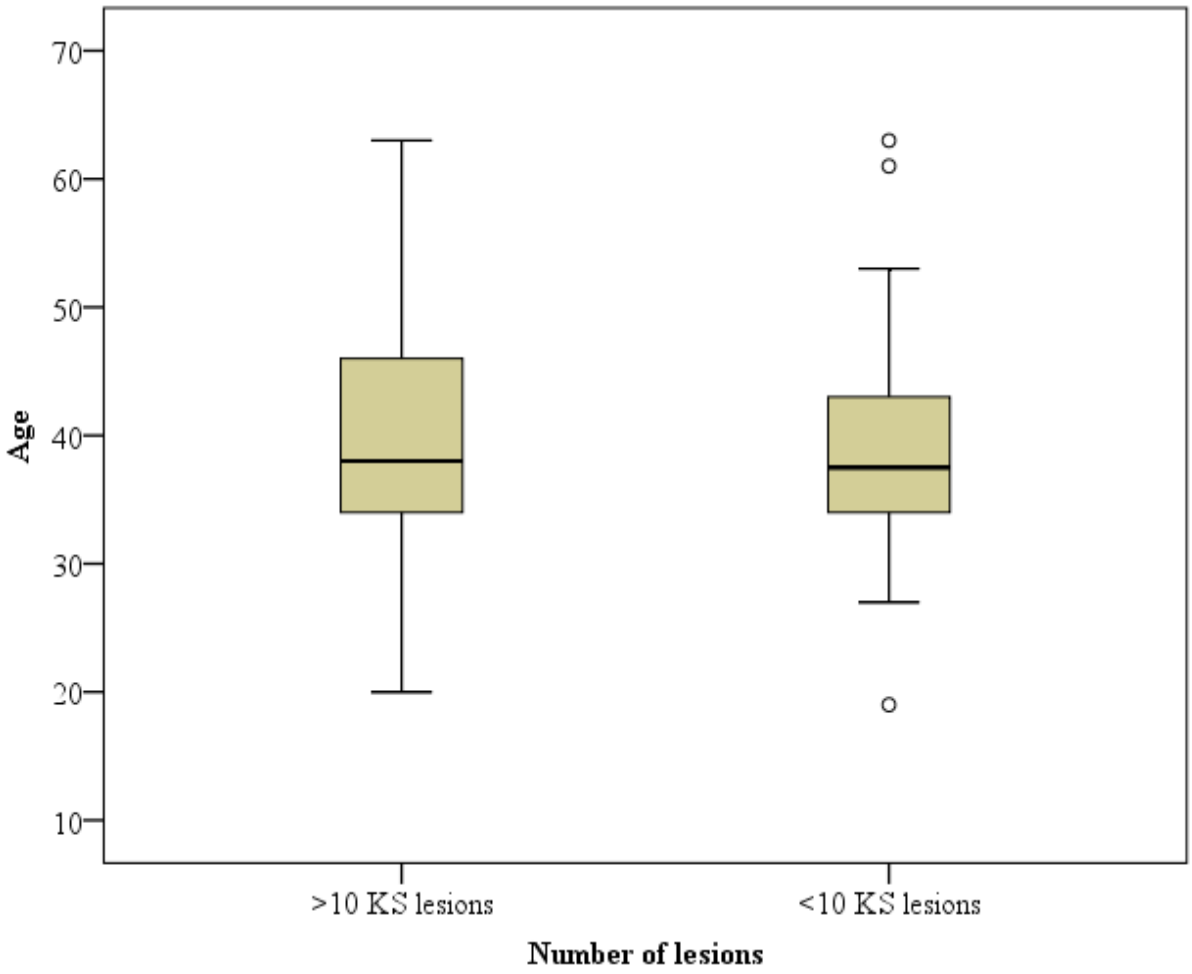


Figure 4.16: Age and number of lesions on cases

Eighteen (40.0%) cases with >10 KS lesions and 21 (58.3%) <10 KS lesions were age group 30-39 years. Thirteen (28.9%) with >10 KS lesions and 10 (27.8%) <10 KS lesions were age group 40-49 years (Table 4.6). The Pearson's chi-square statistic for age and number of KS lesions was 5.1, p=0.27.

Table 4.6: Age and number of KS lesions

	Number of Lesions n (%)	
	<10 KS lesions 36 (44.4)	>10 KS lesions 45 (55.6)
Age Group		
18-29 years	2 (5.6)	7 (15.6)
30- 39 years	21 (58.3)	18 (40.0)
40- 49 years	10 (27.8)	13 (28.9)
50- 59 years	1 (2.8)	5 (11.1)
60- 69 years	2 (5.6)	2 (4.4)

The observation made was that most cases 55.6% (45) had more than ten KS lesions and 44.4% (36) presented with less than ten KS lesions at the affected sites (Table 4.6). The distribution of > 10 KS lesions were more in the age group 18-29 years, 40-49 years and 50-59 years compared to <10 KS lesions among their counterparts in the same age group (Figure 4.17).

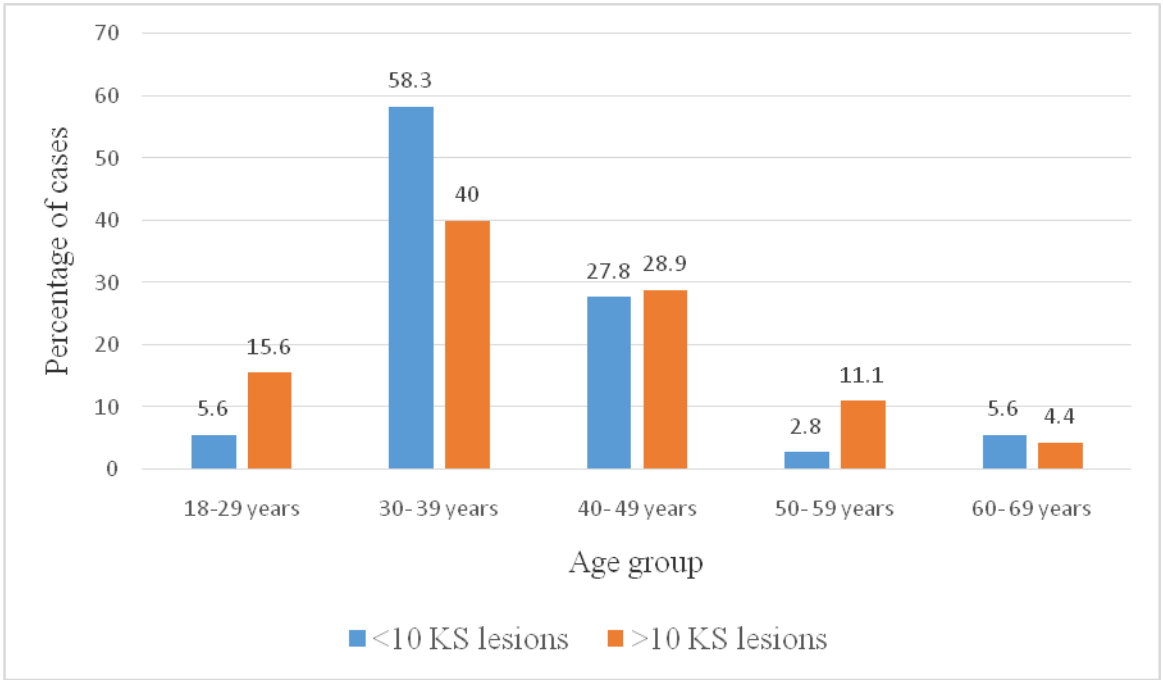


Figure 4.17: Age group and number of KS lesions

4.3.5 Age and Distribution of KS Lesions

The mean age of the studied cases with generalized lesions was 40.3 (SD=9.7) years, range 19-63 years, and a median of 39 years. Among the studied cases with localized lesions, the mean age was 40.9 (SD=9.0), range 22-61 years, and median 36 years. The mean difference in age of studied cases with generalized and localized lesions was 2.8, t=1.3, p=0.19 (Figure 4.18).

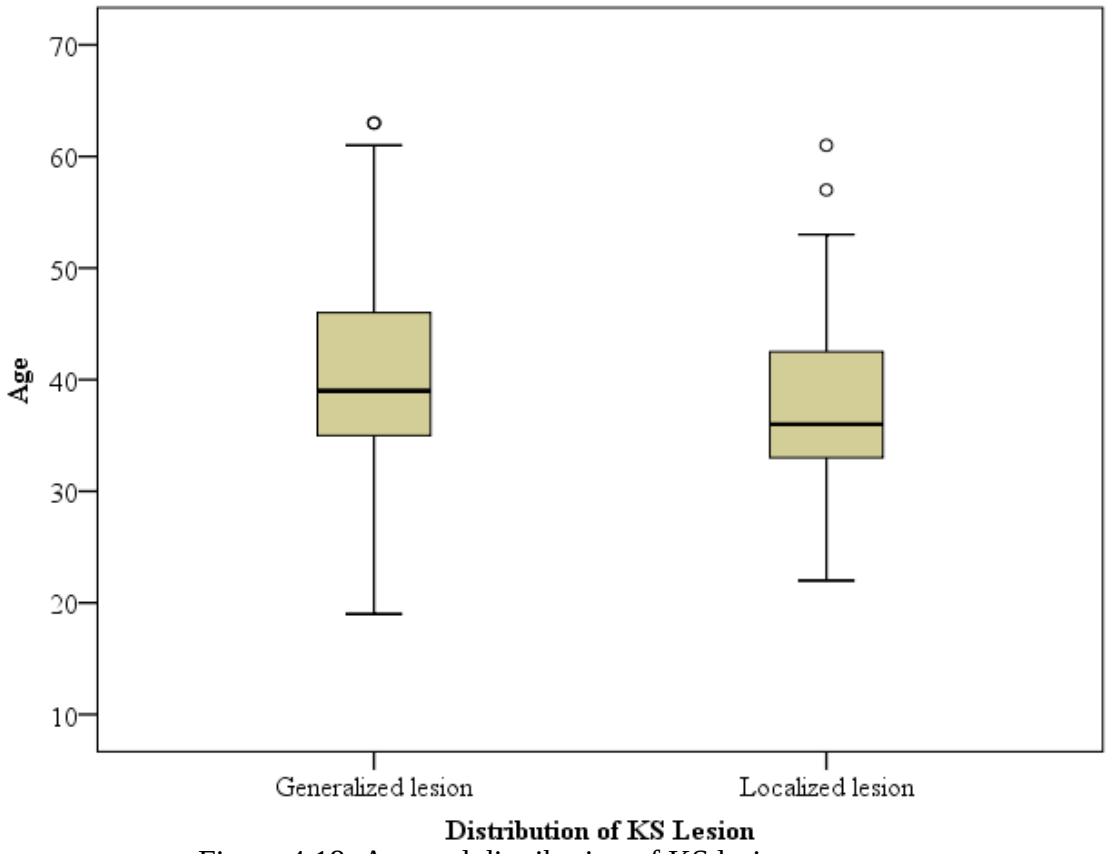


Figure 4.18: Age and distribution of KS lesions on cases

Twenty-two (41.5%) cases with a generalized lesion and 17 (60.7%) with a localized lesion were age group 30-39 years. Eighteen (34.0%) had generalized lesion and 5 (17.9%) presented with a localized lesion in the age group of 40-49 years (Table 4.7). The Pearson's chi-square statistic for age of studied cases and the distribution of KS lesions was 3.2, p=0.51.

Table 4.7: Age and distribution of KS lesions

	Distribution of KS Lesion n (%)	
	Generalized 53 (65.4)	Localized 28 (34.6)
Age Group		
18-29 years	6 (11.3)	3 (10.7)
30- 39 years	22 (41.5)	17 (60.7)
40- 49 years	18 (34.0)	5 (17.9)
50- 59 years	4 (7.5)	2 (7.1)
60- 69 years	3 (5.7)	1 (3.6)

Both generalized and localized KS lesion followed a normal distribution curve in the studied age groups. In all the age groups, generalized KS lesions was observed to be high except among the cases of 30-39 years, where localized KS lesions was witnessed to be more (Figure 4.19).

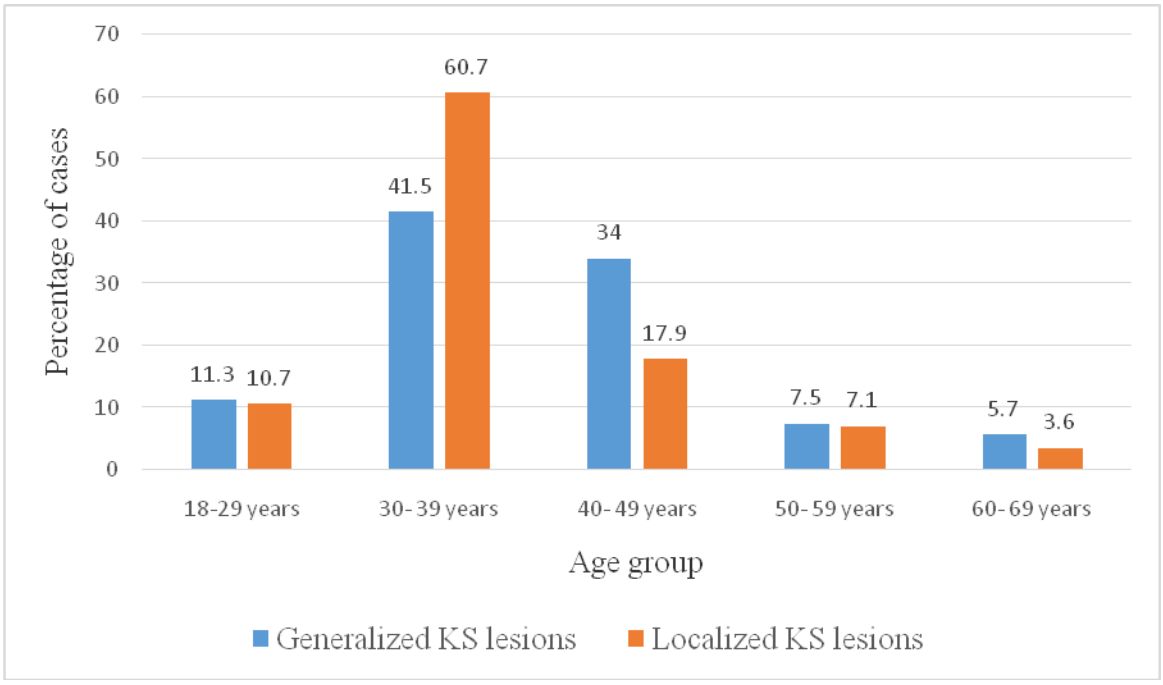


Figure 4.19: Age and distribution of KS lesions

4.3.6 Age and Site of Lesion

The mean age of the studied cases with KS lesions on their lower limbs was 38 (SD=8.8) years, range 20-61 years, and median 38 years. Cases with lesions on the trunk/chest/back had a mean age of 43 years; range 22-63 years, and median 38 years, while cases with KS lesions on their upper limbs has a mean age 39.1 years, range 28-56 years, and median 38 years (Table 4.8). The F statistic for age and site of KS lesion was 2.2, p=0.05*.

Table 4.8: Age and site of lesion

	Age					
Site of lesion	N	Mean	SD	Minimum	Maximum	Median
Lower limbs	33	38.0	8.8	20	61	38
Trunk/Chest/Back	25	43.0	11.2	22	63	38
Upper limbs	17	39.1	6.5	28	56	38
Palate/Mouth	4	34.0	6.5	26	42	34
Genitalia	1	33.0	-	33	33	-
Eyelid	1	19.0	-	19	19	-

4.3.7 Age and Morphology of Lesions

Patchy: Mean age 38.3 (SD=7.5) years, range 20-49 years, and median 39 years.

Nodular: Mean age 40.1 (SD=10.0) years, range 19-63 years, and median 36 years.

Plaque: Mean age 42.5 (SD=9.6) years, range 22-61, and median 36 years.

The F statistic for age of studied cases and morphology of KS lesion was 0.46, p=0.63 (Figure 4.20).

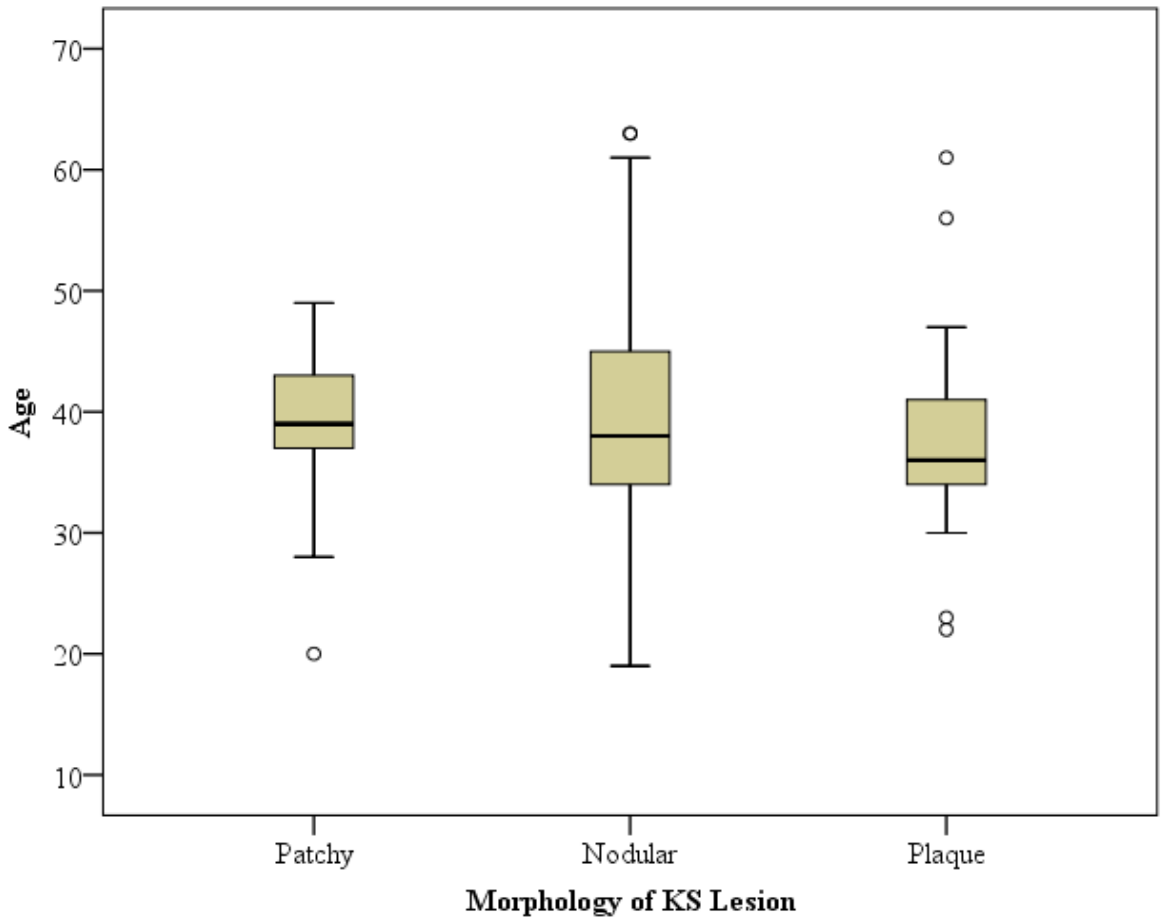


Figure 4.20: Age and morphology of KS lesions

Five (13.5%) cases with a patchy lesion, 22 (44.5%) with nodular lesions, and 12 (63.2%) with plagues were age group 30-39 years. Six (46.2%) cases with patchy lesions, 14 (28.6%) with nodular lesions, and 3 (15.8%) with plagues were age group 40-49 years (Figure 4.21). The Pearson's chi-square statistic for age of studied cases and the distribution of KS lesions was 6.3, p=0.60.

Table 4.9: Age and morphology of KS lesion

	Morphology of KS		
	Patchy n (%) 13 (16.0)	Nodular n (%) 49 (60.5)	Plaque n (%) 19 (23.5)
Age Group			
18-29 years	2 (15.4)	5 (10.2)	2 (10.5)
30- 39 years	5 (13.5)	22 (44.5)	12 (63.2)
40- 49 years	6 (46.2)	14 (28.6)	3 (15.8)
50- 59 years	0 (0.0)	5 (10.2)	1 (5.3)
60- 69 years	0 (0.0)	3 (6.1)	1 (5.3)

The nodular morphological type was greater in number 60.5% (49) compared to plaque that came second at 23.5% (19) and patchy 16% (13) (Table 4.9). It was observed that the nodular and plaque morphological type of KS were distributed in the studied cases from age group 18- 69 years. Among the studied cases, patchy morphological type of KS was seen to be present in 18-49 years of age.

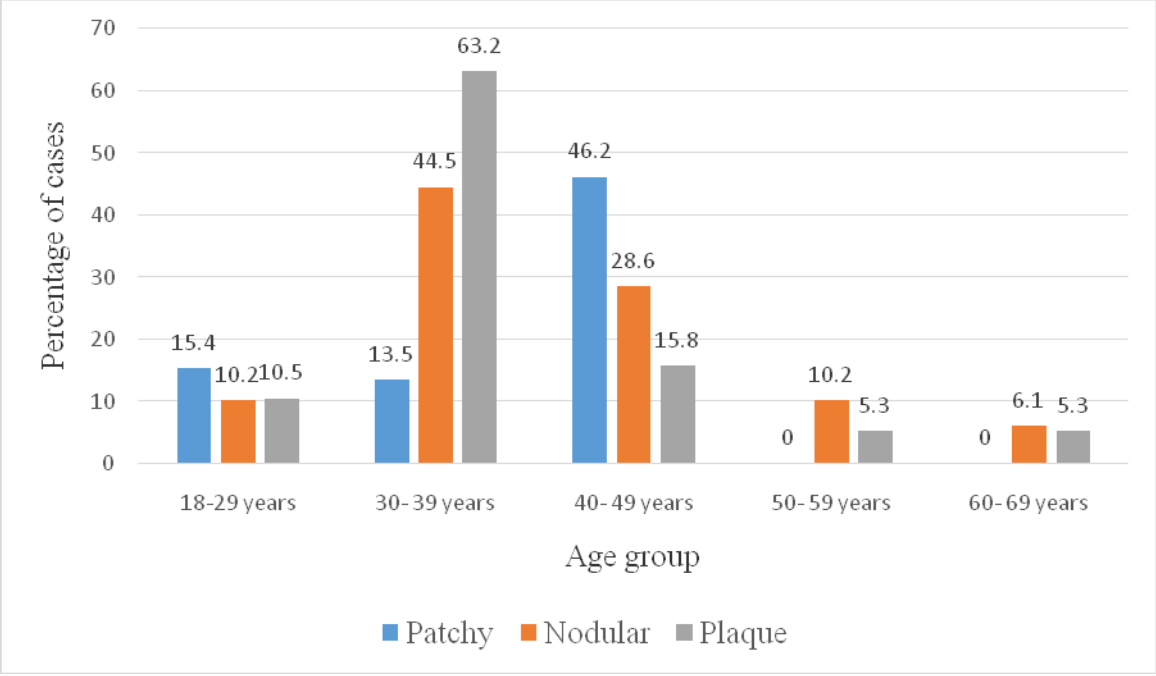


Figure 4.22: Age group and morphology of KS lesions

4.3.8 Age and KS Histology

The mean age of the studied cases with a KS histology was 39.9 (SD=9.3) years, range 19-63 years and a median of 38 years. The mean difference in age of studied cases with KS histology was 2.8, t=1.3, p=0.18 (Figure 4.23).

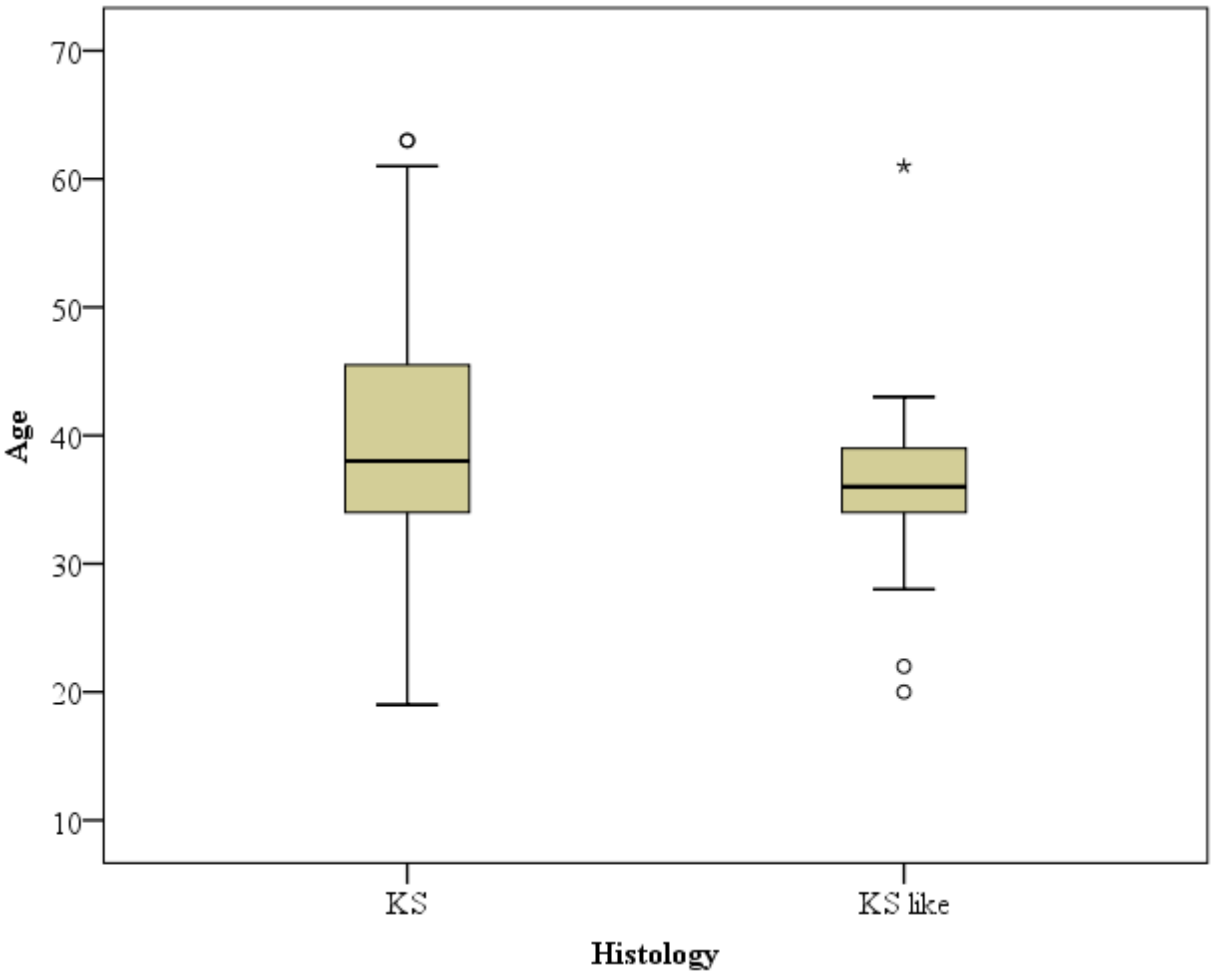


Figure 4.23: Age and KS histology

Thirty-two (47.1%) cases with a KS histology and 7 (53.8%) with KS like histology were age group 30-39 years. Twenty-one (30.9%) cases with a KS histology and 2 (15.4%) with KS like histology were age group 40-39 years (Table 4.10). The Pearson's chi-square statistic for age and histology of KS lesions was 4.4, p=0.35.

Table 4.10: Age and histology of KS lesion

	Histology	
	KS n (%) 68 (84)	KS like n (%) 13 (16)
Age Group		
18-29 years	6 (8.8)	3 (23.1)
30- 39 years	32 (47.1)	7 (53.8)
40- 49 years	21 (30.9)	2 (15.4)
50- 59 years	6 (8.8)	0 (0.0)
60- 69 years	3 (4.4)	1 (7.7)

Majority of the histology results showed that 84% (68) of the cases had KS and 16% (13) presented with KS like features. The KS histology was present across the age group of 18-69 years. While KS like was noted to be absent among the cases in the age group of 50-59 years but present in other age groups studied (Figure 2.24).

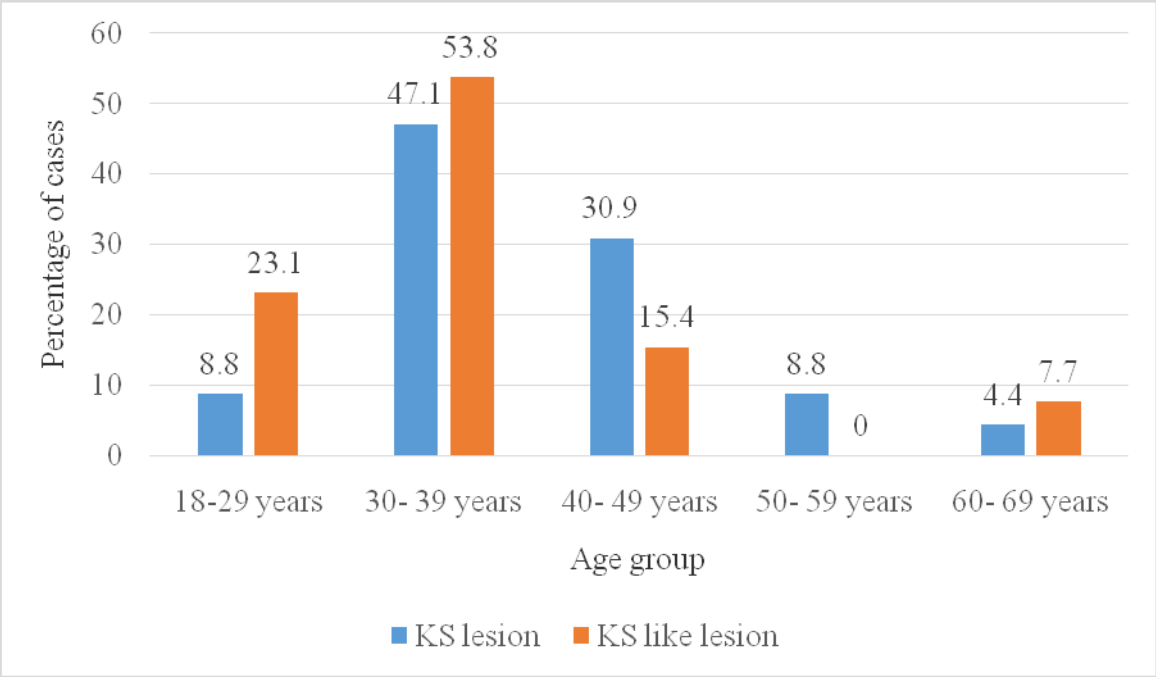


Figure 4.24: Age group and KS histology

4.3.9 Age and K1 Gene

The mean age of the studied cases with the K1 gene was 39.8 (SD=9.7) years, range 19-63 years, and a median of 38 years. Among the studied cases without a K1 gene, the mean age was 35.3 (SD=7.1), range 20-43 years, and median 37 years. The mean difference in age of studied with and without the K1 gene was 4.4 years, t=1.3, p=0.18 (Figure 4.25).

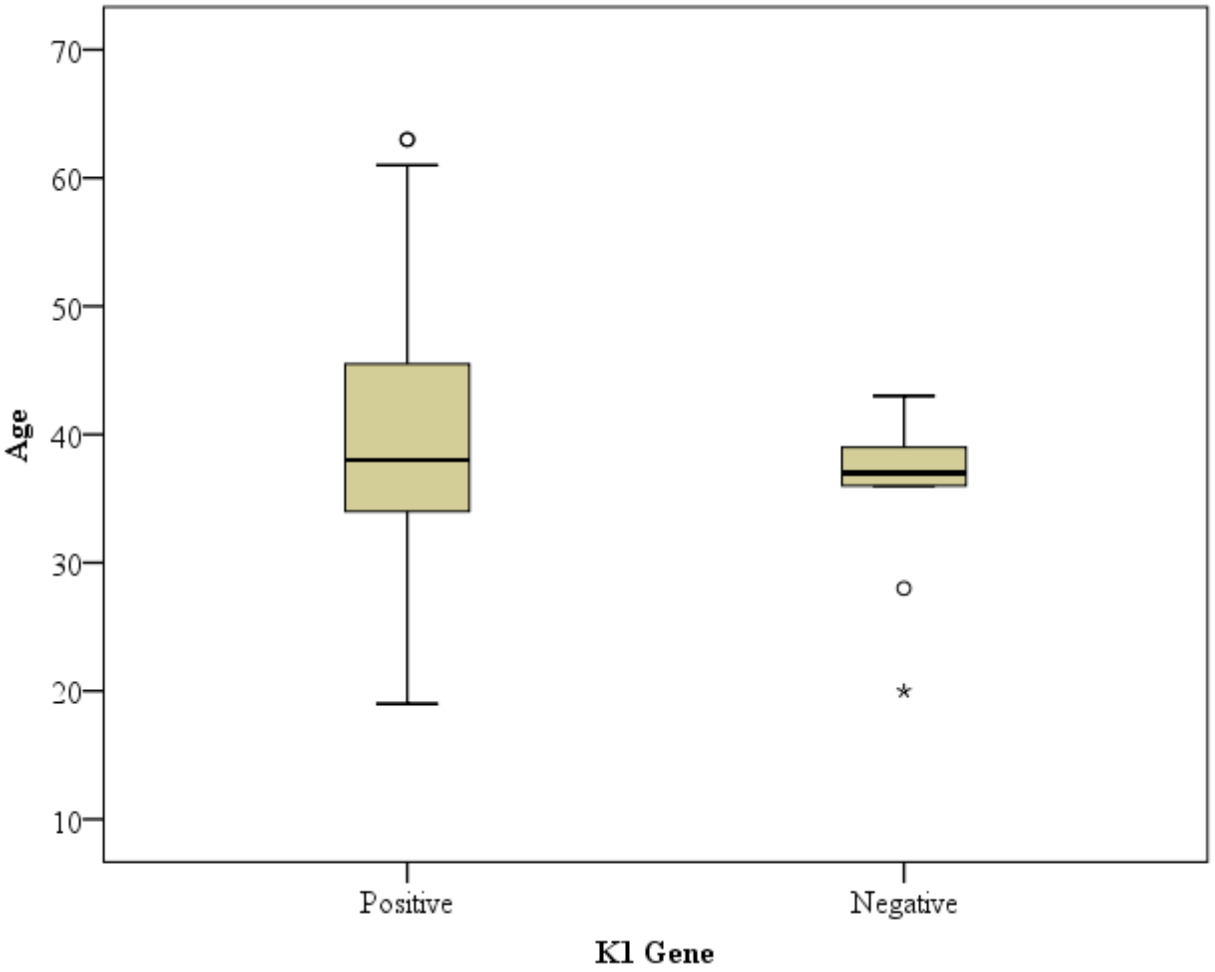


Figure 4.25: Age and K1 gene status

Thirty-four (47.2%) cases with a K1 gene and 5 (55.6%) without a K1 gene were age group 30-39 years. Twenty-one (29.2%) cases with a K1 gene and 2 (22.2%) without a K1 gene were age

group 40-39 years (Figure 4.26). The Pearson's chi-square age and K1 gene status was 2.6, p=0.62.

Table 4.11: Age and Status of K1 Gene

	K1 Gene	
	Positive n (%) 72 (88.9)	Negative n (%) 9 (11.1)
Age Group		
18-29 years	7 (9.7)	2 (22.2)
30- 39 years	34 (47.2)	5 (55.6)
40- 49 years	21 (29.2)	2 (22.2)
50- 59 years	6 (8.3)	0 (0.0)
60- 69 years	4 (5.6)	0 (0.0)

Majority 88.9% (72) of the studied cases were found to be positive implying the existence of K1 gene, while 11.1% (9) indicated negative (Table 4.11). The presence of K1 gene was seen across all the age groups that were studied. Among the cases that were negative for K1 gene, it was notable that their age groups were from 50-69 years.

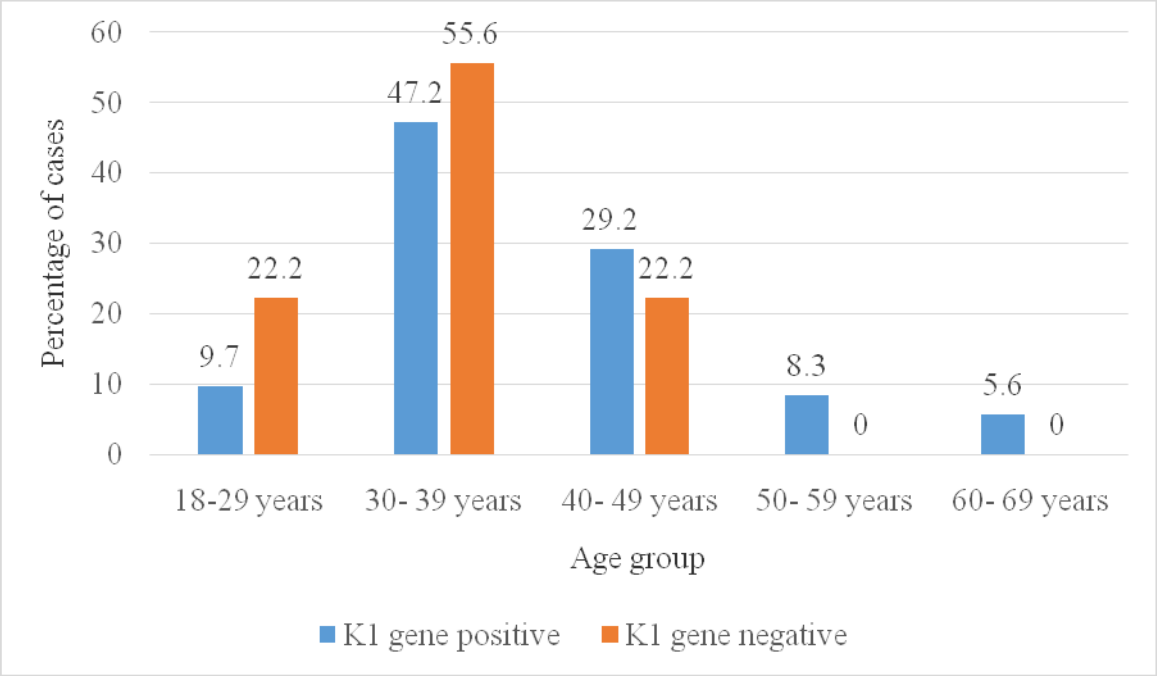


Figure 4.27: Age group and K1 gene status

4.3.10 Age and K15P Gene

The mean age of the studied cases with the K15P gene was 39.8 (SD=9.7) years, range 19-63 years, and a median of 38 years. Among the studied cases without a K15P gene, the mean age was 35.3 (SD=7.1), range 20-43 years, and median 37 years. The mean difference in age of studied cases with and without the K15P gene was 4.4 years, t=1.3, p=0.18 (Figure 4.28).

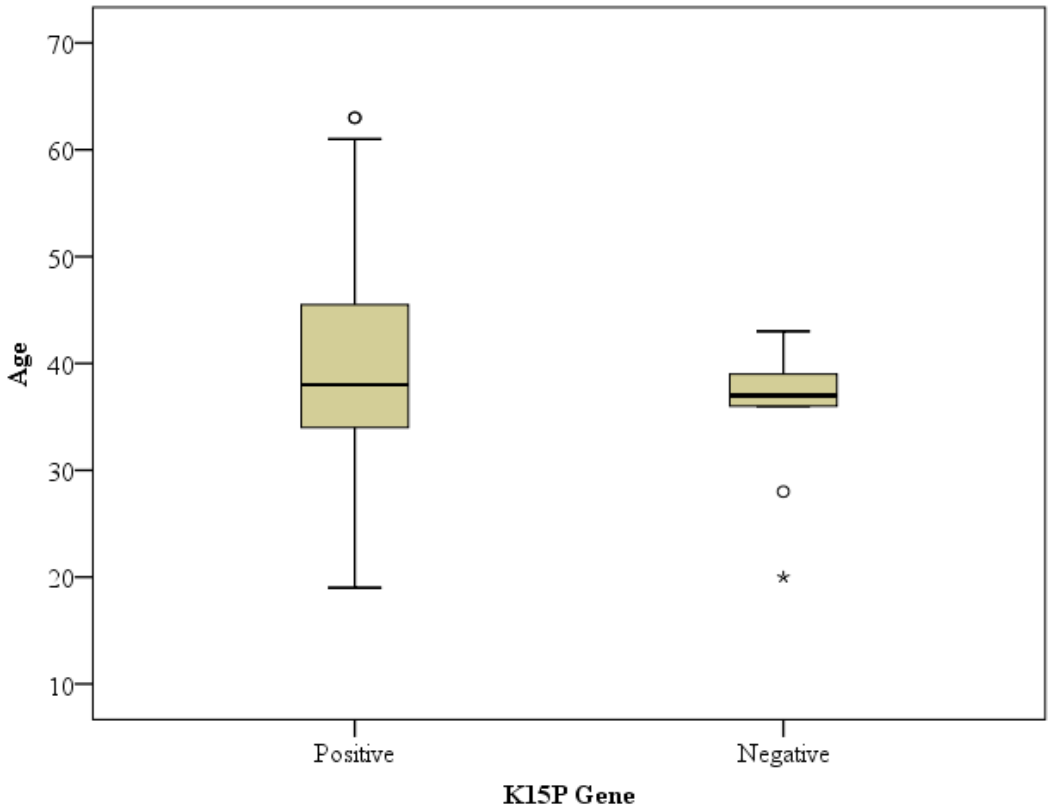


Figure 4.28: Age and K15P status

Thirty-four (47.2%) cases with a K15P gene and 5 (55.6%) without a K15P gene were age group 30-39 years. Twenty-one (29.2%) cases with a K15P gene and 2 (22.2%) without a K15P gene were age group 40-39 years (Figure 4.29). The Pearson's chi-square age and K15P gene was 2.6, p=0.62.

Table 4.12: Age and K15P gene status

	K15P Gene	
	Positive n (%) 72(88.9)	Negative n (%) 9 (11.1)
Age Group		
18-29 years	7 (9.7)	2 (22.2)
30- 39 years	34 (47.2)	5 (55.6)
40- 49 years	21 (29.2)	2 (22.2)
50- 59 years	6 (8.3)	0 (0.0)
60- 69 years	4 (5.6)	0 (0.0)

Majority 88.9% (72) of the studied cases were found to be positive implying the existence of K15P gene, while 11.1% (9) indicated negative (Table 4.12). The presence of K15P gene was seen across all the age groups that were studied. Among the cases that were negative for K15P gene, it was notable that their age groups were from 50-69 years.

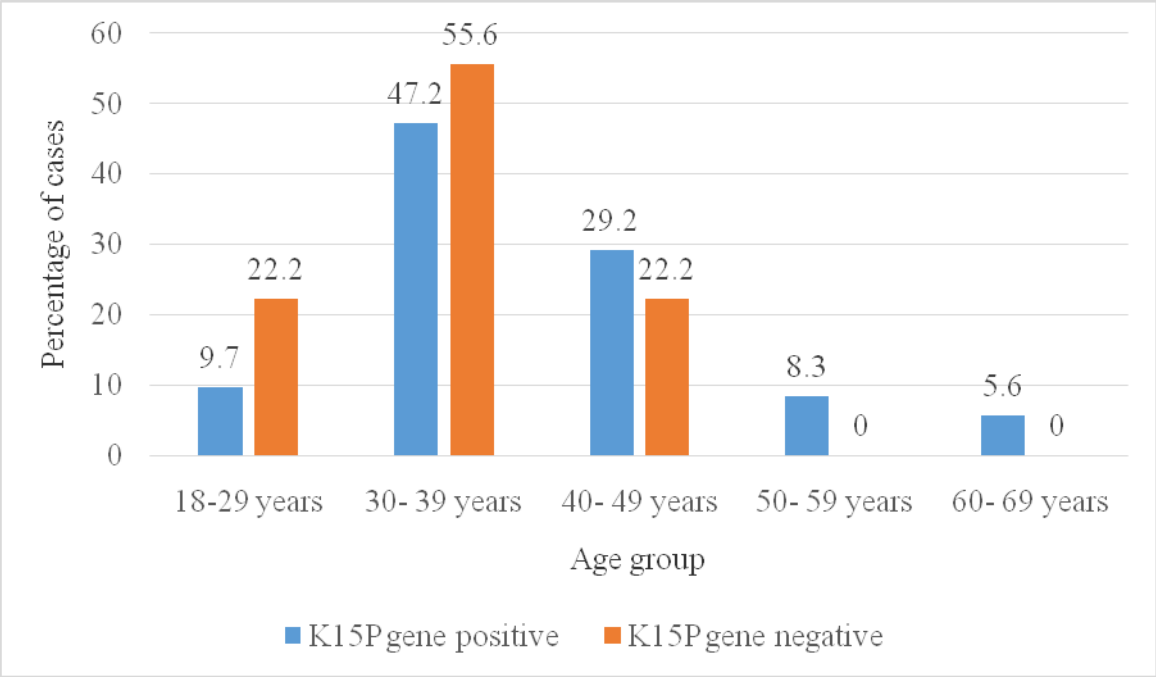


Figure 4.29: Age group and K15P status

4.3.11 Age and ORF75 Gene

The mean age of the studied cases with an ORF75 gene was 38.8 (SD=9.8) years, range 19-63 years, and a median of 37 years. Among the studied cases without an ORF75 gene, the mean age was 40.0 (SD=9.2), range 20-61 years, and median 41 years. The mean difference in age of studied cases with and without an ORF75 gene was -1.1 years, t=-0.51, p=0.60 (Figure 4.30).

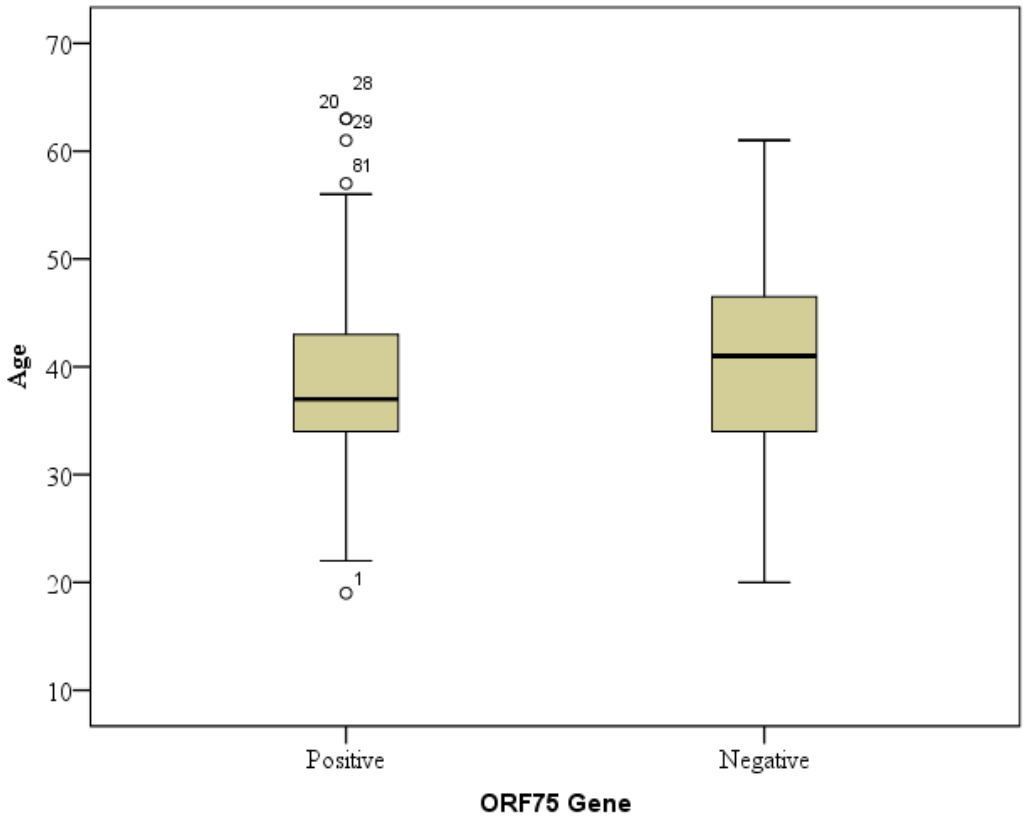


Figure 4.30: Age and ORF75 gene status

Twenty-eight (57.1%) cases with an ORF75 gene and 11 (34.4%) without an ORF75 gene were age group 30-39 years. Nine (18.4%) cases with an ORF75 gene and 14 (43.8%) without an ORF75 gene were 40-39 years (Figure 4.31). The Pearson's chi for age and ORF75 gene was 7.0, p=0.13.

Table 4.13: Age and ORF75 Gene status

	ORF75 Gene	
	Positive n (%) 49 (60.5)	Negative n (%) 32 (39.5)
Age Group		
18-29 years	5 (10.2)	4 (12.2)
30- 39 years	28 (57.1)	11 (34.4)
40- 49 years	9 (18.4)	14 (43.8)
50- 59 years	4 (8.2)	2 (6.3)
60- 69 years	3 (6.1)	1 (3.1)

The presence of ORF75 gene was found to be 60.5% (49) and 39.5% (32) cases indicated negative results (Table 4.13). Both positive and negative ORF75 gene results were seen across all the age groups that were studied. The distribution of positive and negative ORF75 gene results followed a normal curve across the studied age groups.

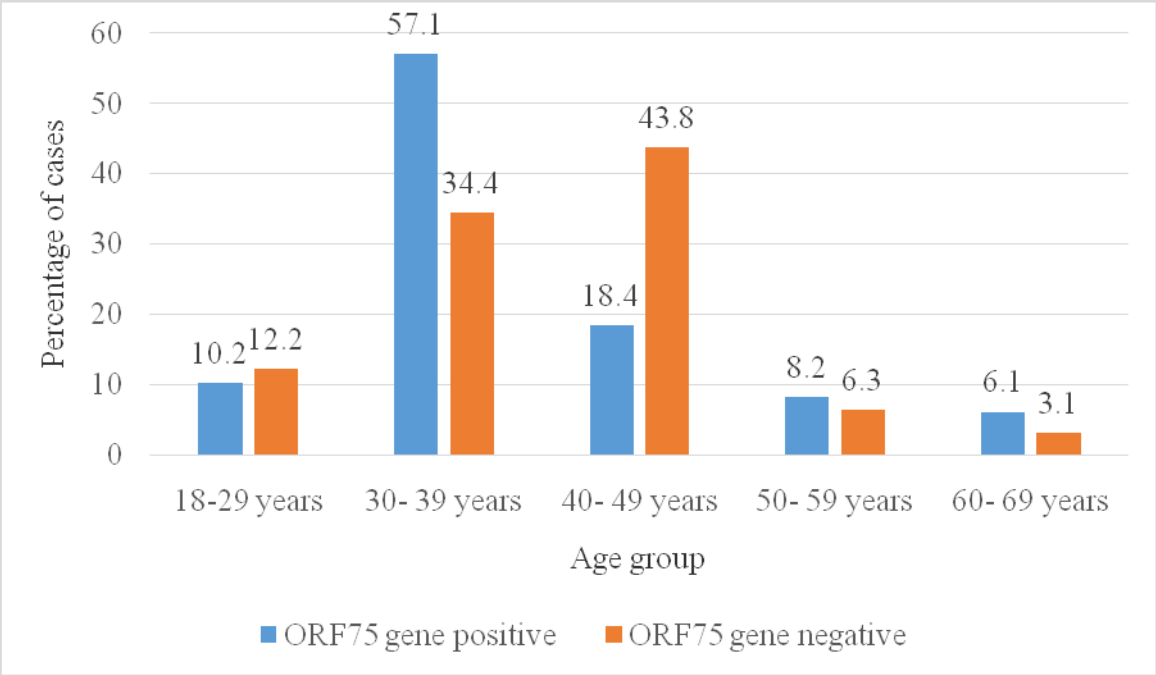


Figure 4.31: Age group and ORF75 gene status

4.3.12 Gender and Treatment

Forty-five (97.8%) males and 32 (91.4%) females were using ARV, OR=4.2 (0.42-42.4), p=0.18.

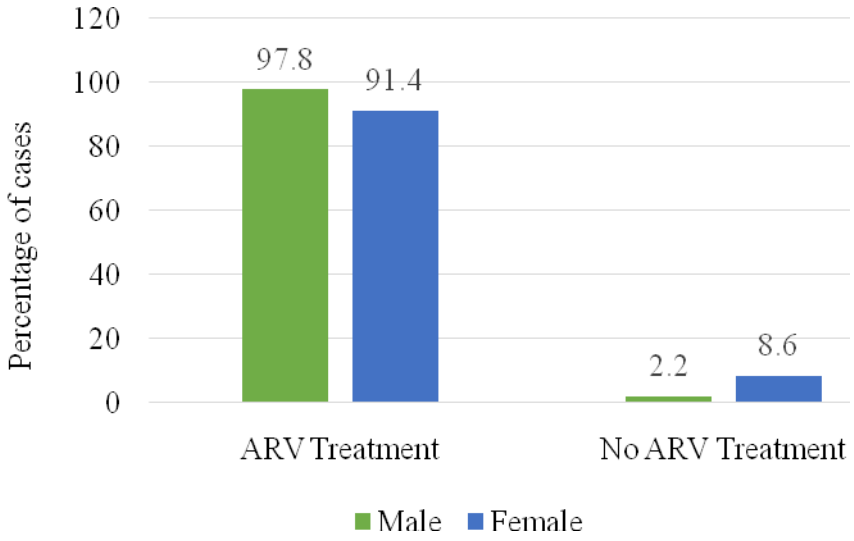


Figure 4.32: Gender and Treatment

The odd of being on ARVs was 4.2 times regardless of the gender. The male 97.8% (45) and female 91.4% (32) cases that were on ARV treatment was found to be comparable (Figure 4.32). There were greater number 8.6% (3) females who were not on ARV treatment compared to males 2.2% (1).

4.3.13 Gender and CD_4 Cell Count

Twenty (43.5%) males and 9 (25.7%) females had a CD_4 cell count 0-200 Cells/mm3, OR=2.2 (0.8-5.7), p=0.09.

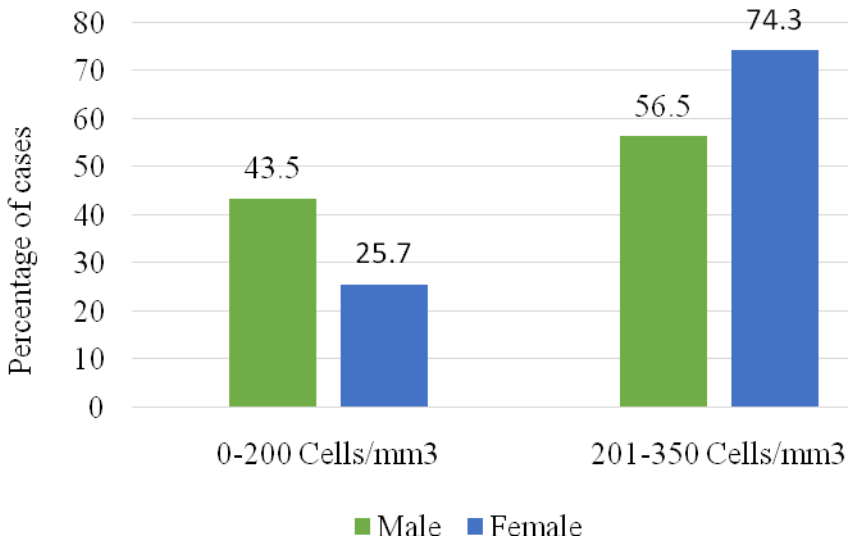


Figure 4.33: Gender and CD_4 Cell Count

The p=0.09 is suggestive of a strong association between gender and the CD_4 Cell Count. The males 43.5% (20) and females 25.7% (9) cases had CD_4 Cell Count ranging from 0-200 cells/mm^3 (Figure 4.33). Whereas, the CD_4 Cell Count ranging from 201-350 cells/mm^3 was higher in females at 74.3% (26) and 56.5% (26) in males.

4.3.14 Gender and Number of Lesions

Twenty-nine (63.0%) males and 16 (45.7%) females had >10 KS lesions, OR=2.0 (0.8-4.9), p=0.12. The number of KS lesions was not significantly p=0.12 influenced by gender. The odds that either of the gender in the studied cases would have >10 or <10 KS lesions was 2 times.

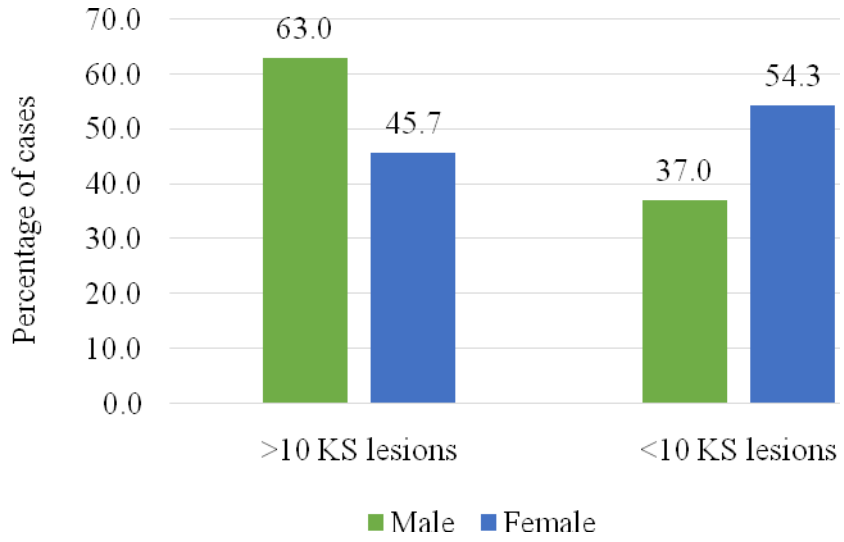


Figure 4.34: Gender and Number of Lesions

There is a striking difference in the distribution among the studied cases. For instance, the males 63% (29) and females 45.7% (16) cases had >10 KS lesions (Figure 4.34). Whereas, <10 KS lesions was higher in females at 54.3% (19) and 37% (17) in males.

4.3.15 Gender and Distribution of KS Lesions

Thirty-one (67.4%) males and 22 (62.9%) females had a generalized lesion, OR=1.2 (0.48-3.07), p=0.67. In both generalized and localized lesions the distribution of KS was comparable in males and females (Figure 4.35).

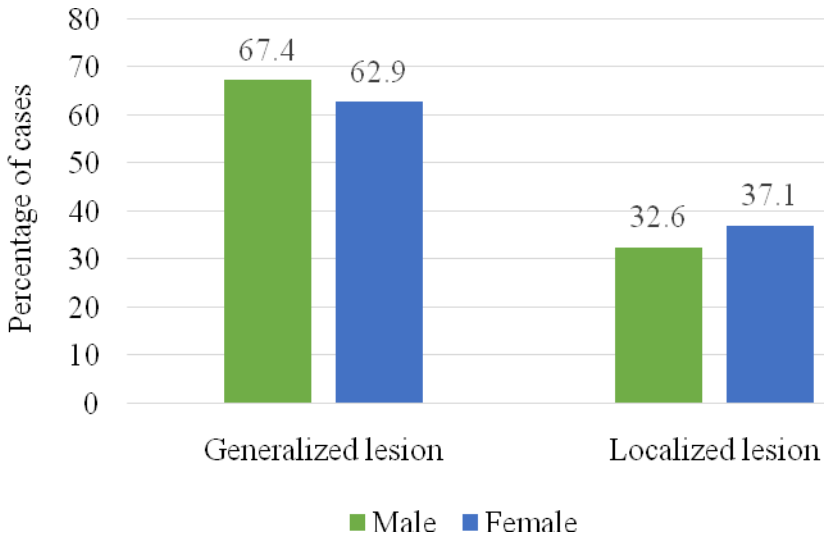


Figure 4.35: Gender and Distribution of KS Lesions

Generalized KS lesion was found to be 67.4% (31) and 62.9% (22) in males and females respectively. While localized KS lesion was 32.6% (15) among males and 37.1% (13) in females (Figure 4.35).

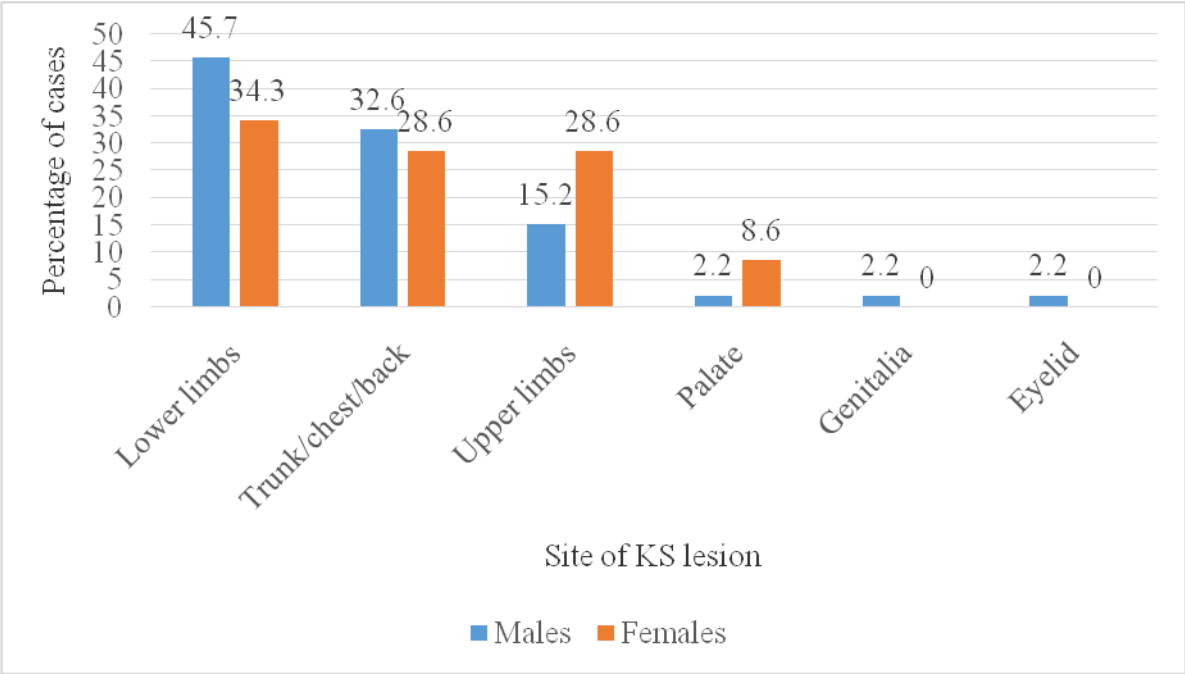
4.3.16 Gender and Site of KS Lesion

Twenty-one (45.7%) males and 12 (34.3%) females had lesions on the lower limbs. Fifteen (32.6%) males and 10 (28.5%) females had lesions on their back/chest/trunk, while 7 (15.2%) males and 10 (28.6%) females had lesions on the upper limbs. The Pearson's chi-square statistic for gender and site of KS lesions was 5.5, p=0.34.

Table 4.14: Gender and site of KS lesion

	Gender	
	Male n (%) 46 (56.8)	Female n (%) 35 (43.2)
Site		
Lower limbs	21 (45.7)	12 (34.3)
Trunk/Chest/Back	15 (32.6)	10 (28.6)
Upper limbs	7 (15.2)	10 (28.6)
Palate/Mouth	1 (2.2)	3 (8.6)
Genitalia	1 (2.2)	0 (0.0)
Eyelid	1 (2.2)	0 (0.0)

It was remarkable observed that prominence of KS lesion was more 45.7% (21) in the lower limbs among male's cases (Table 4.14). In the cases that were studied, there was no record of KS lesions on the eyelids and genitalia in females. Anatomical site location of KS lesion where female predominate was the upper limbs 10 (28.6) and palate/mouth 3 (8.6) (Figure 4.36).



4.36: Gender and site of KS lesion

4.3.17 Gender and Morphology of KS Lesion

Thirty (65.2%) male and 8 (54.3%) females had nodular lesions. Eleven (23.9%) and 8 (22.9%) respectively had plaques, while 5 (10.9%) and 8 (22.9%) respectively had patchy lesions (Table 4.15). The Pearson's chi-square statistic for gender and morphology of lesion was 2.1, p=0.33.

Table 4.15: Gender and morphology of KS lesion

	Gender	
	Male 46 (56.8)	Female 35 (43.2)
Morphology		
Patchy	5 (10.9)	8 (22.9)
Nodular	30 (65.2)	19 (54.3)
Plaque	11 (23.9)	8 (22.9)

Patchy type of KS was more 22.9% (8) in females than males 10.9% (5) (Figure 4.37). Among the studied cases, the number of plaque was more in males 23.9% (11) compared to females 22.9% (8).

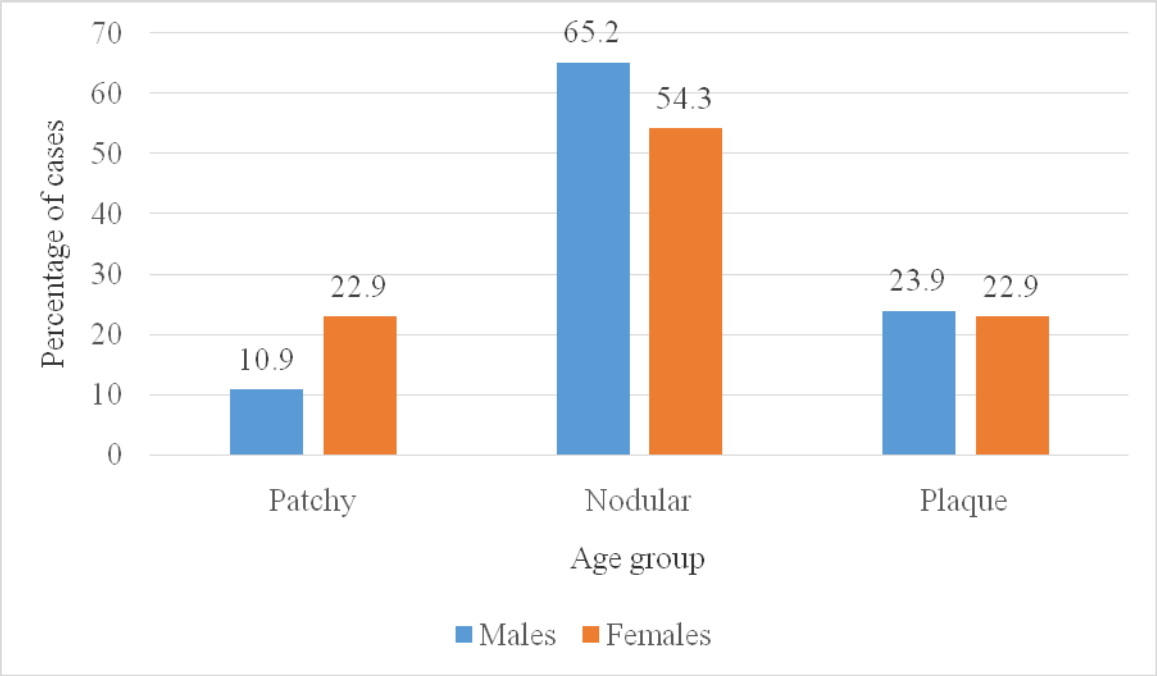


Figure 4.37: Gender and morphology of KS lesion

4.3.18 Gender and Histology of Lesions

Forty (87.0%) males and 28 (80.0%) females had a KS histology, OR=1.6(0.5-5.4), p=0.39.

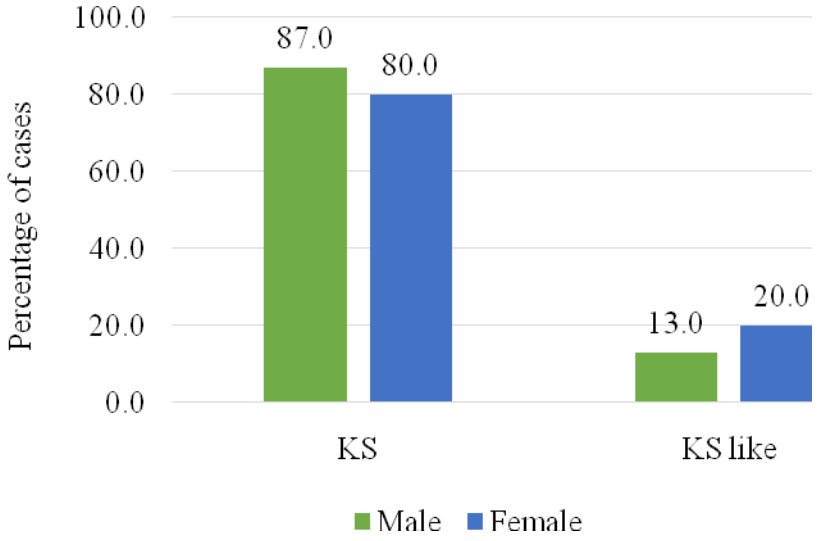


Figure 4.38: Gender and Histology of Lesions

The KS like features was seen more 20% (7) in females compare to males 13% (6) (Figure 4.41). Among the studied cases males 87% (40) and females 80% (28) had typical features of KS.

4.3.19 Gender and K1 Gene

The K1 gene was present in 42 (91.3%) males and 30 (85.7%) females, OR=1.7(0.4-7.0), p=0.42.

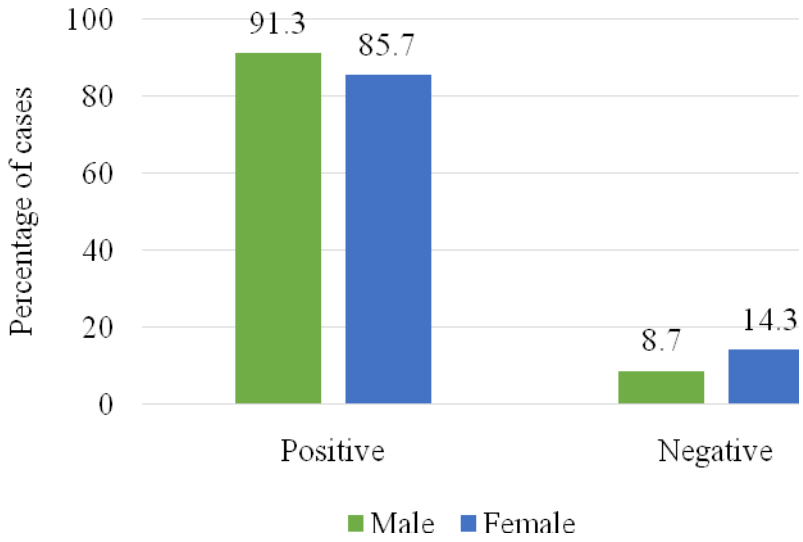


Figure 4.39: Gender and K1 Gene

The odds of either gender testing positive for K1 gene was 1.7 times. The observation made was that 91.3% (42) males and 85.7% (30) females had K1 genes (Figure 4.39). The negativity of K1 was inclined in females compared to males.

4.3.20 Gender and K15P Gene

The K15P gene was present in 42 (91.3%) males and 30 (85.7%) female, OR=1.7(0.4-7.0), p=0.42.

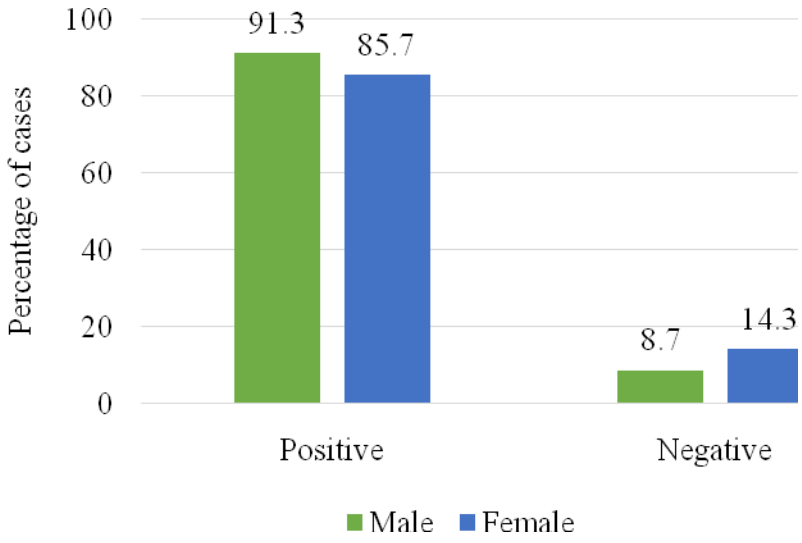


Figure 4.40: Gender and K15P Gene

The odds that gender would turn out positive for K15P gene was 1.7 times. The observation made was that 91.3% (42) males and 85.7% (30) females had K15P genes (Figure 4.40). The negativity of K15P was inclined in females compared to males.

4.3.21 Gender and ORF75 Gene

The ORF75 gene was present in 28 (60.9%) males and 21 (60.0%) females, OR=1.0 (4.2-2.5), p=0.93.

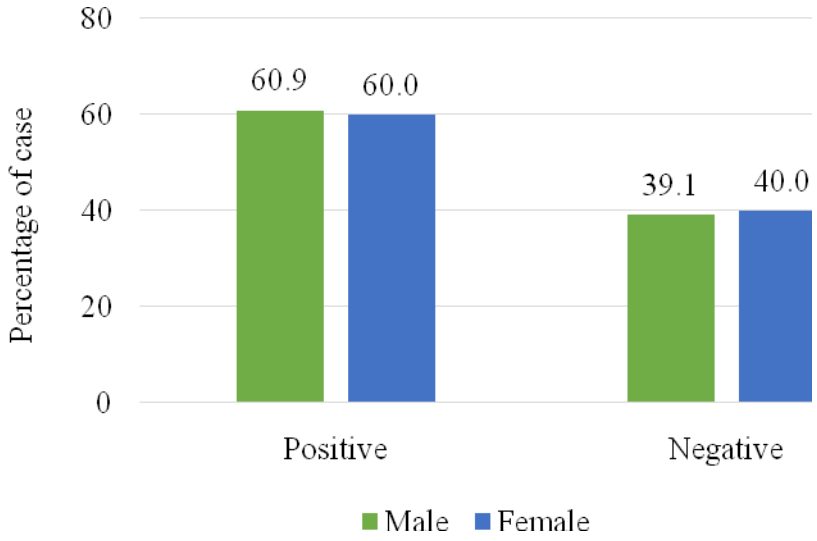


Figure 4.41: Gender and ORF75 Gene

In the studied cases that were positive and negative for ORF75 gene, observation made was that these results were comparable among the gender (Figure 4.41)

4.3.22 Treatment and CD_4 Count

Twenty-six (33.8%) cases under ARV treatment and 3 (75.0%) cases not under ARV treatment has a CD4 cell count of 0-200 Cells/mm3, OR=0.1(0.01-1.7), p=0.09. However, P=0.09 is an indication of a strong association between being on ARV treatment and the CD_4 count.

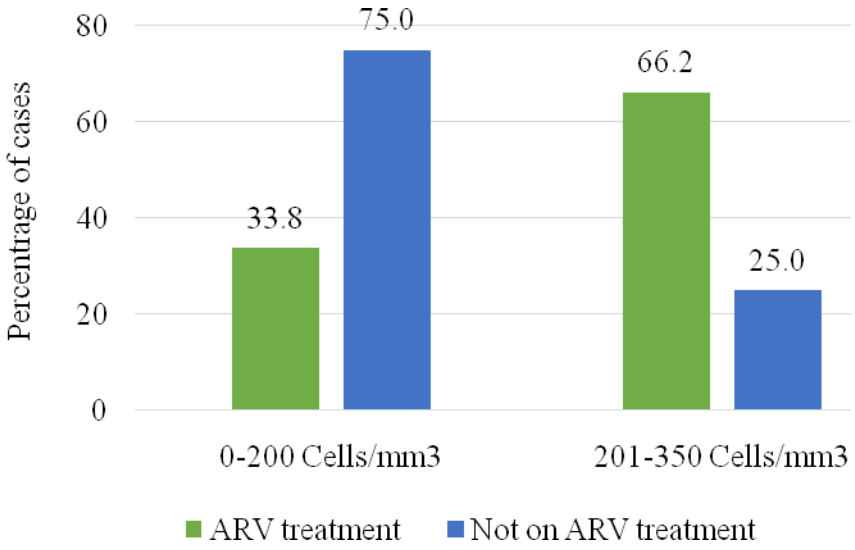


Figure 4.42: Treatment and CD4 Count

The results on CD_4 cell count of 0-200 Cells/mm^3 indicated that 33.8% (26) were on ARV treatment and 75% (3) not under ARV treatment (Figure 4.42). Among the cases that had CD_4 cell count of 201-350 Cells/mm^3 indicated that 66.2% (51) were on ARV treatment and 25% (1) not under ARV treatment

4.3.23 ARV Treatment and Number of Lesions

Forty-four (57.1%) cases under ARV treatment and 1 (25.0%) cases not under ARV treatment has >10 KS lesions, OR=4.0(0.3-40.2), p=0.20.

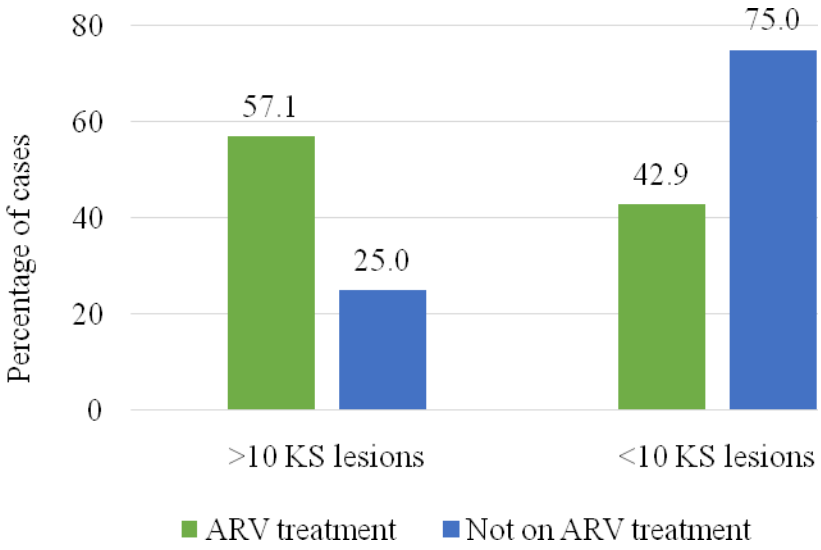


Figure 4.43: ARV Treatment and Number of Lesions

The odds of developing KS lesions when one is on ARVs were 4 times. The number of cases that presented with >10 KS lesions 57.1% (44) were on ARVs and 25% (1) not under treatment (Figure 4.43). The cases that had <10 KS lesions 75% (3) were not on ART and 42.9% (33) on treatment.

4.3.24 Treatment and Distribution of KS

Fifty-two (67.5%) cases under ARV treatment and 1 (25.0%) cases not under ARV treatment had a generalized lesion, OR=6.2(0.6-63.2), p=0.08.

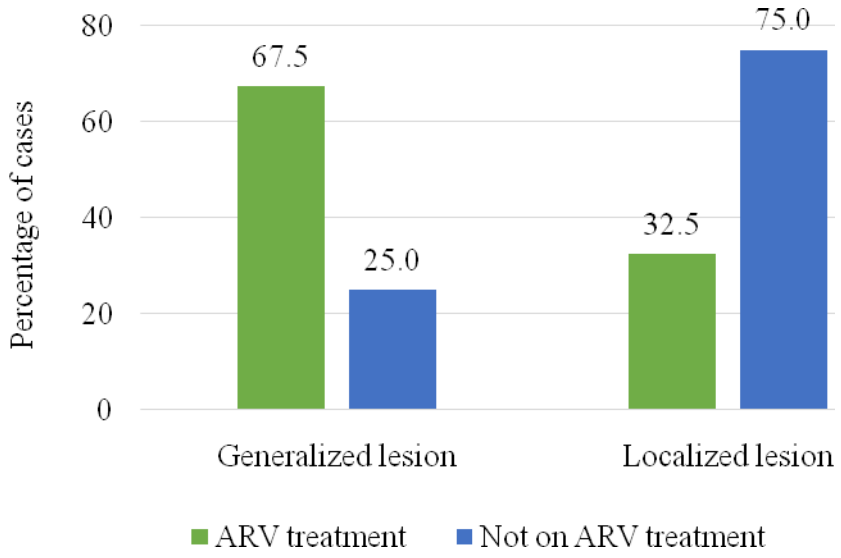


Figure 4.44: Treatment and Distribution of KS

The p=0.08, however implies that there was a strong association between being on ARVs and the distribution pattern of KS. The odds of the distribution pattern of KS lesion whether one is on ARVs or not is 6.2 times. Generalized lesions was observed as follows; 67.5% (52) on ARV treatment 25% (1) not under ARV treatment (Figure 4.44). Localized lesions were as follows; 32.5% (25) on ARV treatment and 75% (3) not on ARV treatment.

4.3.25 ARV Treatment and Site of KS Lesion

Thirty-two (41.6) cases under ARV treatment and 1 (25.0%) not under treatment had lesions on the lower limbs, while 24 (31.2%) and 1 (25.0%) has lesions on the trunk/chest/back. The Pearson's chi-square for ARV treatment status and location of lesion was 18.5, $p<0.01$*.

Table 4.16: ARV treatment and site of KS lesion

	Treatment	
	ARV treatment n (%)	Not on ARV treatment n (%)
Site	77 (95.1)	4 (4.9)
Lower limbs	32 (41.6)	1 (25.0)
Trunk/Chest/Back	24 (31.2)	1 (25.0)
Upper limbs	17 (22.1)	0 (0.0)
Palate/Mouth	2 (2.6)	2 (50.0)
Genitalia	1 (1.3)	0 (0.0)
Eyelid	1 (1.3)	0 (0.0)

There was a significant p=0.01* association between the being on ARV treatment and the anatomical site where the KS lesions are located. The eyelid, genitalia and upper limbs are among the anatomical sites that had no KS lesions manifested by the cases that were not on ARV treatment (Table 4.16).

4.3.26 ARV Treatment and Morphology of KS

Forty-seven (61.0%) cases under ARV treatment and 1 (25.0%) not under treatment had a nodular morphology of KS, while 18 (23.4%) and 1 (25.0%) respectively had plaques. The Pearson's chi-square for ARV treatment status and morphology of KS was 0.29, p=0.86.

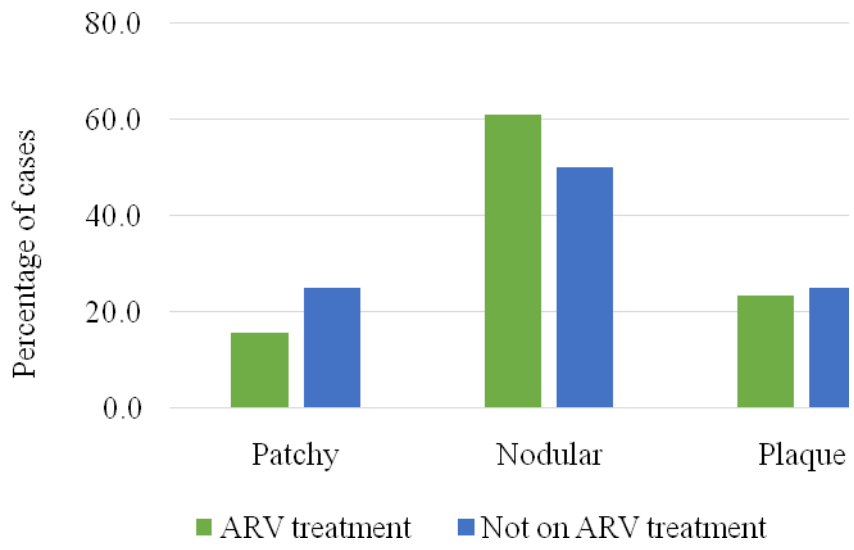


Figure 4.45: ARV Treatment and Morphology of KS

The following was observed on Nodular morphology, 61% (47) under ARV treatment and 25% (1) of the cases were not under treatment (Figure 4.45).

4.3.27 ARV Treatment and Histology

Sixty-five (84.4%) cases under ARV treatment and 3 (75.0%) not under treatment had a KS histology, OR=1.8 (0.1-18.8), p=0.61.

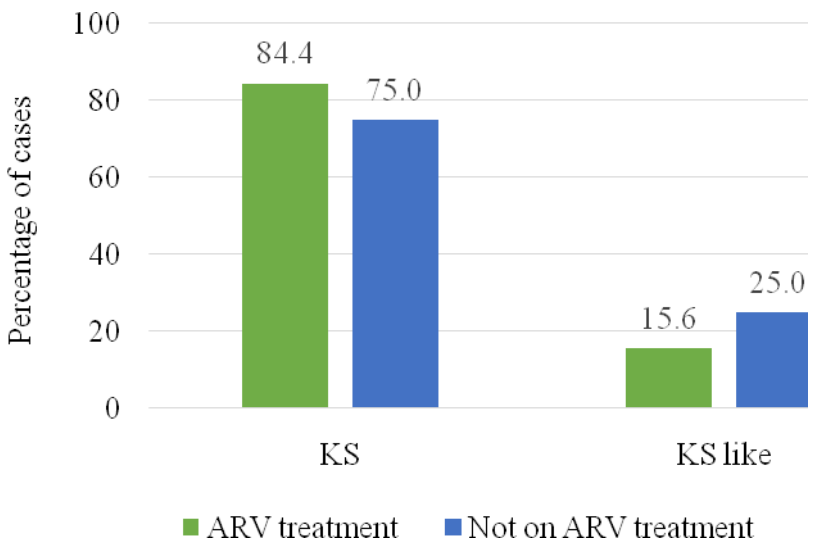


Figure 4.46: ARV Treatment and Histology

The odds of identifying KS or KS like when on ARV or not were 1.8 times. The KS cases, 84.4% (65) were under ARV treatment and 75% (3) not under treatment (Figure 4.46). Results on KS like showed that 15.6% (12) were on ARV treatment and 25% (1) was not.

4.3.28 ARV Treatment and K1 Gene

The KI gene was present in 69 (89.6%) cases under ARV treatment and 3 (75.0%) not under treatment, OR=2.8 (0.2-31.0), p=0.36.

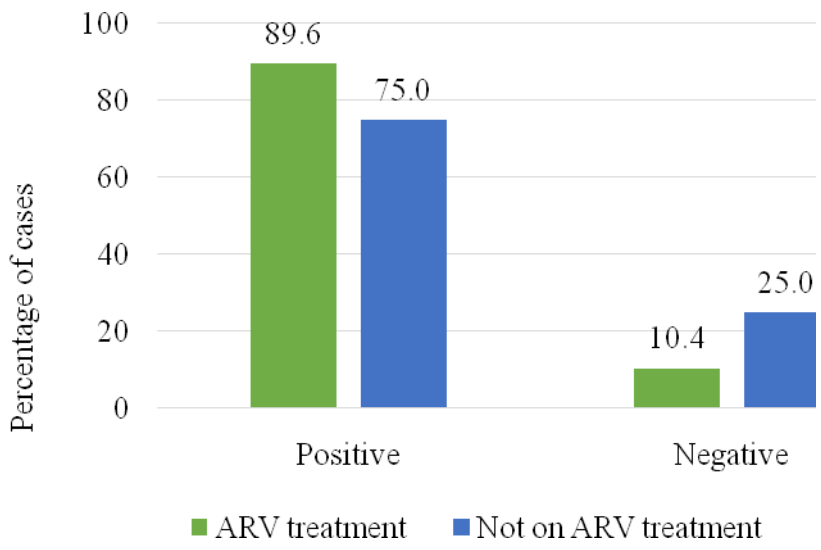


Figure 4.47.ARV Treatment and K1 Gene

The odds for positivity of KI gene whether on ARV treatment or not was 2.8 times. The KI gene was present in 89.6% (69) under ARV treatment and 75% (3) not under treatment (Figure 4.47). The cases that were negative for KI gene 25% (1) was not on ARV treatment and 10.4% (8) on treatment.

4.3.29 ARV Treatment and K15P Gene

The K15P gene was present in 69 (89.6%) cases under ARV treatment and 3 (75.0%) not under treatment, OR=2.8 (0.2-31.0), p=0.36.

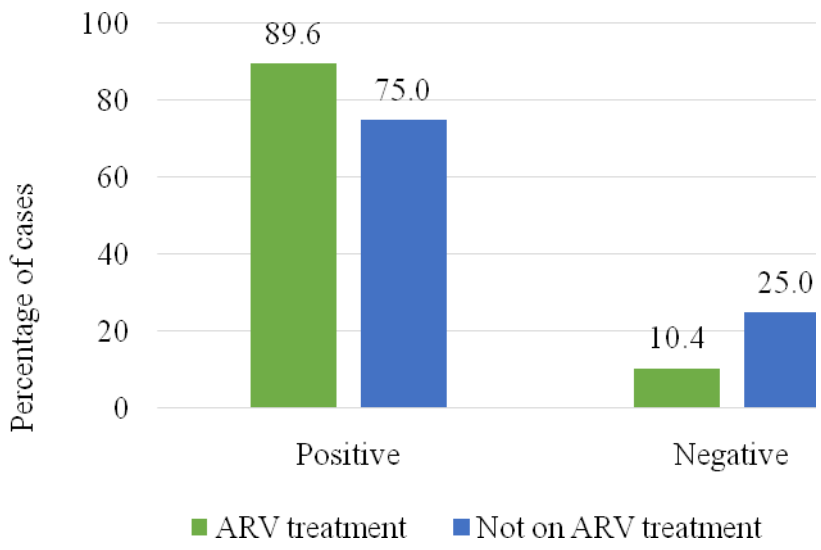


Figure 4.48.ARV Treatment and K15P Gene

The odds for positivity of K15P gene whether on ARV treatment or not was 2.8 times. The K15P gene was present in 89.6% (69) under ARV treatment and 75% (3) not under treatment (Figure 4.48). The cases that were negative for K15P gene 25% (1) was not on ARV treatment and 10.4% (8) on treatment.

4.3.30 ARV Treatment and ORF75 Gene

The ORF75 gene was present in 47 (61.0%) cases under ARV treatment and 2 (50.0%) not under treatment, OR=1.5 (0.2-11.7), p=0.66.

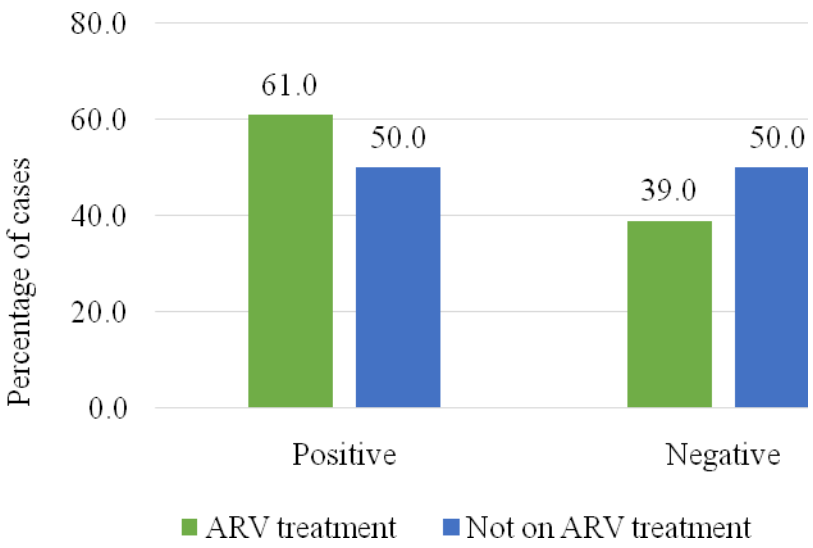


Figure 4.49: ARV Treatment and ORF75 Gene

The odds of ORF75 gene turning out to be positive whether on treatment was 1.5 times. The positive cases for ORF75 gene, 61% (47) were on ARV treatment and 50% (2) not under treatment (Figure 4.49).

4.3.31 CD_4 Cell Count and Number of KS Lesions

Sixteen (55.2%) cases with a CD4 count 0-200 Cells/mm^3 and 29 (55.8%) 201-350 Cells/mm^3 had >10 KS lesions, OR=0.9 (0.3-2.4), p=0.95.

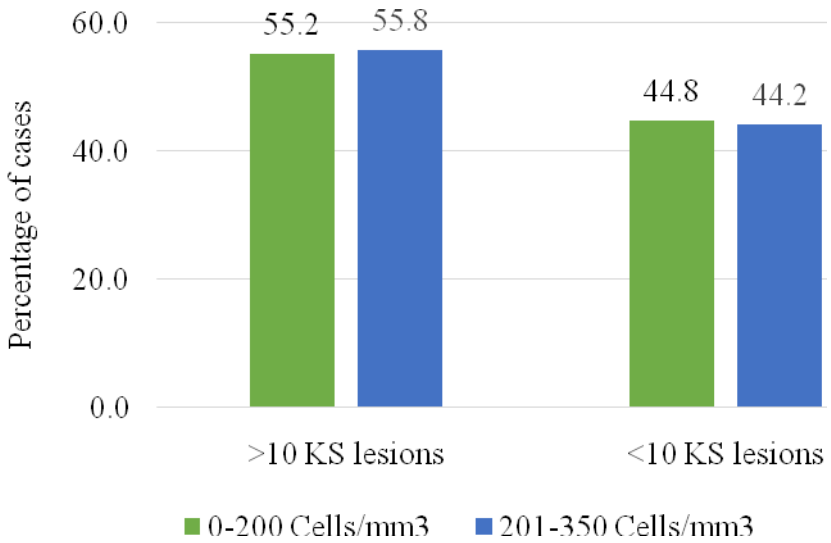


Figure 4.50: CD_4 Cell Count and Number of KS Lesions

The odds of developing >10 or <10 KS lesions when one has a CD_4 count 0-200 Cells/mm^3 or 201-350 Cells/mm^3 was 0.9 times. The category of CD_4 cell count ranging from 0-200 Cells/mm^3, 55.2% (16) presented with >10 KS lesions and 44.8% (13) of the cases had <10 KS lesions (Figure 4.50). The CD_4 cell count ranging from 201-350 Cells/mm^3, 55.8% (29) presented with >10 KS lesions and 44.2% (23) of the cases had <10 KS lesions.

4.3.32 CD_4 Cell Count and distribution of KS Lesions

Nineteen (65.5%) cases with a CD_4 count 0-200 Cells/mm^3 and 34 (65.4%) 201-350 Cells/mm^3 had a generalized lesion, OR=1.0 (0.3-2.6), p=0.99.

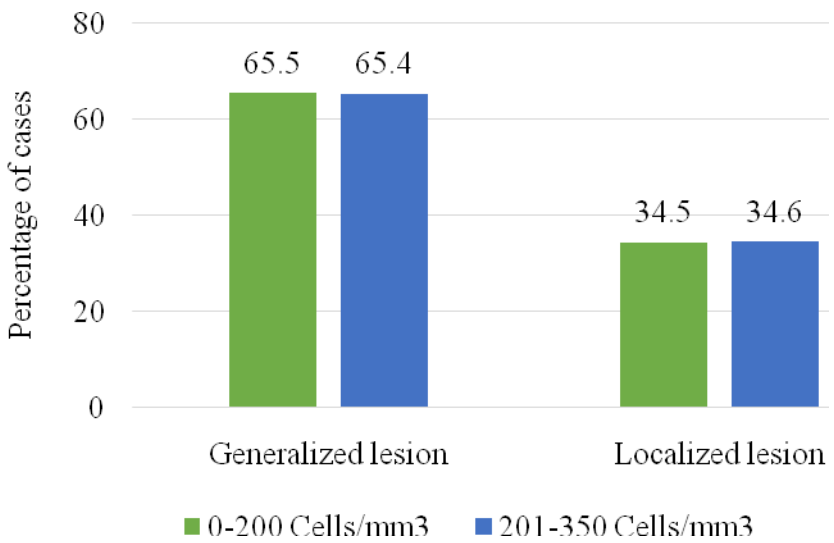


Figure 4.51: CD_4 Cell Count and Distribution of KS Lesions

The odds of developing generalized or localized KS lesion whether one had CD_4 count of 0-200 Cells/mm^3 or 201-350 Cells/mm^3 was 1 times. The CD_4 count of 0-200 Cells/mm^3, 65.5% (19) had generalized KS lesions and 34.5% (10) presented with localized lesions (Figure 4.51). The CD_4 count of 201-350 Cells/mm^3, 65.4% (34) had generalized KS lesions and 34.6% (18) presented with localized lesions.

4.3.33 CD_4 Count and Site of KS Lesion

Ten (34.5%) cases with 0-200 Cells/mm3 and 23 (44.2%) 201-350 Cells/mm3 had lesions on lower limbs, and 10 (34.5%) and 15 (28.8%) respectively had lesions on the trunk/chest/back (Figure 4.54). The Pearson's chi-square for CD4 cell count and site of KS lesion was 3.3, p=0.64

Table 4.17: CD_4 cell count and site of KS lesion

	CD_4Countdata	
	0-200 Cells/mm^3 n (%) 29 (35.8)	201-350 Cells/mm^3 n (%) 52 (64.2)
Site		
Lower limbs	10 (34.5)	23 (44.2)
Trunk/Chest/Back	10 (34.5)	15 (28.8)
Upper limbs	6 (20.7)	11 (21.2)
Palate/Mouth	2 (6.9)	2 (3.8)
Genitalia	0 (0.0)	1 (1.9)
Eyelid	1 (3.4)	0 (0.0)

Majority 34.5% (10) of the KS lesions witnessed in cases that had CD_4 cell count in the range of 0-200 Cells/mm^3were located on the lower limbs and Trunk/chest/back (Table 4.17). The lower limb had the majority 44.2% (23) of the KS lesions among the cases with CD_4 cell count in the range of 201-350 Cells/mm^3.

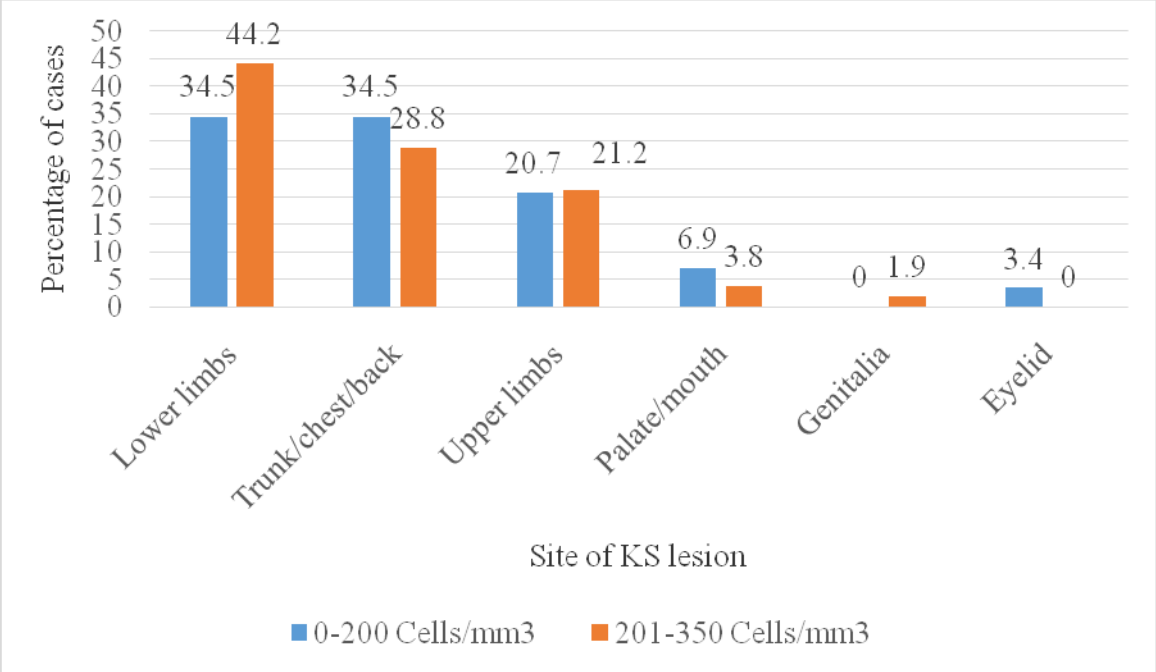


Figure 4.52: CD_4 cell count and site of KS lesion

4.3.34 CD_4 Cell Count and Morphology of KS Lesions

Four (13.8%) cases with a CD_4 count 0-200 Cells/mm3 and 9 (17.3%) 201-350 Cells/mm3 had a patchy morphology, and 17 (58.6%) and 32 (61.5%) respectively had a nodular morphology. The Pearson's chi-square for CD_4 cell count and morphology of KS lesion was 0.4, p=0.78.

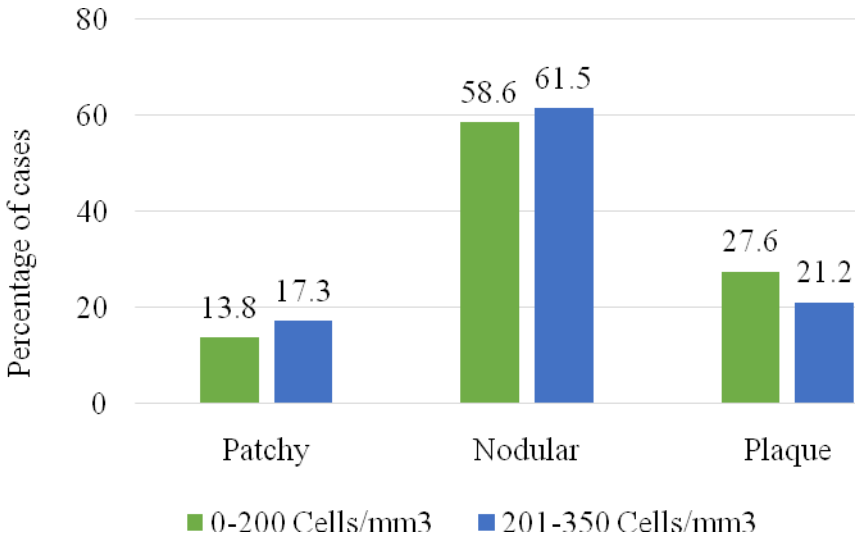


Figure 4.53: CD_4 Cell Count and Morphology of KS Lesions

The distribution of KS morphology among cases that had CD_4 cell count ranging from 0-200 Cells/mm^3 was as follows nodular 58.6% (17), 13.8% (4) patchy and 27.6% (8) plaque (Figure 4.53). The morphological distribution of KS among cases that had CD_4 cell count ranging from 201-350 Cells/mm^3 was as follows nodular 61.5% (32), 17.3% (9) patchy and 21.2% (11) plaque.

4.3.35 CD_4 Cell Count and Histology

Twenty-six (89.7%) cases with a CD4 count 0-200 Cells/mm3 and 42 (80.8%) 201-350 Cells/mm3 had a confirmed KS histology, OR=2.0(0.5-8.2), p=0.29.

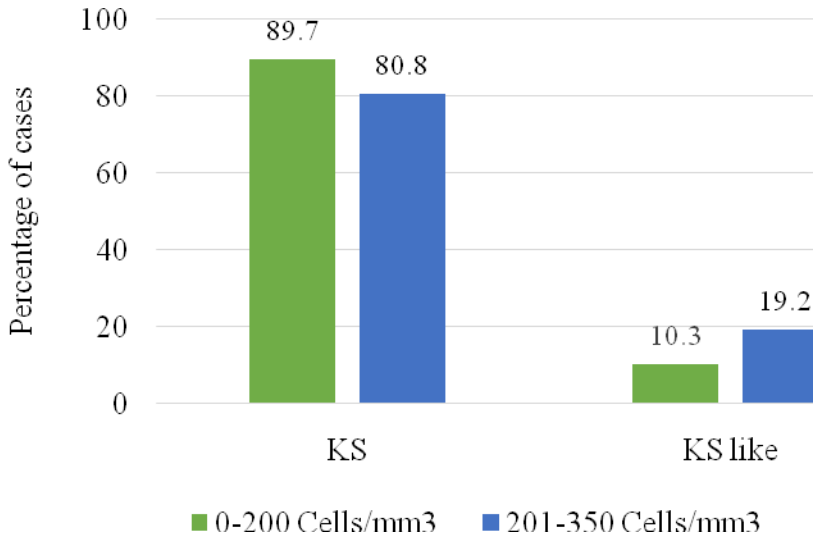


Figure 4.54: CD_4 Cell Count and Histology

The odds of identifying KS or KS like whether one has a CD_4 Cell Count of 0-200 Cells/mm^3 or 201-350 Cells/mm^3 is two times. The cases that had CD_4 Cell Count of 0-200 Cells/mm^3, histology results were as follows; 89.7% (26) had KS and 10.3% (3) presented with KS like (Figure 4.54). The cases that had CD_4 Cell Count of 201-350 Cells/mm^3, histology results were as follows; 80.8% (42) had KS and 19.2% (10) presented with KS like.

4.3.36 CD4 Cell Count and K1 Gene

The K1 gene was present in 27 (93.1%) cases with a CD4 count 0-200 Cells/mm^3 and 45 (88.9%) 201-350 Cells/mm^3, OR=2.1(0.4-10.8), p=0.37.

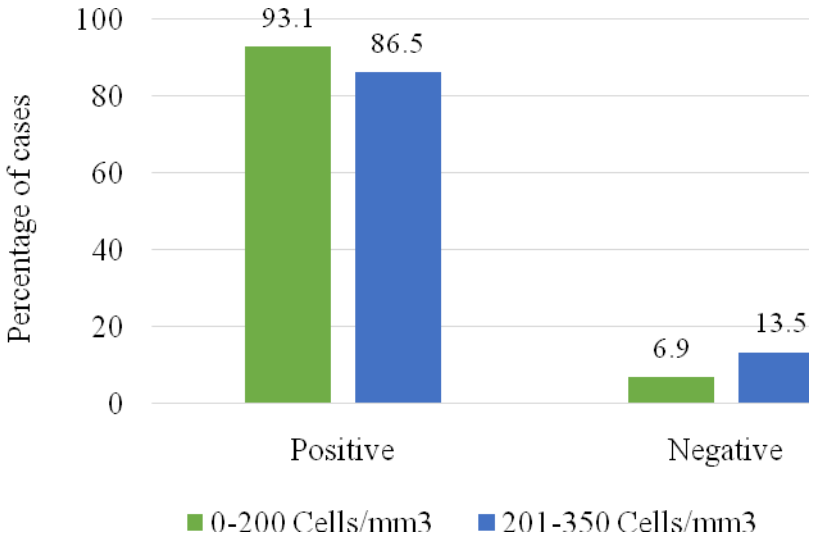


Figure 4.55: CD_4 Cell Count and K1 Gene

The odds of K1 gene being positive whether one has a CD_4 count of 0-200 Cells/mm^3 or 201-350 Cells/$mm^3$was 2.1 times. The cases that had CD_4 count of 0-200 Cells/mm^3, 93.1% (27) was positive and 6.9% (2) negative for K1 gene (Figure 4.55). The cases that had CD_4 count of 201-350 Cells/mm^3, 86.5% (45) was positive and 13.5% (7) negative for K1 gene.

4.3.37 CD_4 Cell Count and K15P Gene

The K15P gene was present in 27 (93.1%) cases with a CD_4 count 0-200 Cells/mm^3 and 45 (88.9%) 201-350 Cells/mm^3, OR=2.1(0.4-10.8), p=0.37.

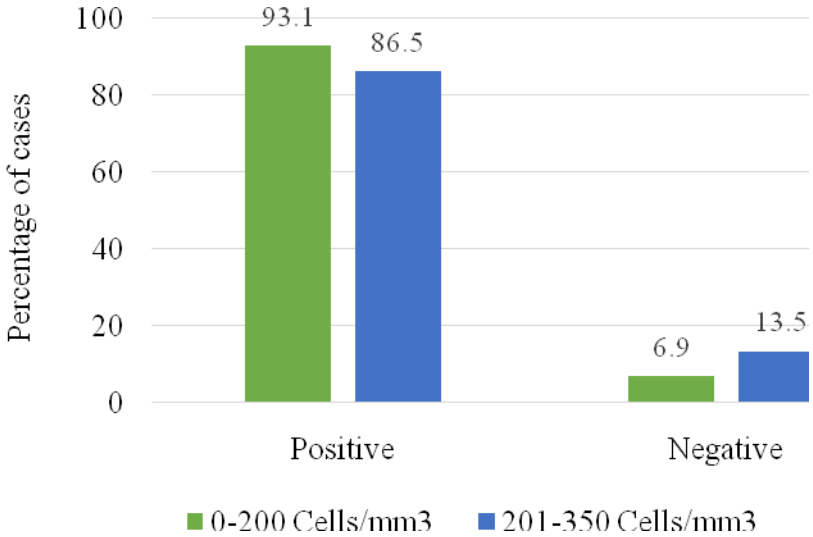


Figure 4.56: CD_4 Cell Count and K15P Gene

The odds of K15P gene being positive whether one has a CD_4 count of 0-200 Cells/mm^3 or 201-350 Cells/$mm^3$was 2.1 times. The cases that had CD_4 count of 0-200 Cells/mm^3, 93.1% (27) was positive and 6.9% (2) negative for K15P gene (Figure 4.56). The cases that had CD_4 count of 201-350 Cells/mm^3, 86.5% (45) was positive and 13.5% (7) negative for K15P gene.

4.3.38 CD_4 Cell Count and ORF75 Gene

The ORF75 gene was present in 20 (69.0%) cases with a CD_4 count 0-200 Cells/mm^3 and 29 (55.8%) 201-350 Cells/mm^3, OR=1.7(0.6-4.5), p=0.24.

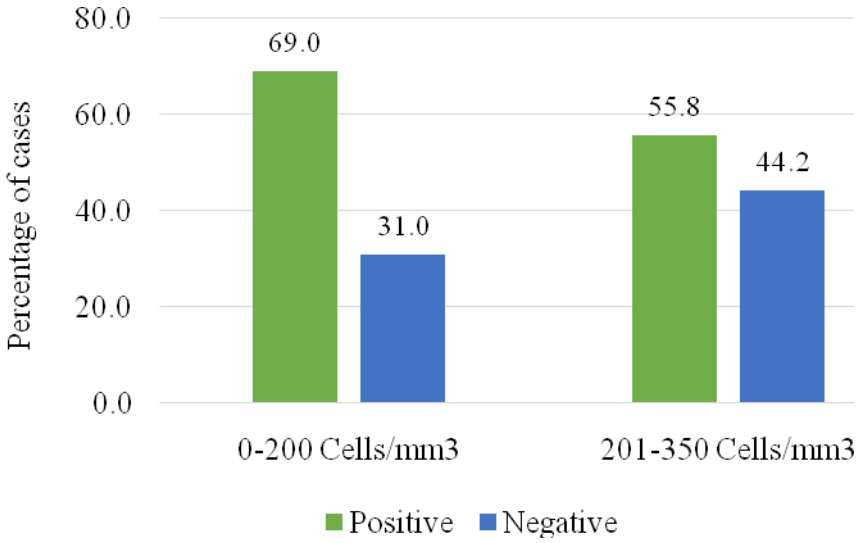


Figure 4.57: CD_4 Cell Count and ORF75 Gene

The odds of ORF75 gene being positive whether one has a CD_4 count of 0-200 Cells/mm^3 or 201-350 Cells/$mm^3$was 1.7 times. The cases that had CD_4 count of 0-200 Cells/mm^3, 69.0% (20) was positive and 31.0% (9) negative for ORF75 gene (Figure 4.57). The cases that had CD_4 count of 201-350 Cells/mm^3, 55.8% (29) was positive and 44.2% (23) negative for ORF75 gene.

4.3.39 Number of Lesions and Distribution of Lesions

Thirty-two (71.1%) cases with >10 KS lesions and 21 (58.3%) <10 KS lesions had generalized lesions, OR=1.7(0.6-4.4), p=0.23.

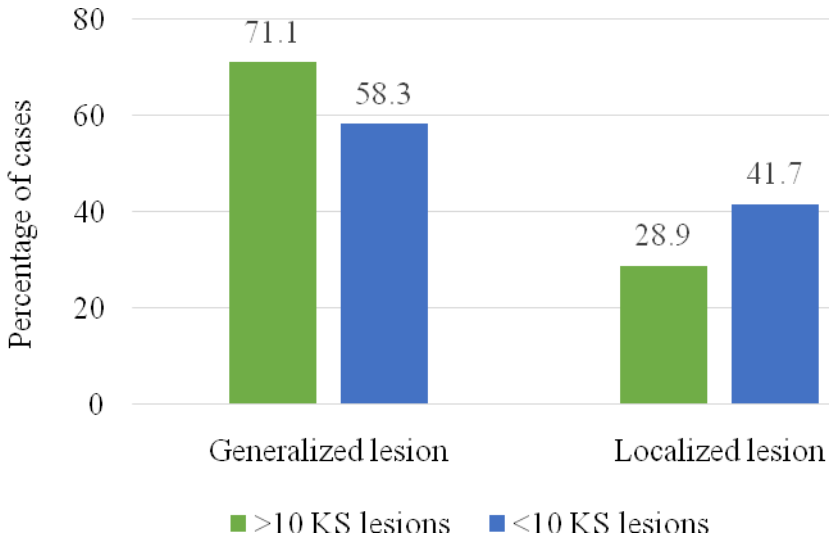


Figure 4.58: Number of Lesions and Distribution of Lesions

The odds of KS lesions manifesting as generalized or localized whether one has <10 KS lesions or >10 KS lesion was 1.7 times. The cases that had <10 KS lesion, generalized lesions was 58.3% (21) and 41.7% (15) localized (Figure 4.58). The cases that had >10 KS lesions, generalized lesions was 71.1% (32) and 28.9% (13) localized.

4.3.40 Number of Lesions and Site of KS Lesion

Twenty-one (46.7%) cases with >10 KS lesions and 12 (33.3%) <10 KS lesions had lesions on lower limbs, while 13 (28.9%) and 12 (33.3%) respectively had lesions on the trunk/chest/back. Pearson's chi-square for KS lesions and site was 5.0, p=0.40.

Table 4.18: Number of KS lesions and site

	Number of Lesions	
	<10 KS lesions n (%) 36 (44.4)	>10 KS lesions n (%) 45 (55.6)
Site		
Lower limbs	12 (33.3)	21 (46.7)
Trunk/Chest/Back	12 (33.3)	13 (28.9)
Upper limbs	7 (19.4)	10 (22.2)
Palate/Mouth	3 (8.3)	1 (2.2)
Genitalia	1 (2.8)	0 (0.0)
Eyelid	1 (2.8)	0 (0.0)

Cases that had >10 KS lesions, majority 46.7% (21) presented with lesions on the lower limbs (Table 4.17). Cases that had <10 KS lesions, majority 33.3% (12) presented with lesions on the lower limbs and trunk/chest/back. Among the cases that had >10 KS lesion there was no case with lesions in/on the genitalia or eyelid.

4.3.41 Number of KS Lesions and Morphology

Twenty-six (57.8%) cases with >10 KS lesions and 23 (63.9%) <10 KS lesions had nodular lesions, while 11 (24.4%) and 8 (22.2%) respectively had plaques. The Pearson's chi-square for number of KS lesions and morphology was 0.3, p=0.83.

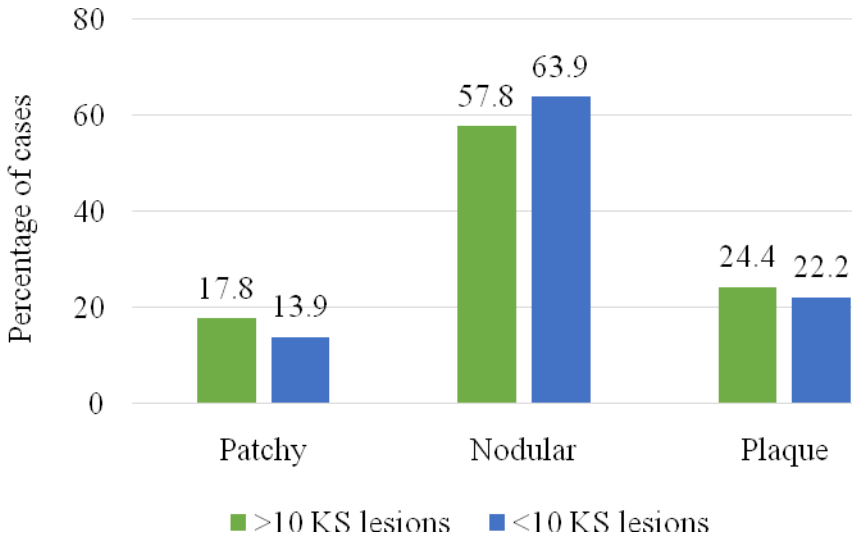


Figure 4.61: Number of KS Lesions and Morphology

The cases that had <10 KS lesions, nodular was 63.9% (23), 13.9% (5) patchy and 22.2% (8) plaque (Figure 4.59). The cases that had >10 KS lesions, nodular was 57.8% (26), 17.8% (8) patchy and 24.4% (11) plaque (Figure 4.59).

4.4.42 Number of KS Lesions and Histological Type

Thirty-seven (82.2%) cases with >10 KS lesions and 31 (86.1%) <10 KS lesions had a confirmed KS histology, OR=0.7(0.2-2.5), p=0.63.

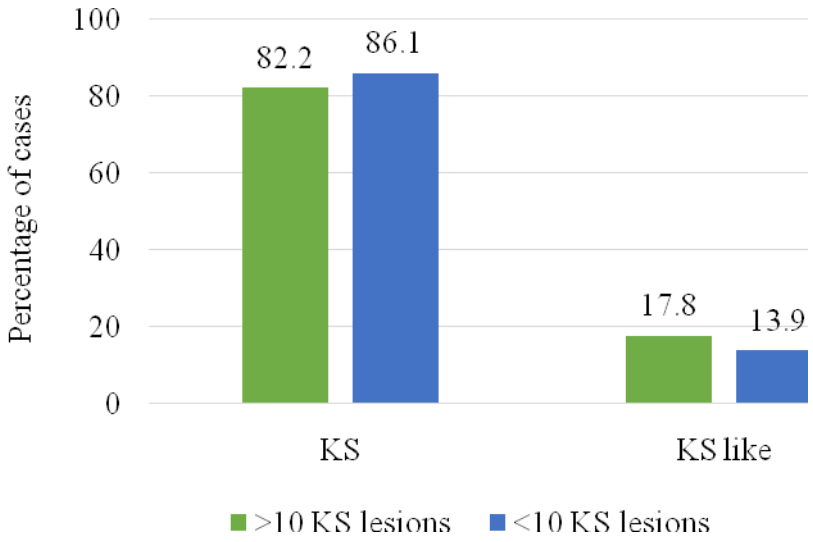


Figure 4.60: Number of KS Lesions and Histological Type

The number of KS lesions that were <10, observation made was KS 86.1% (31) and KS like 13.9% (5) (Figure 4.60). The number of KS lesions >10, 82.2% (37) was KS and 17.8% (8) KS like.

4.3.42 Number of KS Lesions and K1 Gene

The K1 gene was present in 40 (88.9%) cases with >10 KS lesions and 32 (88.9%) <10 KS lesions, OR=1.0(0.2-4.0), p=1.0.

Figure 4.61: Number of KS Lesions and K1 Gene

The odds of K1 gene to be present were 1 times regardless of the number of KS lesions. The number of KS lesions that had <10 and positive for K1 gene was 88.9% (32) (Figure 4.61). The number of KS lesions that had >10 and positive for K1 gene was 88.9% (40).

4.3.43 Number of KS Lesions and K15P Gene

The K15P gene was present in 40 (88.9%) cases with >10 KS lesions and 32 (88.9%) <10 KS lesions, OR=1.0(0.2-4.0), p=1.0.

Figure 4.62: Number of KS Lesions and K15P Gene

The odds of K15P gene to be present were 1 times regardless of the number of KS lesions. The number of KS lesions that had <10 and positive for K15P gene was 88.9% (32) (Figure 4.62). The number of KS lesions that had >10 and positive for K15P gene was 88.9% (40).

4.3.44 Number of KS Lesions and ORF75 Gene

The ORF75 gene was present in 28 (62.2%) cases with >10 KS lesions and 21 (58.3%) <10 KS lesions, OR=1.1(0.4-2.8), p=0.72.

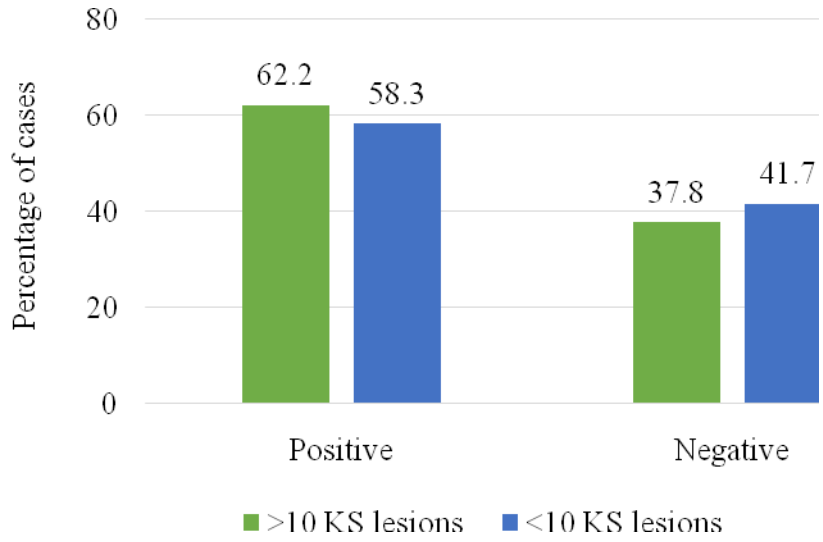


Figure 4.63: Number of KS Lesions and ORF75 Gene

The odds of ORF75 gene to be present were 1.1 times regardless of the number of KS lesions. The number of KS lesions that had <10 and positive for ORF75 gene was 58.3% (21) (Figure 4.63). The number of KS lesions that had >10 and positive for K15P gene was 62.2% (45).

4.3.45 Distribution of KS lesions and Site of Lesions

Twenty-four (45.3%) cases with a generalized lesion and 9 (32.1%) localized lesion had lesions on lower limbs, while 17 (32.1%) and 8 (28.6%) respectively had lesions on the trunk/chest/back. The Pearson's chi-square for KS lesions and site was 10.8, p=0.05*.

Table 4.19: Distribution of KS lesions and Site of Lesions

	Distribution of KS	
	Generalized lesion n (%)	Localized lesion n (%)
Site	53 (65.4)	28 (34.6)
Lower limbs	24 (45.3)	9 (32.1)
Trunk/Chest/Back	17 (32.1)	8 (28.6)
Upper limbs	11 (20.8)	6 (21.4)
Palate/Mouth	0 (0.0)	4 (14.3)
Genitalia	0 (0.0)	1 (3.6)
Eyelid	1 (1.9)	0 (0.0)

Majority 45.3% (24) of the generalized KS lesions were located on the lower limbs. The localized KS lesions had lower limbs are the most 32.1% (9) observed lesions. Generalized KS lesions was found to be absent in the genitalia and eyelid. Localized KS lesion was absent in the eyelid.

4.3.46 Distribution of Lesions and Morphology

Twenty-eight (52.8%) cases with generalized lesions and 21 (75.0%) localized lesions had nodular lesions, while 10 (18.9%) and 3 (10.7%) respectively had patchy lesions. The Pearson's chi-square for distribution of lesions and morphology was 3.7, p=0.15.

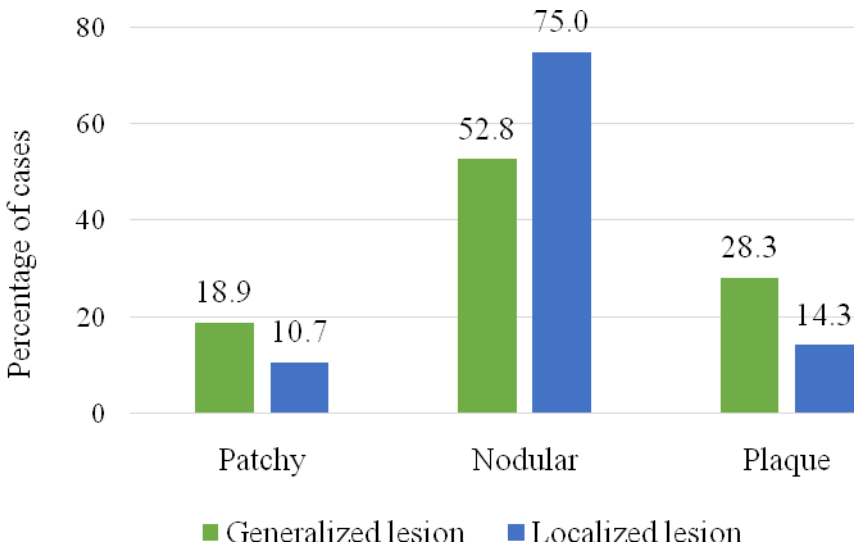


Figure 4.64: Distribution of Lesions and Morphology

Generalized KS lesions had the following results on morphology; nodular 52.8% (28), 18.9% (10) patchy and 28.3% (15) plaque (Figure 4.64). Localized KS lesions had the following results on morphology; nodular 75% (21), 10.7% (3) patchy and 14.3% (4) plaque.

4.3.47 Distribution of Lesions and Histological Type

Forty-two (79.2%) cases with generalized lesions and 26 (92.9%) localized lesions had a confirmed KS histology, OR=0.29(0.06-1.4), p=0.11.

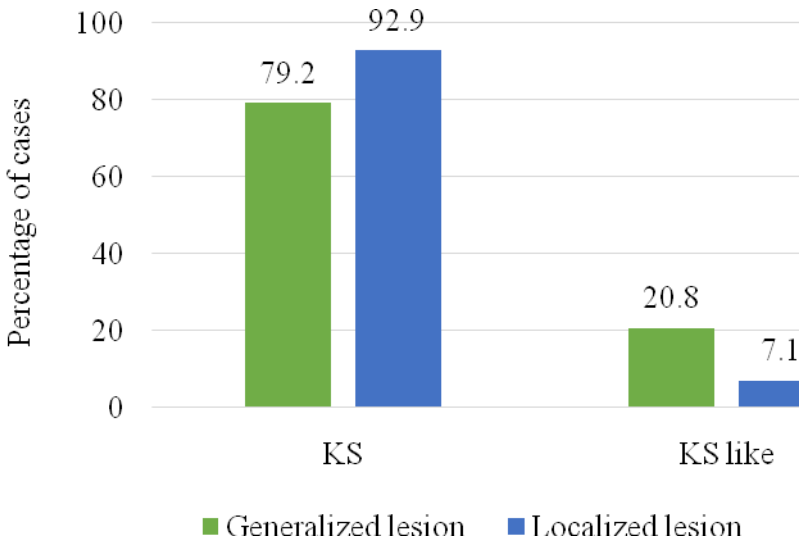


Figure 4.65: Distribution of Lesions and Histological Type

Odds of getting a histology results as KS or KS like is 0.29 times regardless of the distribution. The following histology results were observed on Localized KS lesion 92.9% (26) KS and 7.1% (2) KS like (Figure 4.65). The following histology results were observed on generalized KS lesion 79.2% (42) KS and 20.8% (11) KS like

4.3.48 Distribution of Lesions and K1 Gene

The K1 gene was present in 46 (86.8%) cases with generalized lesions and 26 (92.2%) localized lesions, OR=0.5(0.09-2.6), p=0.40.

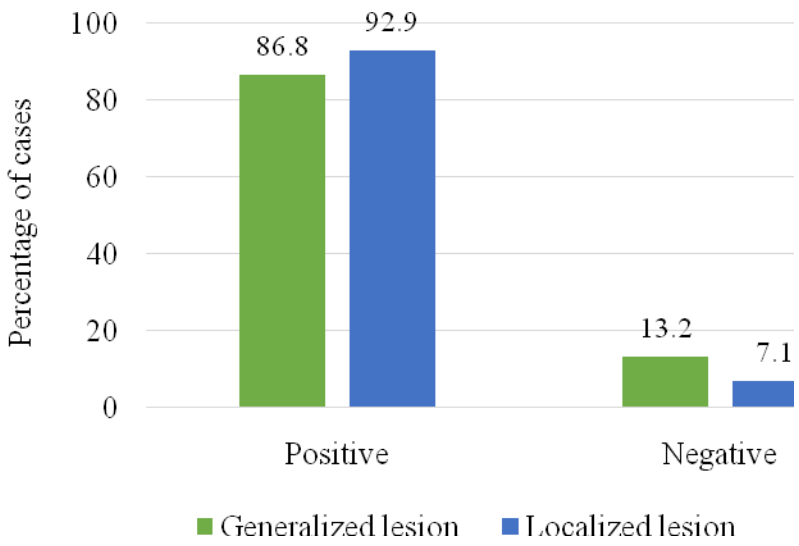


Figure 4.66: Distribution of Lesions and K1 Gene

The odds of K1 gene was 0.5 times regardless of the distribution. The K1 gene was positive at 86.8% (46) among the generalized lesion and 92.9% (26) localized lesions (Figure 4.66).

4.3.49 Distribution of Lesions and K15P Gene

The K15P gene was present in 46 (86.8%) cases with generalized lesions and 26 (92.2%) localized lesions, OR=0.5(0.09-2.6), p=0.40.

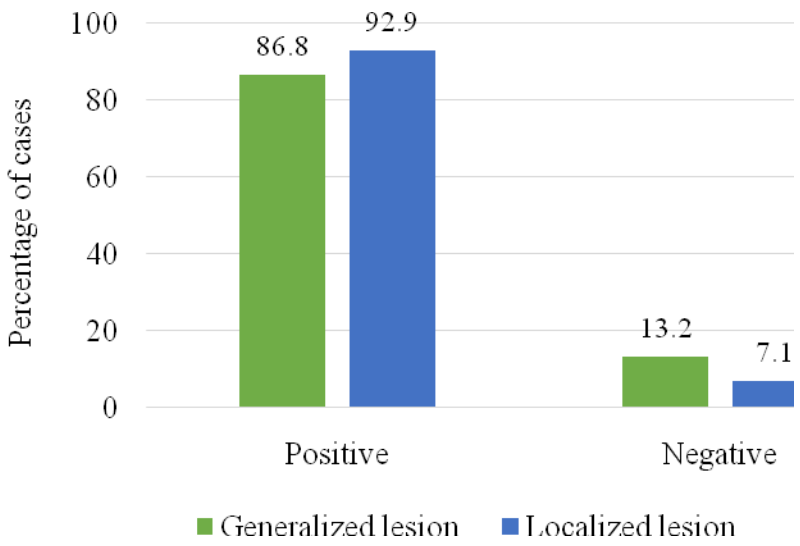


Figure 4.67: Distribution of Lesions and K15P Gene

The odds of K15P gene was 0.5 times regardless of the distribution. The K15P gene was positive at 86.8% (46) among the generalized lesion and 92.9% (26) localized lesions (Figure 4.67).

4.3.50 Distribution of Lesions and ORF75 Gene

The ORF75gene was present in 28 (52.8%) cases with generalized lesions and 21 (75.0%) localized lesions, OR=0.3(0.1-1.0), p=0.05*.

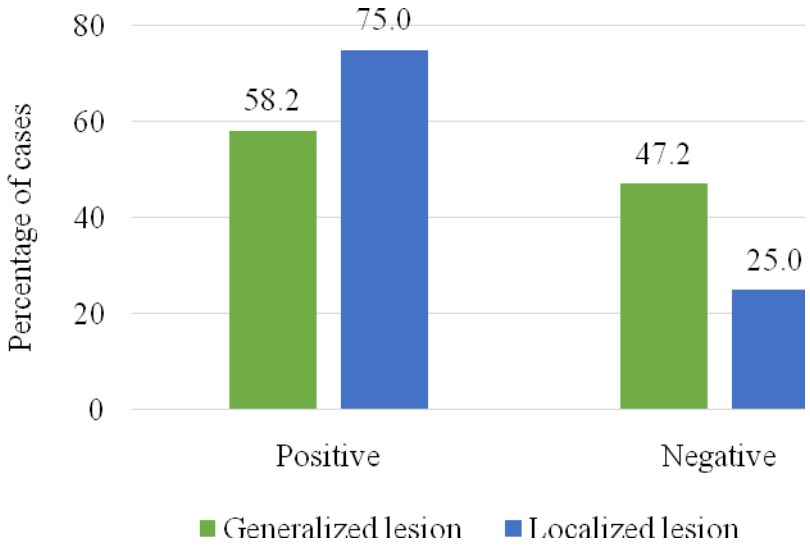


Figure 4.68: Distribution of Lesions and ORF75 Gene

There was a significant p=0.05* association between the distribution of KS lesions and ORF75 gene. The odds of ORF75 gene was 0.3 times regardless of the distribution. The ORF75 gene was positive at 52.8% (28) among the generalized lesion and 75% (21) localized lesions (Figure 4.68).

4.3.51 Morphology and Confirmed Histology

Six (46.2%) cases with a patchy morphology, 49 (100%) with a nodular morphology, and 13 (68.4%) with plaques had a confirmed KS histology, p<0.01*.

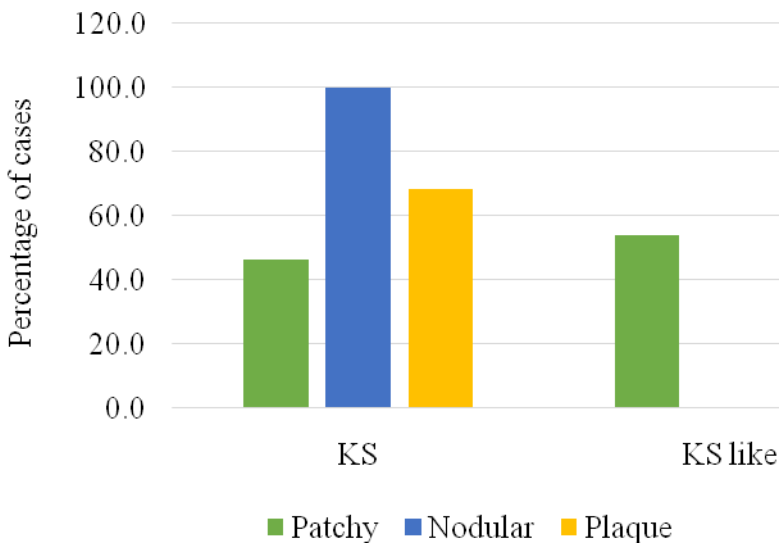


Figure 4.69: Morphology and Confirmed Histology

Histology results that were reported as KS had the following morphological presentations 100% (49) nodular, 46.2% (6) patchy and 68.4% (13) plaque (Figure 4.69). Histology results that were reported as KS like had the following morphological presentations 53.8% (7) patchy and 31.6% (6) plaque.

4.3.52 Morphology and K1 Gene Status

TheK1gene was present in 5 (38.5%) cases with patchy lesions, 49 (100%) with nodular lesions, and 18 (94.7%) with plaques, $p<0.01$*.

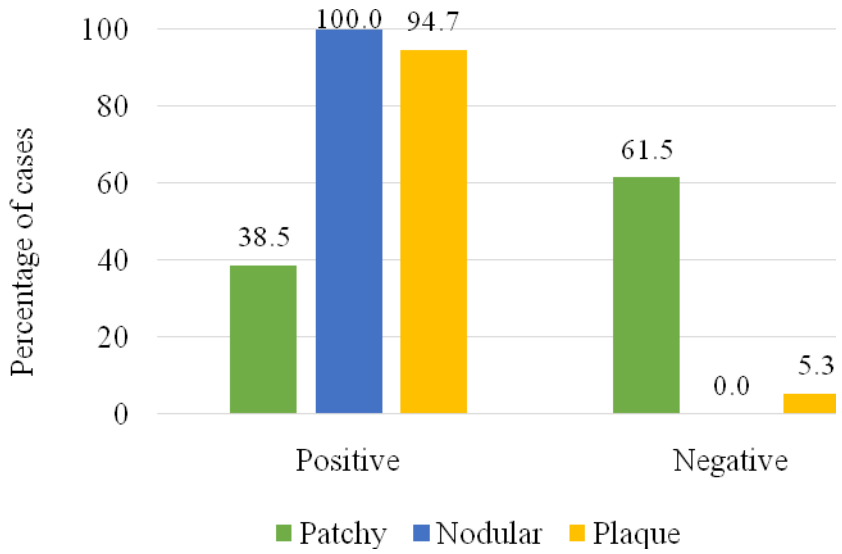


Figure 4.70: Morphology and K1 Gene Status

The following results on morphological types of KS were observed on K1 positive genes; nodular 100% (49), 38.5% (5) patchy and 94.7% (18) plaque (Figure 4.70). Morphological types of KS that was observed on K1 negative genes were; 61.5% (8) patchy and 5.3% (1) plaque.

4.3.53 Morphology and K15P Gene Status

The K15P gene was present in 5 (38.5%) cases with patchy lesions, 49 (100%) with nodular lesions, and 18 (94.7%) with plaques, $p<0.01$*.

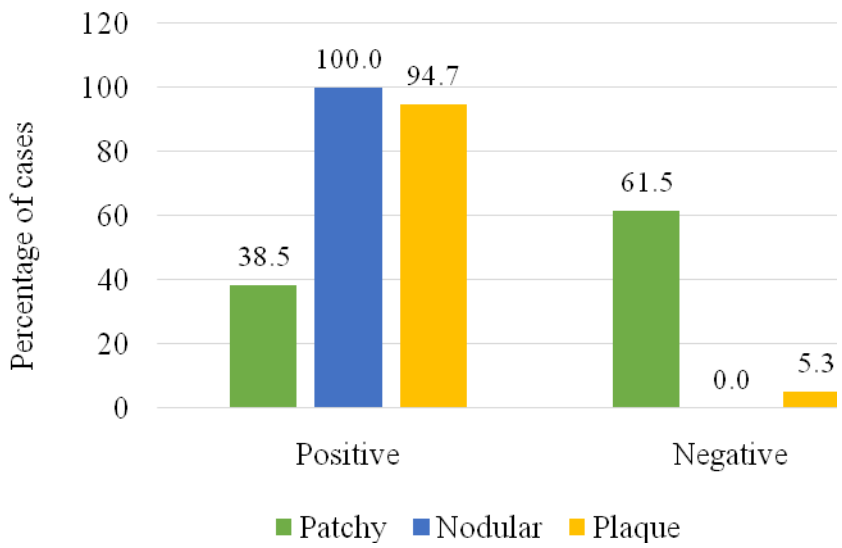


Figure 4.71: Morphology and K15P Gene Status

The following results on morphological types of KS were observed on K15P positive genes; nodular 100% (49), 38.5% (5) patchy and 94.7% (18) plaque (Figure 4.71). Morphological types of KS that was observed on K15P negative genes were; 61.5% (8) patchy and 5.3% (1) plaque.

4.3.54 Morphology and ORF75 Gene Status

The ORF75 gene was present in 2(15.4%) cases with patchy lesions, 37 (75.5%) with nodular lesions, and 10 (52.6%) with plaques, $p<0.01$*.

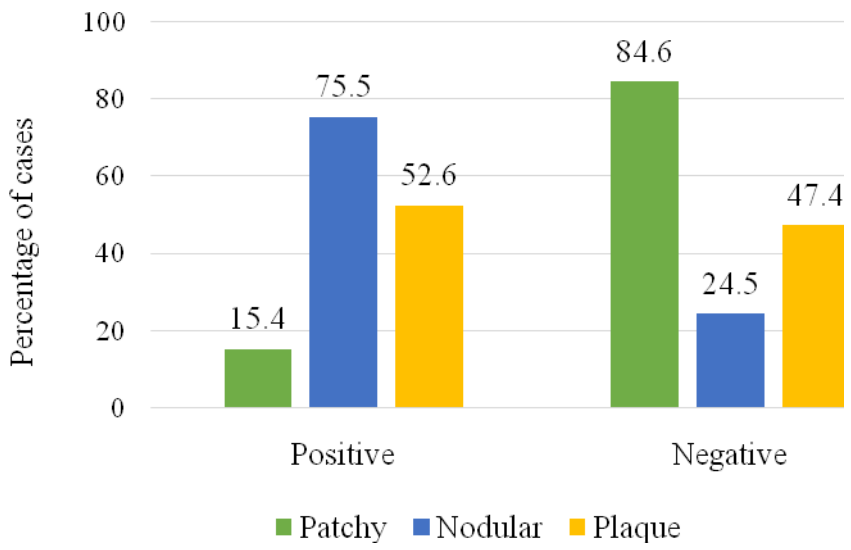


Figure 4.72: Morphology and ORF75 Gene Status

The morphological types of KS that were observed on ORF75 genes that were positive includes; nodular 75.5% (37), 15.4% (2) patchy and 52.6% (10) plaque (Figure 4.72). The ORF75 genes that were negative had the following morphological types of KS; nodular 24.5% (12), 84.6% (11) patchy and 47.4% (9) plaque.

4.3.55 Histology and K1 Gene Status

The K1 gene was present in 67(98.5%) cases with a confirmed KS histology and 5 (38.5%) KS like histology, OR=107, $p<0.01$*.

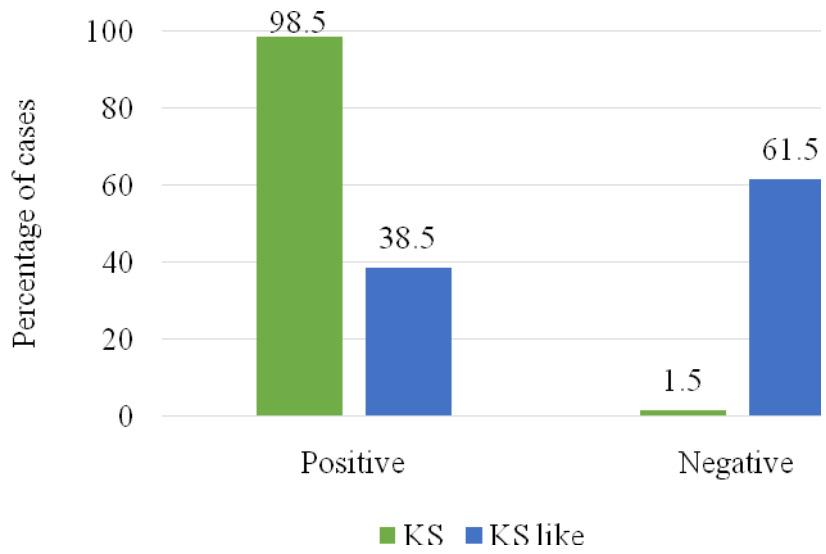


Figure 4.73: Histology and K1 Gene Status

The histology results on K1 genes that were positive includes; 98.5% (67) KS and 38.5% (5) KS like (Figure 4.73). The K1 genes that were negative the following histology results; 1.5% (1) KS and 61.5% (8) KS like.

4.3.56 Histology and K15P Gene Status

The K15P gene was present in 67(98.5%) cases with a confirmed KS histology and 5 (38.5%) KS like histology, OR=107, $p<0.01$*.

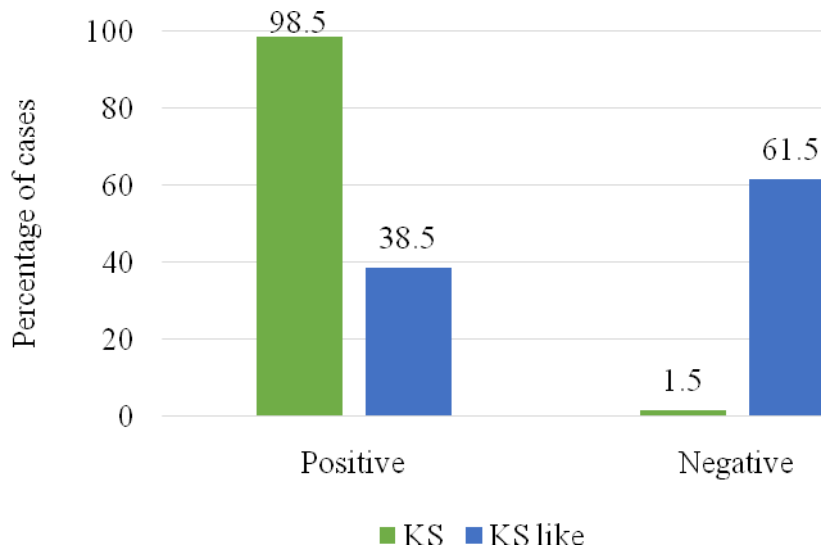


Figure 4.74: Histology and K15P Gene Status

The histology results on K15P genes that were positive includes; 98.5% (67) KS and 38.5% (5) KS like (Figure 4.74). The K15P genes that were negative the following histology results; 1.5% (1) KS and 61.5% (8) KS like.

4.3.57 Histology and ORF75 Gene Status

The ORF75 gene was present in 47 (69.1%) cases with a confirmed KS histology and 2 (15.4%) KS like histology, OR=12.3 (2.5-60.4), p<0.01*.

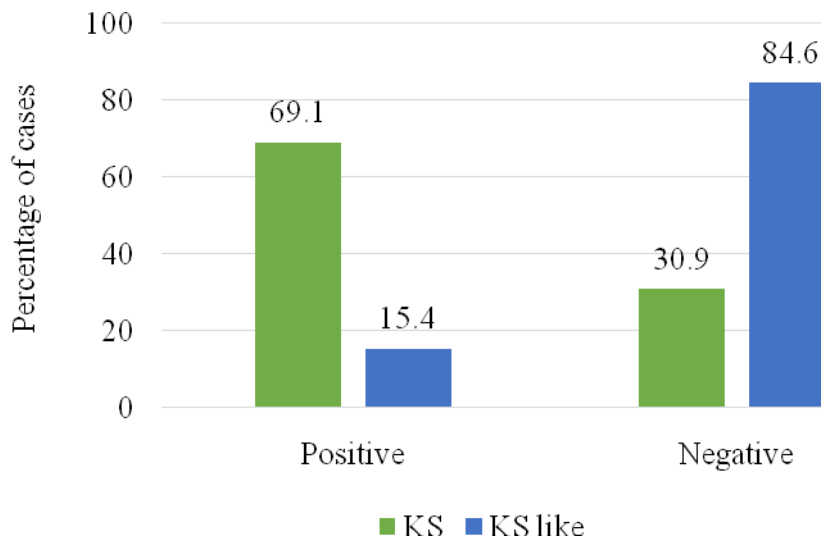


Figure 4.75: Histology and ORF75 Gene Status

The following histology results were seen on the positive cases for ORF75 gene; 69.1% (47) KS and 15.4% (2) KS like (Figure 4.75). The ORF75 genes that were negative had the following histology results; 30.9% (21) KS and 84.6% (11) KS like.

4.3.58 K1 Gene Status and K15P Gene Status

The K15P gene was present in 72(100%) cases with the K1 gene and 0 (0.0%) were K1 gene negative, $p<0.01$*.

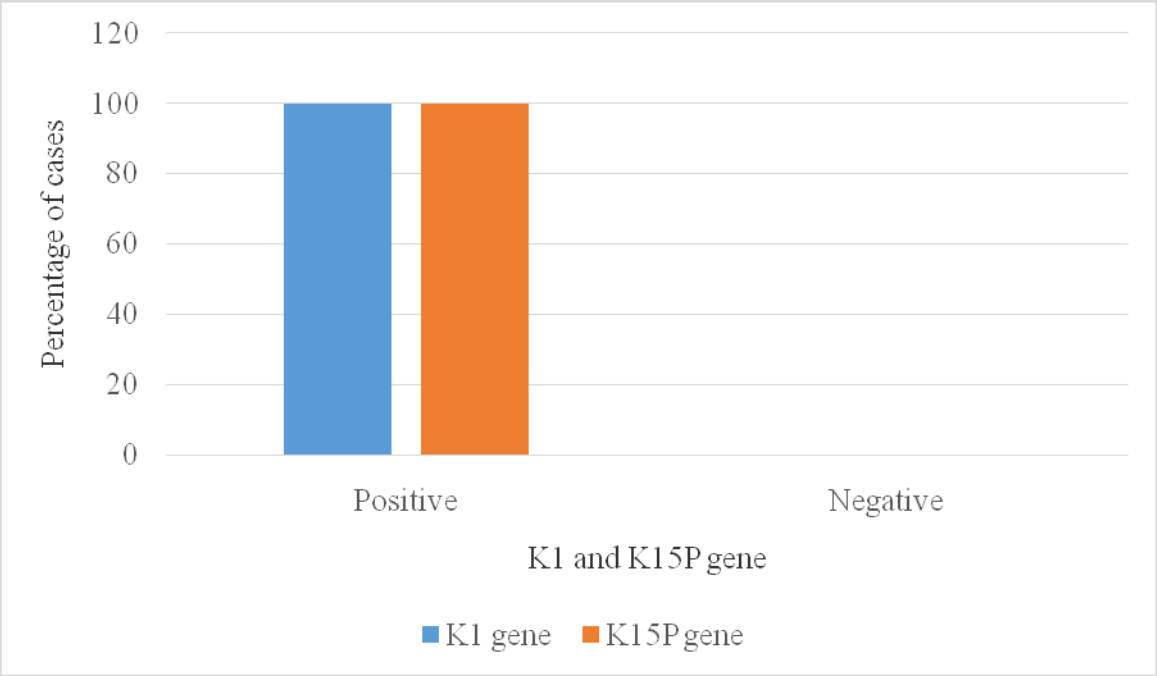


Figure 4.76: K1 Gene Status and K15P Gene Status

All 100% (72) the positive cases for K1 gene were also positive for K15P gene (Figure 4.76).

4.3.59 K1 Gene Status and ORF75 Gene Status

The ORF75 gene was positive in 66.7% (48) and negative in 77.8% (7) of the cases with K1 gene, OR=6.5(1.2-34.0) p=0.01*.

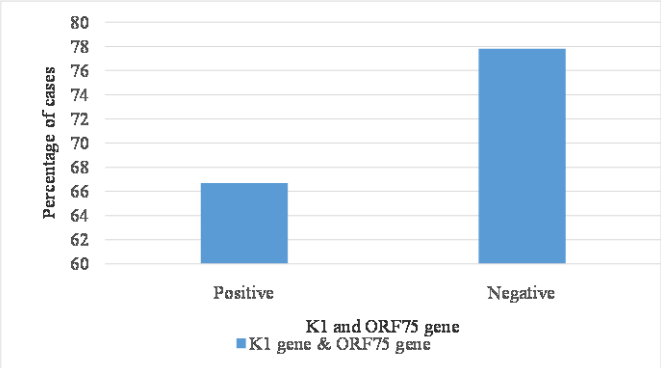


Figure 4.77: K1 Gene Status and ORF75 Gene Status

The cases that were both positive for K1 gene and ORF75 gene was 66.7% (48) and those that were both negative was 77.8% (7) (Figure 4.77). The ORF75 cases that were positive and negative for K1 gene was 22.2% (2). The cases that were positive for K1 gene and negative for ORF75 gene was 33.3% (24).

4.3.59 K15P Gene Status and ORF75 Gene Status

The ORF75 gene was positive in 66.7% (48) and negative in 77.8% (7) of the cases with K15P gene, OR=6.5(1.2-34.0) p=0.01*.

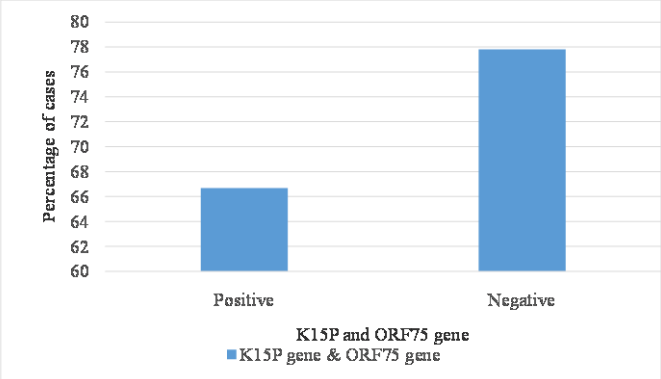


Figure 4.78: K15P Gene Status and ORF75 Gene Status

The cases that were both positive for K15P gene and ORF75 gene was 66.7% (48) and those that were both negative was 77.8% (7) (Figure 4.78). The ORF75 cases that were positive and negative for K15P gene was 22.2% (2). The cases that were positive for K15P gene and negative for ORF75 gene was 33.3% (24).

4.4 PCR patterns

Findings of the three targeted genes of Kaposi's sarcoma herpesvirus were viewed by conventional agarose gel electrophoresis (Figure 4.79). A 1Kbp DNA ladder was used, -ve control being RNase free water, +ve control being a known case of KS (Figure 4.79).

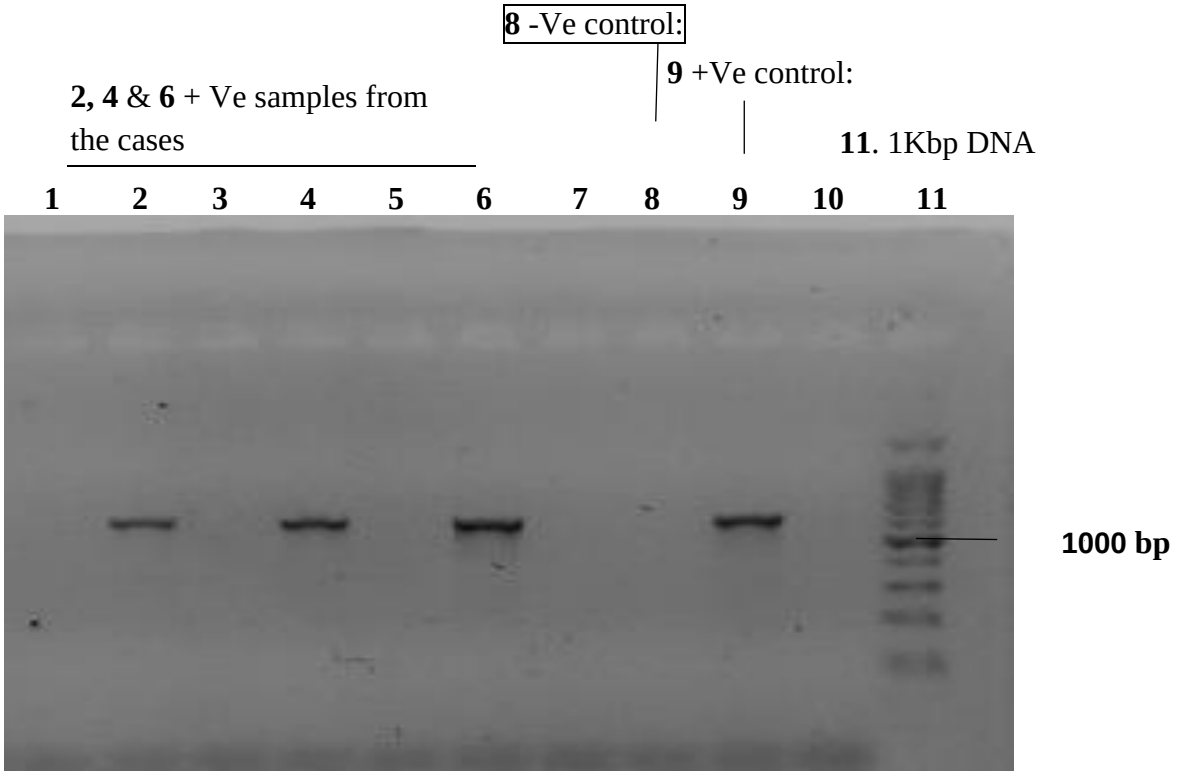


Figure 4.79: PCR patterns of the KSHV genes detected

4.5 Sequencing and phylogenetic results

What influenced the selection for sequencing of the 33 cases out of the 81 was the results gotten from the amplified products of the nested PCR. For sequencing, the study only selected amplified products that gave very good positive band on the agarose gel. What the study considered as a very good positive band on the agarose gel was a thick clear bold band. Thin faint band was also considered positive for the targeted gene, however, they tend to give poor sequencing results due to low viral load. The amplified products of the nested PCR was purified then subjected to direct nucleotide sequencing (next-generation sequencing) using the following primers: forward (5'GGCCCTTGTGTAAACCTGTC3') and reverse (5'CCTGAATGTCAGTACCAATCCA3'). A total of 60 amplified DNA extracts that were positive for K1, K15P and ORF75 KSHV genes were sequenced.

Biological aequence alignment Edit (BioEdit) software enabled saving the sequence files in a FASTA (DNA and protein sequence alignment software). Multiple Alignment using Fast Fourier Transform (MAFFT) made it easier to align multiple sequences. Molecular Evolutionary Genetic Analaysis (MEGA) was useful in the drawing phylogentic tree. The products of the sequences were entered into BioEdit software package, aligned using MAFFT version 7, brought back to BioEdit, converted to MEGAversion 6 software package and analyzed. Subsequently, a construction of a phylogenetic tree (Figure 4.80) was done in a MEGA version6 software package by use of neighbor-joining bootstrap value of 1000 replicates and a pairwise comparison. The Bootstrap above 70% represents significant branching and this implied similarity. The alignment of KSHV, K1 K15P and ORF75 gene sequences (Figure 4.81, 4.82 and 4.83 respectively) was done using http://espript.ibcp.fr/ESPript/cgi-bin/ESPript.cgi (Robert and

Gouet, 2014)and had above 80% sequence identity. The observation made was that nucleotide sequences of K1, K15P and ORF75 gene were aligned to other HHV8 gene sequences which have been deposited in the NCBI data bank (http://www.ncbi.nlm.nih.gov).

4.5.1 Phylogenetic tree of the KSHV-K1, K15 (P) & ORF75

Sequence analysis was done on cases that were positive for K1, K15P and ORF75 genes. The following KSHV genes were sequenced, 50% (10/20) ORF75, 40% (8/20) K15P and 75% (15/20) K1gene. The study noted that four samples had twofold genes detected; tissue block P57 (Patient 57) had ORF75 gene and K15P gene, tissue block P17 had ORF75 gene and K15 (P) gene, tissue block P28 had ORF75 gene and K1 gene and tissue block P25 had K15P gene and K1 gene.

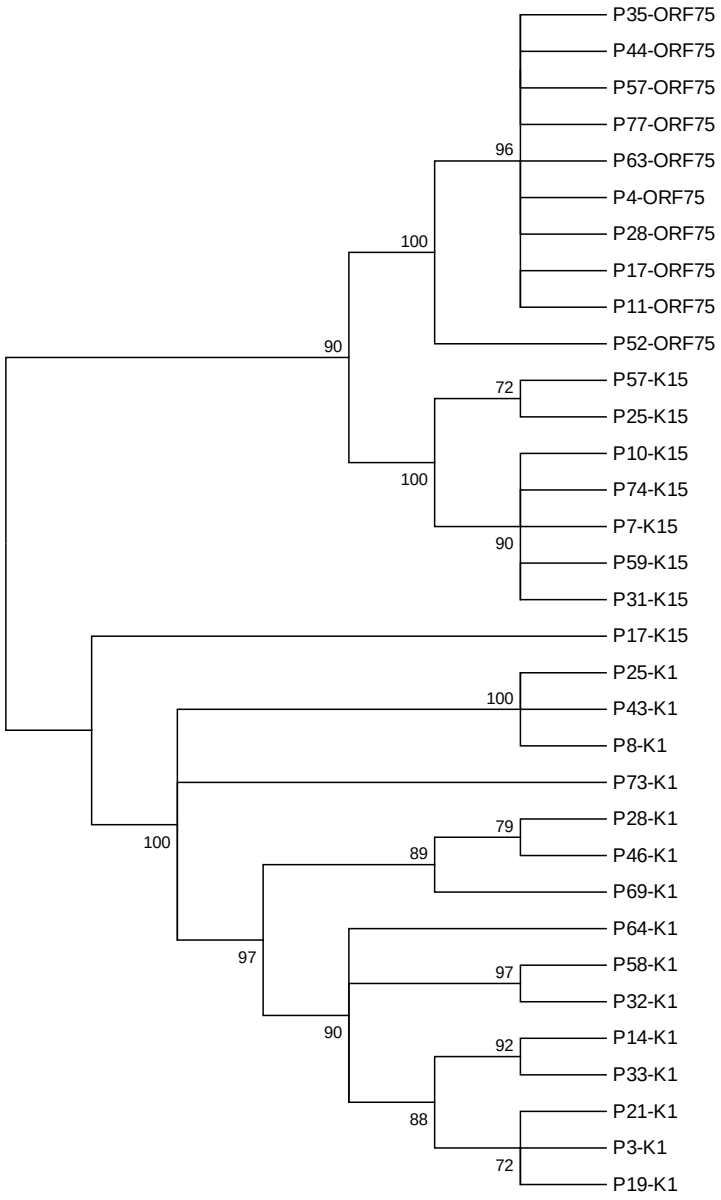
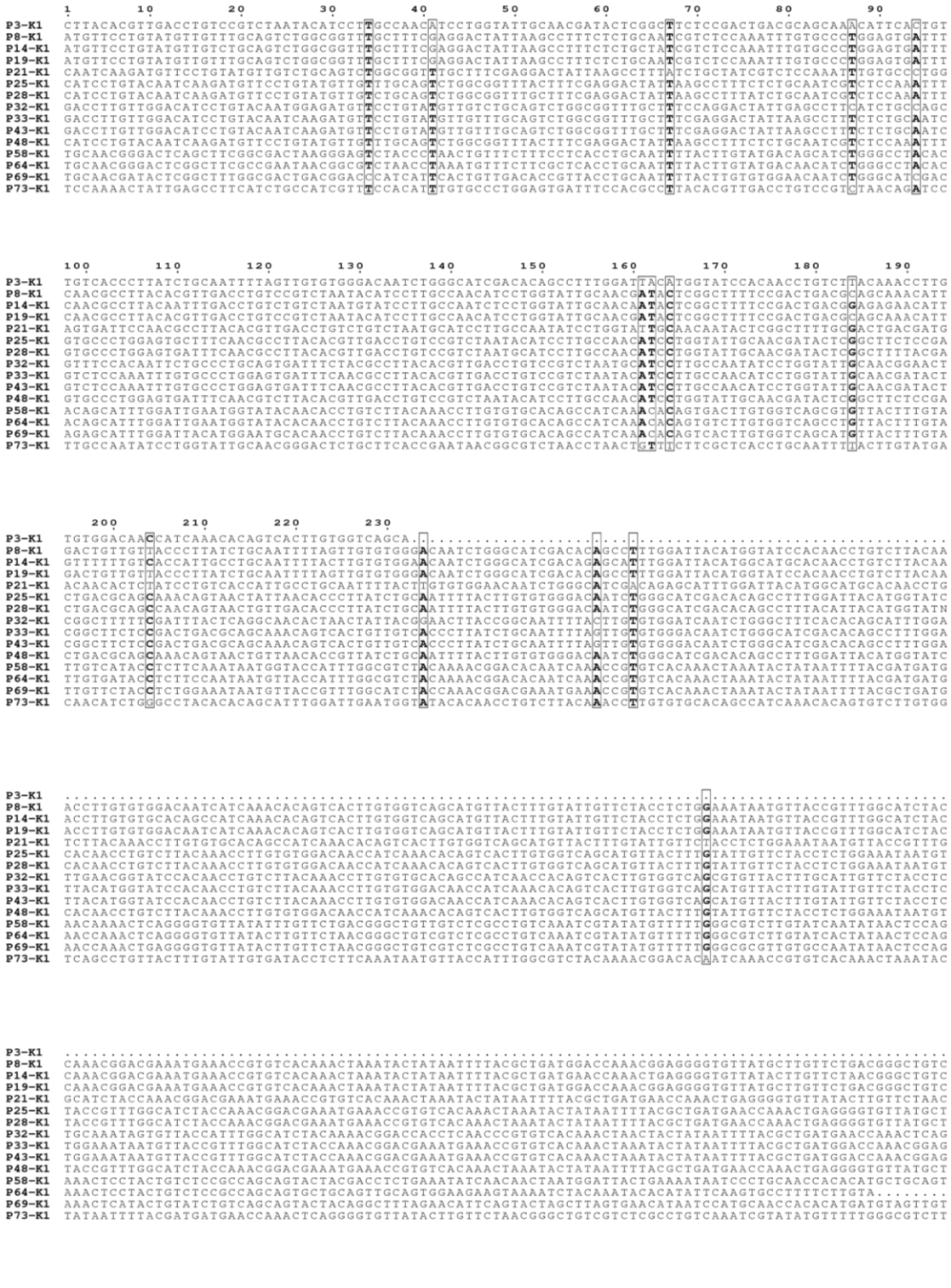


Figure 4.80: Phylogenetic tree of K1, K15 (P) & ORF75 gene

Key: Branches are the lines within the tree, Nodes are tips of branches or point at which branches connect, Root is the basal node and Clade grouping of closely related species. Branch is assigned a percentage of occurrences in the constructed tree. Thirty-three samples were subjected to bootstrap analysis to create the consensus tree. Neighbour-joining tree was reconstructed using MEGA Version 6 software package. The numbers at the nodes represent the percentage bootstrap values for the interior branches after 1000 replications. The Bootstrap that was above 70% represented significant branching.

4.5.2 K1 gene sequence



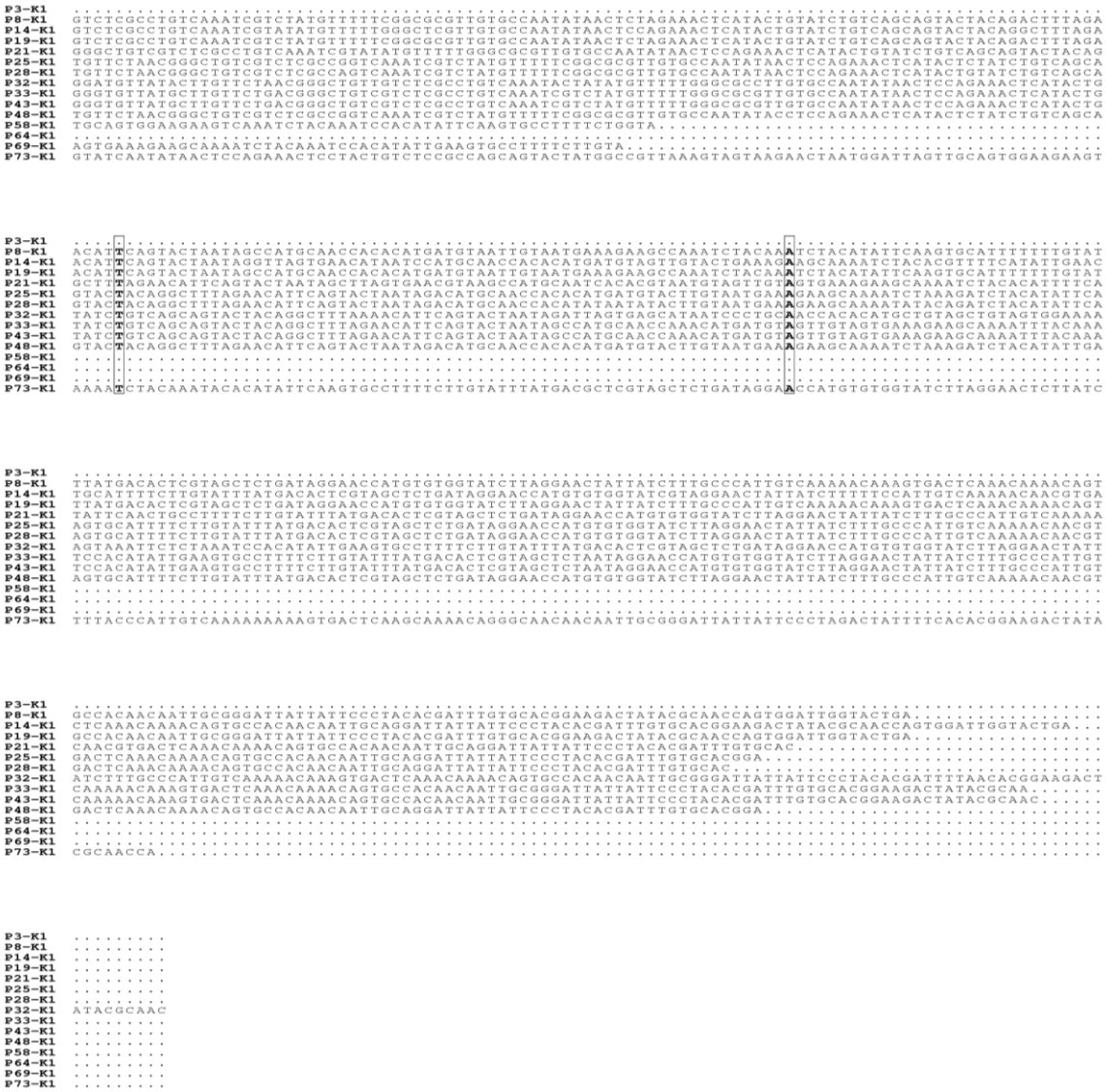
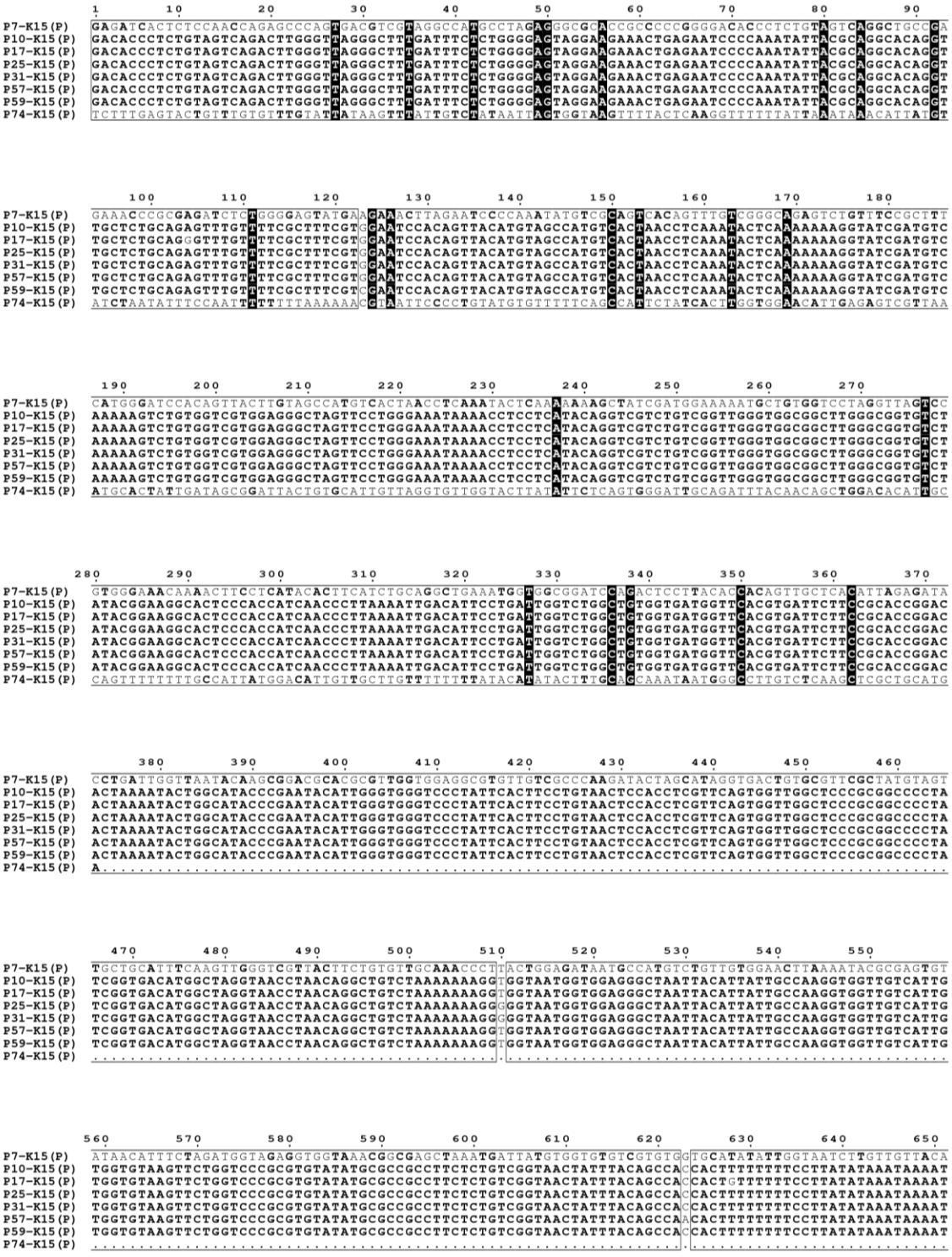
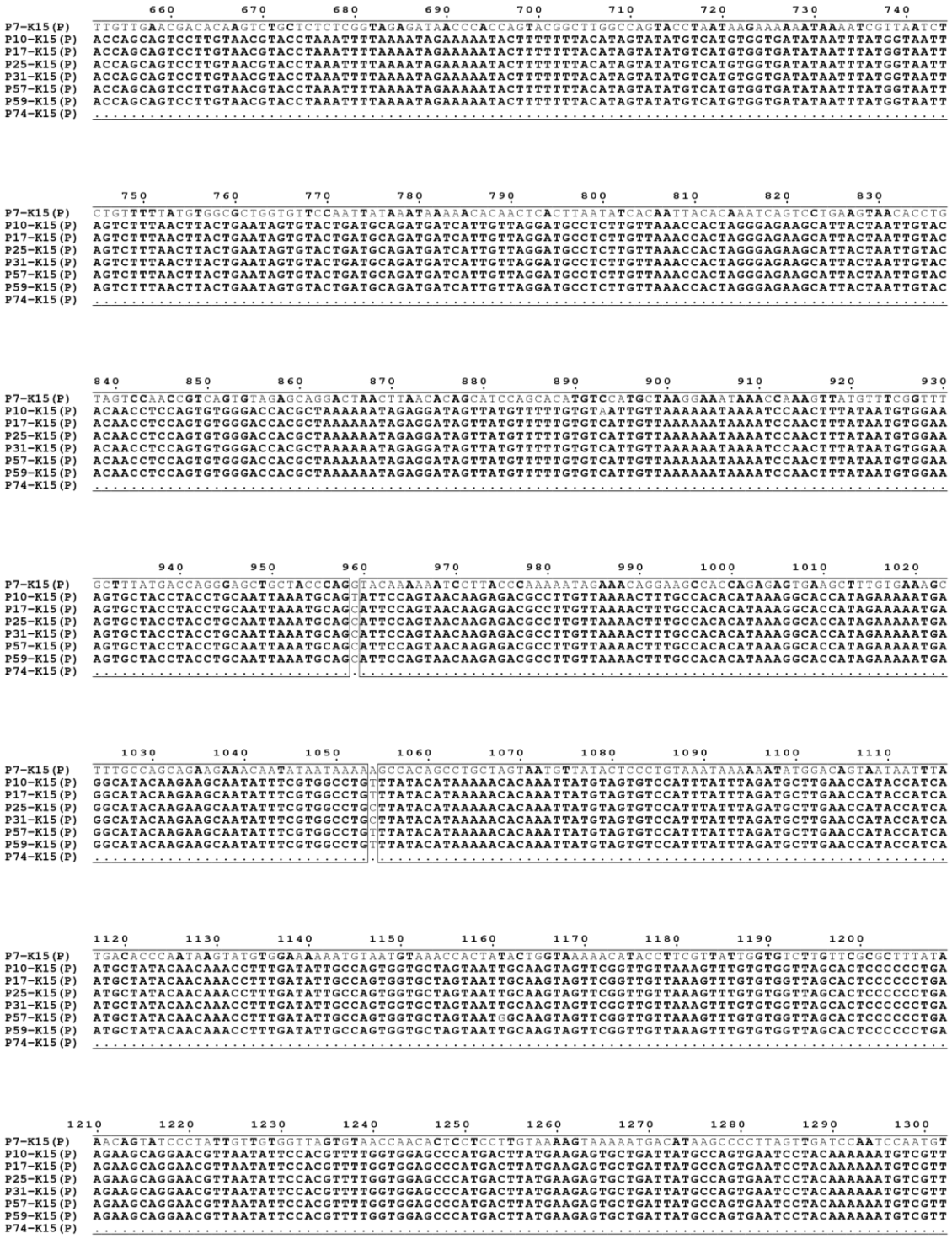


Figure 4.81: K1 gene sequence

Using http://espript.ibcp.fr/ESPript/cgi-bin/ESPript.cgi (Robert and Gouet, 2014) where 80% sequence identity was noted. Alignment of amino acid sequences of K1 gene, generated from nucleotide sequences for each sample investigated. The conserved regions are bolded in black.

4.5.3 K15P gene sequence




660 670 680 690 700 710 720 730 740
P7-K15(P) TTGTTGAACGACACAAGTCTGCTCTCTCGGTAGAGATAACCCACCAGTACGGCTTGGCCAGTACCTAATAAGAAAAAATAAAATCGTTAATCT
P10-K15(P) ACCAGCAGTCCTTGTAACGTACCTAAATTTTAAAATAGAAAAATACTTTTTTTTACATAGTATATGTCATGTGGTGATATAATTTATGGTAATT
P17-K15(P) ACCAGCAGTCCTTGTAACGTACCTAAATTTTAAAATAGAAAAATACTTTTTTTTACATAGTATATGTCATGTGGTGATATAATTTATGGTAATT
P25-K15(P) ACCAGCAGTCCTTGTAACGTACCTAAATTTTAAAATAGAAAAATACTTTTTTTTACATAGTATATGTCATGTGGTGATATAATTTATGGTAATT
P31-K15(P) ACCAGCAGTCCTTGTAACGTACCTAAATTTTAAAATAGAAAAATACTTTTTTTTACATAGTATATGTCATGTGGTGATATAATTTATGGTAATT
P57-K15(P) ACCAGCAGTCCTTGTAACGTACCTAAATTTTAAAATAGAAAAATACTTTTTTTTACATAGTATATGTCATGTGGTGATATAATTTATGGTAATT
P59-K15(P) ACCAGCAGTCCTTGTAACGTACCTAAATTTTAAAATAGAAAAATACTTTTTTTTACATAGTATATGTCATGTGGTGATATAATTTATGGTAATT
P74-K15(P) ....
750 760 770 780 790 800 810 820 830
P7-K15(P) CTGTTTTTATGTGGCGCTGGTGTTCCAATTATAAATAAAACACAACTCACTTAATATCACAATTACACAAATCAGTCCTGAAGTAACACCTG
P10-K15(P) AGTCTTTAACTTACTGAATAGTGTACTGATGCAGATGATCATTGTTAGGATGCCTCTTGTTAAACCACTAGGGAGAAGCATTACTAATTGTAC
P17-K15(P) AGTCTTTAACTTACTGAATAGTGTACTGATGCAGATGATCATTGTTAGGATGCCTCTTGTTAAACCACTAGGGAGAAGCATTACTAATTGTAC
P25-K15(P) AGTCTTTAACTTACTGAATAGTGTACTGATGCAGATGATCATTGTTAGGATGCCTCTTGTTAAACCACTAGGGAGAAGCATTACTAATTGTAC
P31-K15(P) AGTCTTTAACTTACTGAATAGTGTACTGATGCAGATGATCATTGTTAGGATGCCTCTTGTTAAACCACTAGGGAGAAGCATTACTAATTGTAC
P57-K15(P) AGTCTTTAACTTACTGAATAGTGTACTGATGCAGATGATCATTGTTAGGATGCCTCTTGTTAAACCACTAGGGAGAAGCATTACTAATTGTAC
P59-K15(P) AGTCTTTAACTTACTGAATAGTGTACTGATGCAGATGATCATTGTTAGGATGCCTCTTGTTAAACCACTAGGGAGAAGCATTACTAATTGTAC
P74-K15(P) ....
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P7-K15(P) TAGTCCAACCGTCAGTGTAGAGCAGGACTAACTTAACACAGCATCCAGCACATGTCCATGCTAAGGAAATAAACCAAAGTTATGTTTCGGTTT
P10-K15(P) ACAACCTCCAGTGTGGGACCACGCTAAAAAATAGAGGATAGTTATGTTTTTGTGTAATTGTTAAAAAATAAAATCCAACTTTATAATGTGGAA
P17-K15(P) ACAACCTCCAGTGTGGGACCACGCTAAAAAATAGAGGATAGTTATGTTTTTGTGTCATTGTTAAAAAATAAAATCCAACTTTATAATGTGGAA
P25-K15(P) ACAACCTCCAGTGTGGGACCACGCTAAAAAATAGAGGATAGTTATGTTTTTGTGTCATTGTTAAAAAATAAAATCCAACTTTATAATGTGGAA
P31-K15(P) ACAACCTCCAGTGTGGGACCACGCTAAAAAATAGAGGATAGTTATGTTTTTGTGTCATTGTTAAAAAATAAAATCCAACTTTATAATGTGGAA
P57-K15(P) ACAACCTCCAGTGTGGGACCACGCTAAAAAATAGAGGATAGTTATGTTTTTGTGTCATTGTTAAAAAATAAAATCCAACTTTATAATGTGGAA
P59-K15(P) ACAACCTCCAGTGTGGGACCACGCTAAAAAATAGAGGATAGTTATGTTTTTGTGTCATTGTTAAAAAATAAAATCCAACTTTATAATGTGGAA
P74-K15(P) ....
940 950 960 970 980 990 1000 1010 1020
P7-K15(P) GCTTTATGACCAGGGAGCTGCTACCCAGGTACAAAAAATCCTTACCCAAAAATAGAAACAGGAAGCCACCAGAGAGTGAAGCTTTGTGAAAGC
P10-K15(P) AGTGCTACCTACCTGCAATTAAATGCAGTATTCCAGTAACAAGAGACGCCTTGTTAAAACTTTGCCACACATAAAGGCACCATAGAAAAATGA
P17-K15(P) AGTGCTACCTACCTGCAATTAAATGCAGCATTCCAGTAACAAGAGACGCCTTGTTAAAACTTTGCCACACATAAAGGCACCATAGAAAAATGA
P25-K15(P) AGTGCTACCTACCTGCAATTAAATGCAGCATTCCAGTAACAAGAGACGCCTTGTTAAAACTTTGCCACACATAAAGGCACCATAGAAAAATGA
P31-K15(P) AGTGCTACCTACCTGCAATTAAATGCAGCATTCCAGTAACAAGAGACGCCTTGTTAAAACTTTGCCACACATAAAGGCACCATAGAAAAATGA
P57-K15(P) AGTGCTACCTACCTGCAATTAAATGCAGCATTCCAGTAACAAGAGACGCCTTGTTAAAACTTTGCCACACATAAAGGCACCATAGAAAAATGA
P59-K15(P) AGTGCTACCTACCTGCAATTAAATGCAGCATTCCAGTAACAAGAGACGCCTTGTTAAAACTTTGCCACACATAAAGGCACCATAGAAAAATGA
P74-K15(P) ....
1030 1040 1050 1060 1070 1080 1090 1100 1110
P7-K15(P) TTTGCCAGCAGAAGAAACAATATAATAAAAAAGCCACAGCCTGCTAGTAATGTTATACTCCCTGTAAATAAAAAATATGGACAGTAATAATTTA
P10-K15(P) GGCATACAAGAAGCAATATTTCGTGGCCTGTTTATACATAAAAACACAAATTATGTAGTGTCCATTTATTTAGATGCTTGAACCATACCATCA
P17-K15(P) GGCATACAAGAAGCAATATTTCGTGGCCTGTTTATACATAAAAACACAAATTATGTAGTGTCCATTTATTTAGATGCTTGAACCATACCATCA
P25-K15(P) GGCATACAAGAAGCAATATTTCGTGGCCTGCTTATACATAAAAACACAAATTATGTAGTGTCCATTTATTTAGATGCTTGAACCATACCATCA
P31-K15(P) GGCATACAAGAAGCAATATTTCGTGGCCTGCTTATACATAAAAACACAAATTATGTAGTGTCCATTTATTTAGATGCTTGAACCATACCATCA
P57-K15(P) GGCATACAAGAAGCAATATTTCGTGGCCTGTTTATACATAAAAACACAAATTATGTAGTGTCCATTTATTTAGATGCTTGAACCATACCATCA
P59-K15(P) GGCATACAAGAAGCAATATTTCGTGGCCTGTTTATACATAAAAACACAAATTATGTAGTGTCCATTTATTTAGATGCTTGAACCATACCATCA
P74-K15(P) ....
1120 1130 1140 1150 1160 1170 1180 1190 1200
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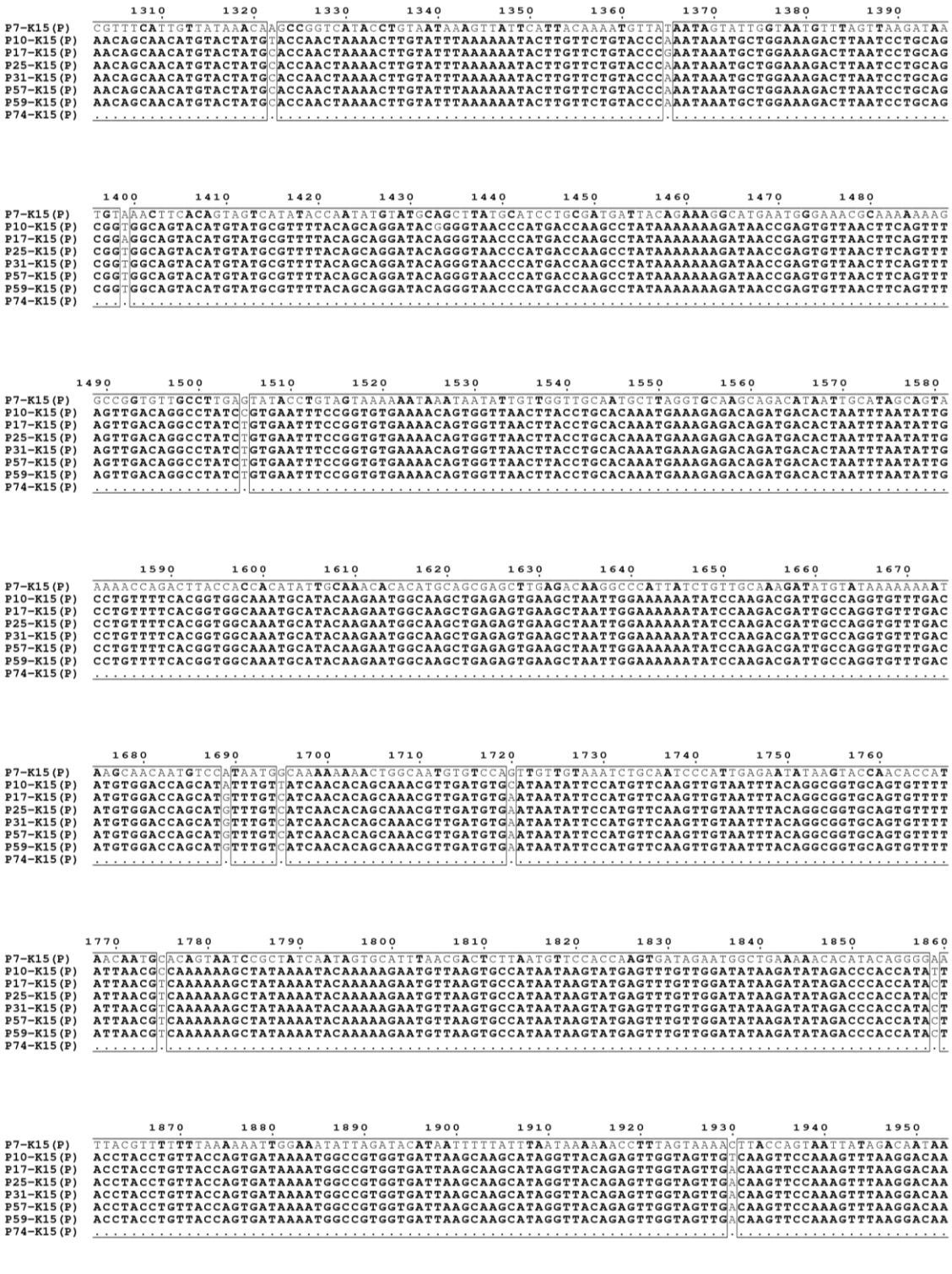
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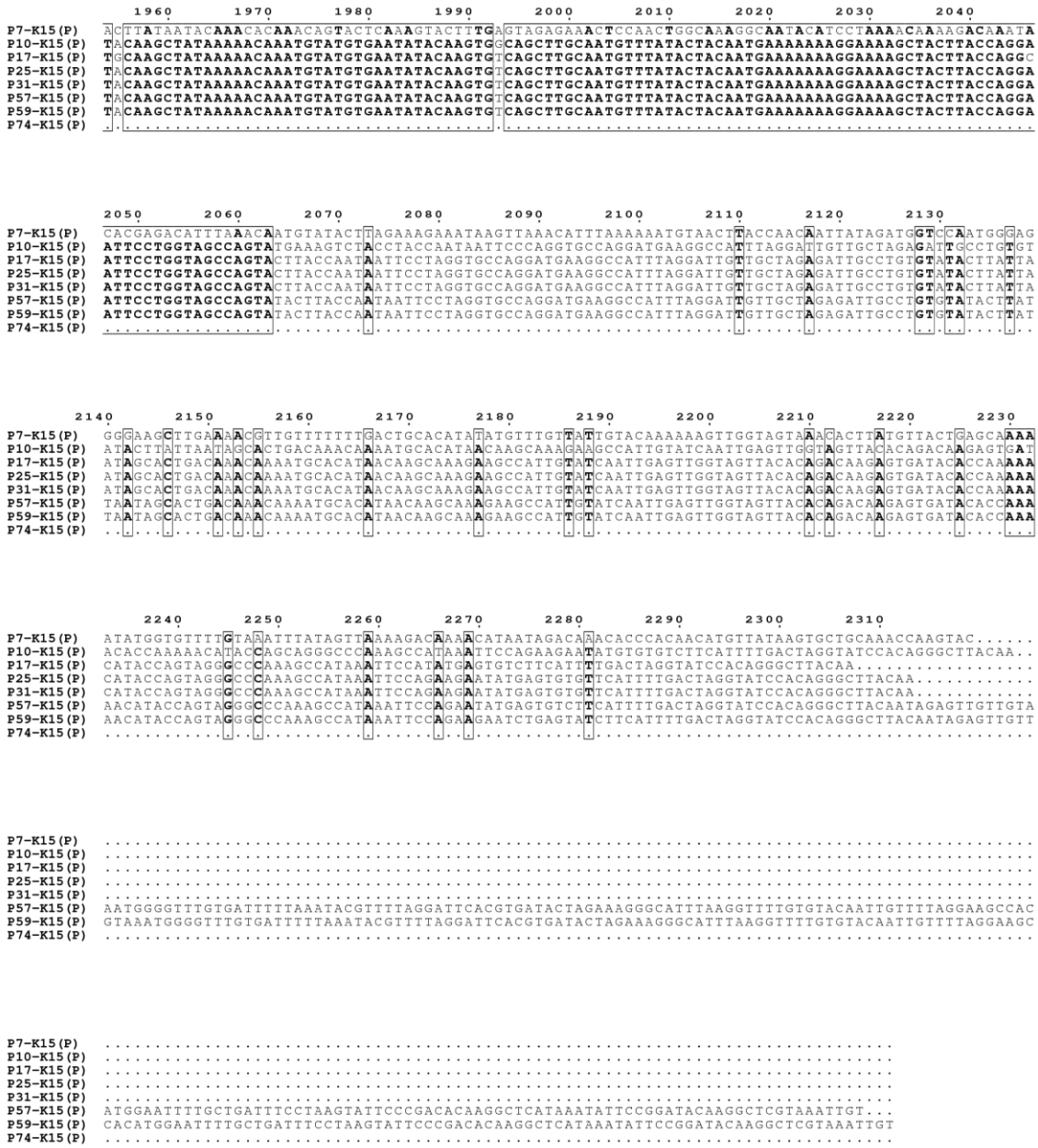
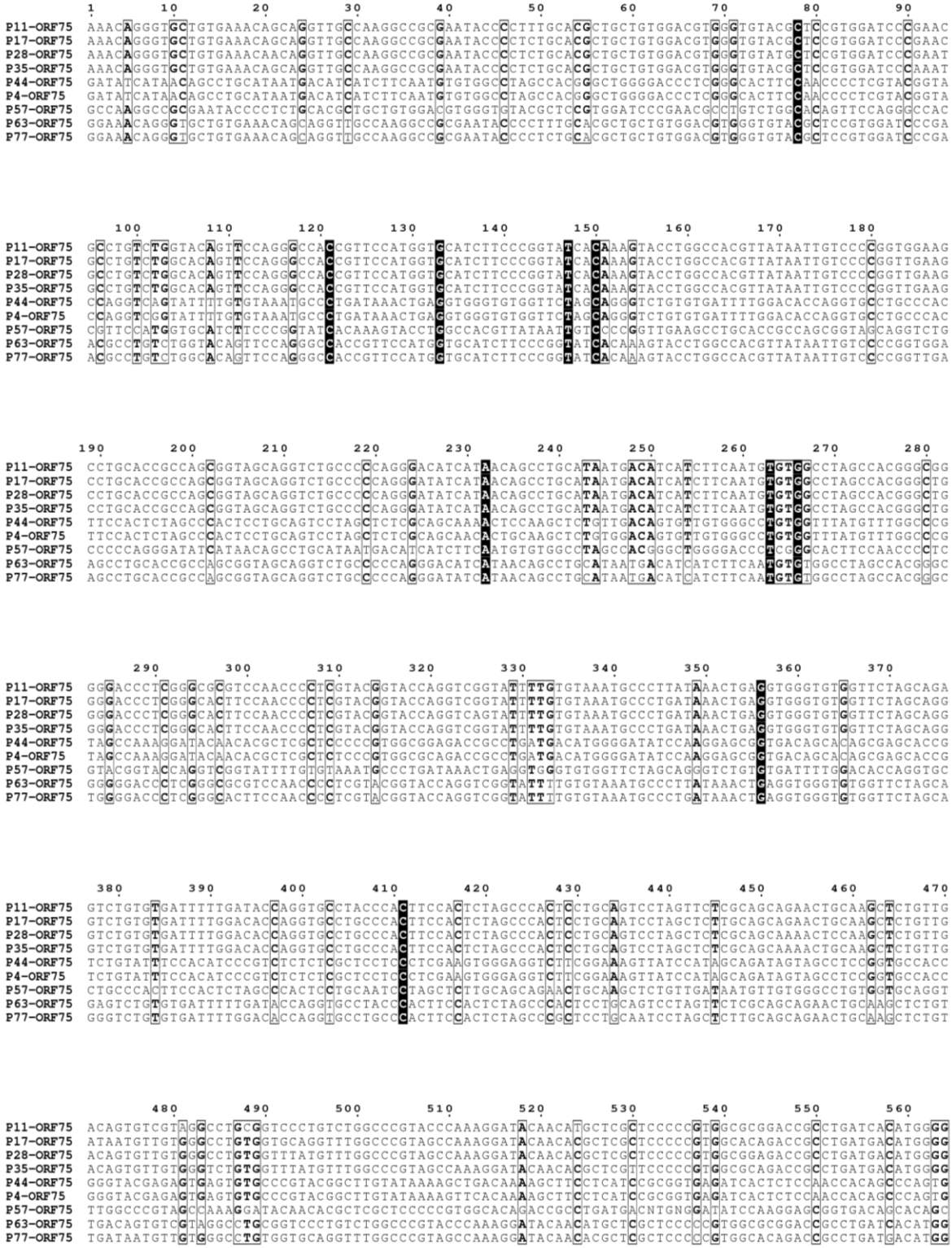


Figure 4.82: K15P gene sequence

Using http://espript.ibcp.fr/ESPript/cgi-bin/ESPript.cgi (Robert and Gouet, 2014) where 80% sequence identity was noted. Alignment of amino acid sequences of K15P gene, generated from nucleotide sequences for each sample investigated. The conserved regions are bolded in black.

4.5.4 ORF75 gene sequence



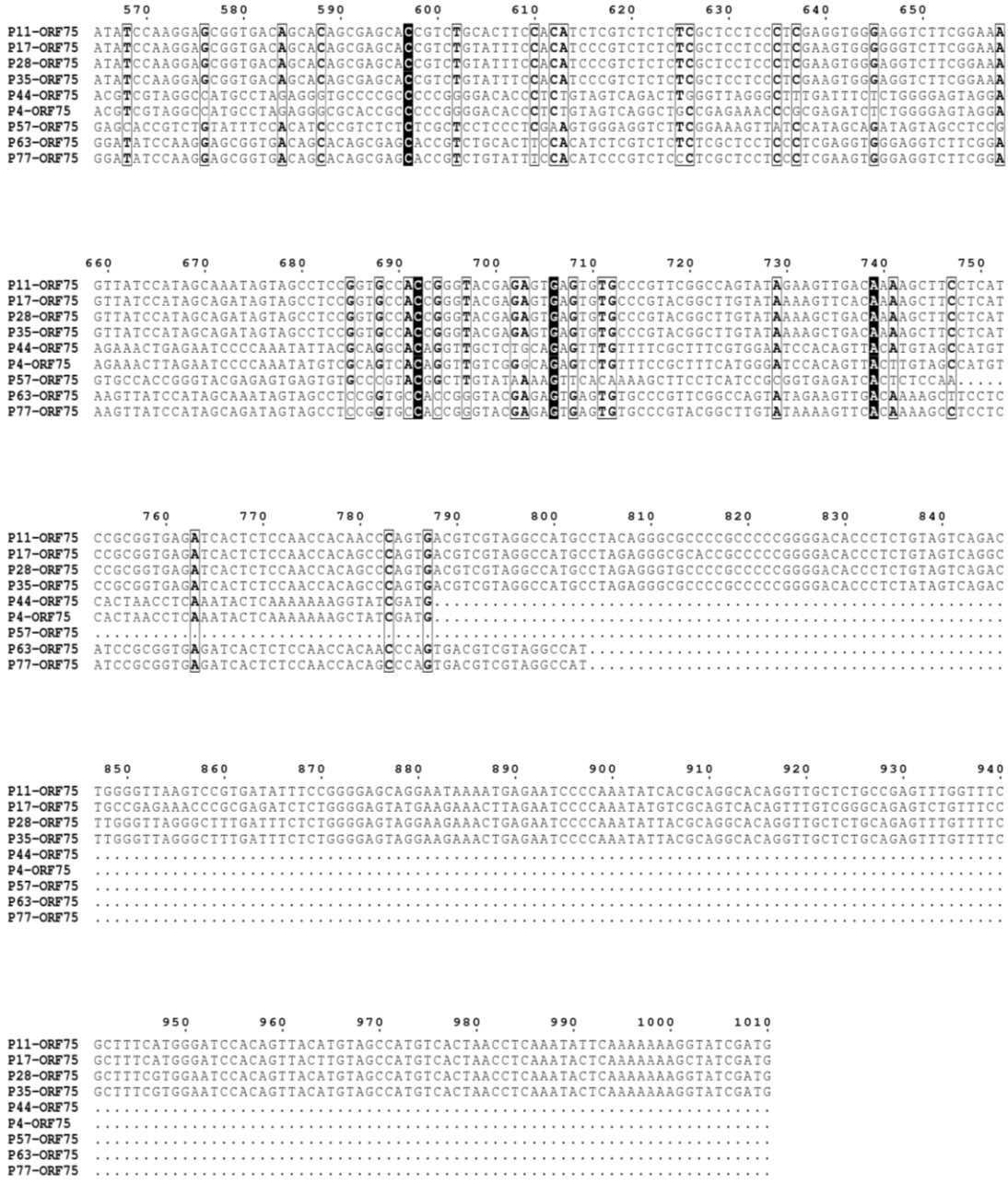


Figure 4.83: ORF75 gene sequence

Using http://espript.ibcp.fr/ESPript/cgi-bin/ESPript.cgi (Robert and Gouet, 2014) where 80% sequence identity was noted. Alignment of amino acid sequences of ORF75 gene, generated from nucleotide sequences for each sample investigated. The conserved regions are bolded in black.

CHAPTER FIVE

DISCUSSION

In this study, molecular epidemiology and characterization of the selected HHV-8 genes associated with AIDS-KS was explored. In spite of a decrease in the incidence of AIDS-related KS worldwide since introduction of cART, KS continues to occur in HIV infected patients. In Africa, the prevalence of KSHV is reported to be very high especially among those co-infected with HIV (Malope-Kgokong *et al.,* 2010). Findings of this current study observed that K1, K15P and ORF75 genes of KSHV was prevalent among the cases. The results of this study is in line with the previously reported observation that Kaposi's sarcoma-associated herpesvirus encodes several genes which establishes a lifelong infection that are responsible for significant cancer burden (Lange and Damania, 2019). Therefore, it is conceivable to hypothesize that K1, K15P and ORF75 genes might have played a role in KSHV pathogenesis in infected cases. A study by Moody and Laimins, (2010), alluded that during the lytic KSHV infection, K1 and K15P viral proteins are expressed by cells in KS lesions, have tumorigenic activities and so contributes to the angiogenic and inflammatory phenotypes of KS lesions.

The cross-sectional analysis of this study demonstrated that majority 39 (48.2%) of Kaposi's sarcoma cases fell in the age group of 30-39 years. The high prevalence of KSHV demonstrated in these age groups is comparable with results from similar settings among HIV infected patients (Malope-Kgokong *et al.,* 2010). The high prevalence of KSHV in these age group fuels theories of a sexual route of transmission. A co-infection between KSHV and HIV has also been observed to be associated with poor outcome in HIV infected population. A study by Mandong *et al.,* (2004) reported that KSHV affects people in all ages. This was also observed in the current

study where the age distribution among cases that had KSHV ranged from 19-63 years with a mean age of 39.3 (SD=9.5) years. A study conducted by Pfeiffer *et al.,* (2010), observed that the rate of HHV 8 infection increases with age group within a population. In Tanzania referral hospital it was reported that significant (P=0.007) cases of Kaposi's sarcoma increase with age above 35 years among HIV positive patients (Semango *et al.,* 2018).

In view of gender distribution of KSHV, this study observed male preponderance among the studied cases. Kaposi's sarcoma has been reported to be two times higher in heterosexual men than in women and six times higher in Men who have sex with men than in women (Judd *et al.,* 2017). Male preponderance among the cases in this present study consistent with Tembo *et al.,* (2017). Tembo *et al.,* (2017) hypothesized that gender related factors including hormone, genetic factors and environmental factors could have influenced the outcome of AIDS-KS. In Uganda, Biryahwaho *et al.,* (2010) observed a significant variation in gender among the sero-positive patients that had Kaposi's sarcoma in Uganda.

All the cases that were studied were confirmed to have HIV/AIDS associated Kaposi's sarcoma. In sub-Saharan Africa countries, KS is one of the most prevalent malignancies linked with HIV (Globocan, 2012). Immunosuppression due to HIV sets foundation for herpes virus 8 (Zhang *et al.,* 2012; Rohner *et al.,* 2016; Rohner *et al.,* 2016; Liu *et al.,* 2017). Findings of this study hypothesize that immunosuppression resulting from HIV infection might have contributed to the development of KSHV. To limit KSHV-induced pathogenesis, Sathish and Yuan, (2011) reported that there is need for immune surveillance because of its association with KS

development. Hu and Usherwood, (2014), reported that the precise nature of immune response essential to prevent KS remains unclear, however, KSHV encodes a number of viral proteins that supports its persistence, transmission and replication. Semango *et al.,* (2018) reported that Kaposi's sarcoma was a frequent malignant tumor linked with HIV/AIDS. A study by Ferlay *et al.,* (2015) observed that in sub-Saharan Africa, KS is among the leading cause of cancer in HIV infected people. Liu *et al.,* (2018) also reported that the rising cases of KS in Africa has been attributed to HIV infection. In addition, Ma *et al.,* (2015), also described how clinical opportunistic diseases like Kaposi's sarcoma are expressed resulting from immunosuppression due to HIV. Immunodeficiency due to HIV poses as a risk factor for Kaposi's sarcoma associated herpes (Robbins *et al.,* 2015; Hesamizadeh *et al.,* 2016). Although KSHV infection is necessary for KS to develop, this study hypothesize that HIV infection is an important cofactor. In agreement with the results of this study, Casper *et al.,* (2011) reported KS is co-morbidity afflicting HIV positive patients. Cases of KS have been witnessed by Burger *et al.,* (2018), among AIDS patients. The observation made from these current study findings are useful in generating hypotheses about possible reduction in prevalence of KSHV when a national guidelines changes on ART initiation at higher CD_4 count.

Conventional haematoxylin and eosin (H&E) staining was used in the pathological diagnosis of Kaposi's sarcoma among the studied cases. The characteristic features of KS subtypes were observed as shown in figure 4.4. The patchy KS lesion was characterized by sparse perivascular infiltrate composed of lymphocytes and plasma cells; red blood cell extravasation and siderophages with fascicles of spindle cells. Plaque KS lesion was characterized by a diffuse infiltrate of vessels throughout the dermis with fascicles of spindle cells replacing the dermal

collagen. In addition, the plaque subtype of KS lesion had extravasation of RBCs with siderophages, numerous lymphocytes and plasma cells. The nodular sub-type of KS was the most distinct, characterized by well-defined nodules composed of sheets of spindle cells that replaces the dermal collagen. The vascular space had a honeycomb-like pattern filled with RBCs with interweaving spindle cells. The nodular sub-type of KS morphology was the most predominant, plaque came second and patchy was third. The nodular morphological type was more in males than females among the studied cases. Tembo *et al.,* (2017) in Lusaka, Zambia also observed that nodular morphological type of KS was leading at 60.7% (51/84) and others identified included 22.6% (19/84) patchy and 16.7% (14/84) plaque. A study on morphological distribution of KS by Isaac *et al.,* (2016), showed that majority of the cases were plaque, followed by nodular and patchy came third. The morphology sub-types of KS identified in this study were consistent with Wayne and Liron, (2008), who reported patchy, plaque and nodular as the histopathology. In this study, it was observed that patchy contained spindle cells that were red to naked eye. The histopathology of KS observed in this study were consistent with Judde *et al.,* (2000); Sangüeza and Requena (2003) Grayson, (2006) and Duprez *et al.,* (2007).

Moody and Laimins, (2010) reported that AIDS-associated KS can present as an aggressive disseminated disease affecting skin, lymph nodes and visceral organs. Observation made in this study on the anatomical site location of KS was as follows; lower limbs, trunk/chest/back, upper limbs, palate/mouth, eyelid and genitalia. Among the cases studied, lower limb had majority (40.7%) of KS lesions, trunk/chest/back (30.9%) came second and upper limbs (21.0%) was third. Isaac *et al.,* (2016) observed that KS lesions were located on the face, oral mucosa, trunk, upper limbs, lower limbs and genitalia. The majority of KS lesions were found on the lower

limbs at 81% (70), trunk 58% (50), face and upper limbs were third at 52% (45) as reported by Isaac *et al.,* (2016). In Tanzania, Chalya *et al.,* (2015) observed that KS lesions were located on the skin, limbs, trunks, oropharyngeal, ocular, lymph nodes and genitals. In consistent with the findings of this study Grayson and Pantanowitz, (2008), observed that the lower limbs, upper limbs, and the trunk were the most frequent anatomical site that had KS lesions. In another study Taglioni and Ferrucci, (2003) stated that AIDS-KS are characterized by aggressive manifestations of the disease with a rapid course, multifocal dissemination and at times may extensively involve the internal organs. Based on the observations made on this current study, AIDS associated KS lesions can be manifested anywhere in or on the body of the affected host.

The studied cases had more (65.4%) generalized distribution of KS lesions than localized (34.6%). Consistent with the findings of this study, Isaac *et al.,* (2016) observed that majority of the KS distribution were 76% (65) generalized and 24% (21) localized. The justification for categorizing the KS lesions as localized or generalized is to determine the treatment methods (Schwartz *et al.,* 2008). Localized skin lesion of KS for instance will require surgery, chemotherapy or radiotherapy (Schwartz *et al.,* 2008). The wide spread of KS lesion may be treated by chemotherapy or biologic therapy (Schwartz *et al.,* 2008; National Cancer Institute, 2017; Hoffmann *et al.,* 2017). Similar grouping of KS lesions as either localized or generalized has been reported by Su *et al.,* (2008); Posada, *et al.,* (2008); Mosam *et al.,* (2008), Pantanowitz and Duke, (2008). Remarkable observation in the studied cases was that the lower limbs had majority of KS lesion being generalized.

The observation made from the findings of this study showed that K1 gene was predominantly among the male cases. In consistent with this study finding, the K1 gene was found to be more in men (18) than women (11) according to Betsem *et al.,* (2014). Information about significant association between K1 gene and histology identification is useful in generating hypotheses about possible interactions between the viral gene expression and histopathology of KS. The strongest risk factor for development of KS is having the gene that causes the diseases. Possession of the gene as well as cultural and socioeconomic experiences is thought to be good predictors of developing KS. Many traits associated with a disease are determined by the existence of a gene.

The study hypothesize that K1 gene contributes directly or indirectly to the pathogenesis of KS infection. The K1 gene identified in this study confirmed the presence KSHV. In addition, this current study hypothesizes that environmental factors might influence the presence of KS infection. Furthermore, genetic susceptibility could perhaps explain the significant association between the K1 gene and morphological subtype of KS observed in this study. For a gene to be expresses it can be influenced by environment, other genes, and protein (Blazer and Hernandez, 2006). It is therefore not surprising that in this study a significant association was observed between K1 and K15P gene. Wen and Damania, (2010), observed that there was an association between K1 gene and oncogenesis and this was similarly observed in the present study findings. This study hypothesizes that, K1 gene identified in the cases might have contributed to the pathogenesis of KSHV. The K1 gene is a marker for HHV8, found in HIV positive patients with low CD_4count and its presence may not be influenced by ARV treatment status (Dalla *et al.,*

2019). The observation by Dalla *et al.,* (2019) was consistent with the results of this study where the K1 gene was observed in cases that were on ART and had very low CD_4cells counts.

The nucleotide sequences of K1 gene were aligned to HHV8 sequence in the NCBI PUBMED. This current study observed that the K1 gene had conserve sequences. This study hypothesizes that the similarities in the conserve sequences among the studied cases might have some aspects of geographical variations. Observation on conserve sequences was consistent with Kullberg *et al.,* (2006); Man *et al.,* (2010); Schoch *et al.,* (2012); Harmston *et al.,* (2013); Earl *et al.,* (2014); Hug *et al.,* (2016), who stated that quite often, sets of conserved sequences have similar sequences that are closely related.

Similar molecular epidemiological studies and the importance of genetic variability of K1 gene have been stipulated in published literature (Biggar *et al.,* 2000; Kadyrova *et al.,* 2003 and Kazanji *et al.,* 2005). Molecular characterization studies by Meng *et al.,* (2001); Zong *et al.,* (2002); Whitby *et al*., (2004), have identified five subtypes of the K1 gene as A, B, C, D and E. Furthermore, the molecular epidemiology by several studies recorded that HHV 8 B strain was mainly in Africa, subtype A and C were found in Europe and European-descent residents from United States of America whereas the subtype D was confined to Pacific population. According to Hayward and Zong, (2007); Tornesello *et al.,* (2010), the molecular epidemiology and characterization of KSHV subtypes, defined by K1 gene have definite geographical pattern and are globally distributed. Tornesello *et al.,* (2010), similarly reported that subtypes B and A5 are predominantly from Africa whereas Kasolo *et al.,* (2007); Zong *et al.,* (2007); White *et al*., (2008), have further reported that they found subtype B and A5 outside Africa. It was suggested

by Tania *et al.,* (2016); Tozetto-Mendoza *et al.,* (2016), that genotype B might be linked with better tumor prognosis compared to other subtypes. Historically, genotype B of HHV 8 is thought to have appeared in Africa continent 100,000 years ago (Hayward and Zong, 2007). According to Etta *et al.,* (2018), the African countries that have data on K1 gene are about 33.9% (18/53). The finding of this present study observed that K1 genotype was highly prevalent and this will add to the existing knowledge on the molecular epidemiology of KSHV infection in Africa.

The viral protein K15P was prevalent in the studied cases. Therefore, it is conceivable that K1P might have played a role in subverting the hosts signaling pathways, leading to KSHV-induced sarcoma. Etta *et al.,* (2018), was also able to identify the K15P gene at a frequency of 52.2% (12/23). This current study further observed a significant association between K15P and the histological features of KS. The identification of viral protein K15P from the studied cases is a confirmation that KSHV DNA was present. Other factors such as socioeconomic and cultural experiences might be good predictors for KS development. As earlier alluded, Kaposi's sarcoma herpesvirus has several genes that have different functions. The K15P gene for instance, is an Immunomodulatory and its presence further confirms that KSHV was identified in the studied cases. Genetic susceptibility among the studied cases could perhaps explain the significant association observed in this study between the K15P gene and morphological type of KS. The K15P genotype in the studied cases showed clearly in gender, age and was consistent across the board. The conserve sequences were observed in the nucleotide sequences of K15P gene. According to Harmston *et al.,* (2013); Earl *et al.,* (2014); Hug *et al.,* (2016), conserved sequences are usually similar or identical sequences in the nucleic acid or proteins across species

or within a genome or between donor and receptor. In addition, Harmston *et al.,* (2013); Earl *et al.,* (2014); Hug *et al.,* (2016) stated that conservation is an indication that the sequence has been maintained by natural selection.

Similar molecular epidemiological studies and the importance of genetic variability of K15P gene has been stipulated in published literature by Etta *et al.,* (2018), who reported that K15P are the most prevalent in Africa and are highly transmissible. In Morocco, K15P gene was identified and sequenced by Kakoola *et al.,* (2001). The clustering pattern of KSHV genotype with geographical location has been attributed by previous studies (Cassar *et al.,* (2007); Wojcicki *et al.,* (2008) to be on the rise due to human migration and genetic polymorphisms.

Forty-nine cases out of 81 studied had ORF75 gene identified. A significant association between ORF75 gene and histology identification of KS was also observed among the cases studied. Since ORF75 gene is involved in lytic replication of Kaposi's sarcoma herpesvirus, the identification of this viral protein can be linked to genetic expression of the virus. Other countries that have successfully identified the ORF75 gene include South Africa (Alagiozoglou *et al.,* 2000) and Zambia (Kasolo *et al.,* 1998). The identification of ORF75 gene in this study therefore implies that ORF75 gene was prevalent in the cases studied.

In this current study, it was observed that the nucleotide sequence of ORF75 gene had conserved regions that were denoted by black regions (figure 4.83). The ORF75 gene studied had similarities in their conserve sequences. Nawrocki and Eddy, (2013) documented that a highly

conserved sequence remains unaltered to back up the phylogenetic tree resulting far back in geological time. Additionally, Nawrocki and Eddy, (2013) stated that the nucleic acid sequences in the genome of an evolutionary lineage can change gradually over time due to mutation or deletion. However, conserved sequence persists in the genome despite such forces and has been shown to have slower rates of mutation than the background mutation rate (Nawrocki and Eddy, 2013). In other studies, Bertram and Tanzi, (2009); G*eet al.,* (2009); Pandey and Nichols, (2011), stated that, conserved sequences are used in medical research as starting point for identifying the causative of genetic diseases. Conserved sequences can also be used to predict and discover functional sequences such as gene (Marchler-Bauer *et al.,* 2010).

5.2 Limitations of the study

The current study findings must be considered in light of possible limitation of the study. To start with, the cross-sectional nature of the study design, inferences about causal relationship between KSHV and the factors investigation could not be determined. The sample size used in the present study was small. Reduced sample size have been shown to stray the p value from significance. Furthermore, an increased sample size is hypothetically more representative and should be adopted in an epidemiological study.

The results from this study were relevant to population in Kenya, these findings cannot be used to generalize epidemiological pattern in other countries.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The findings of this study demonstrated that viral proteins K1, K15P and ORF75 was prevalent among the studied cases. With respect to morphological subtypes of Kaposi's sarcoma, the nodular, plaque and patchy histological features of the epidemiological forms of KS were distinguishable. Furthermore, it was observed that there was an aggressive disseminated disease affecting the lower limbs, trunk/chest/back, upper limbs, palate/mouth, genitalia and eyelids. In this study, male preponderance (AIDS-KS) was observed among the cases. There was a significant association between K1, K15P, ORF75 and the morphological subtypes of Kaposi's sarcoma. In addition, a significant association between K1, K15P, ORF75 and the histology of Kaposi's sarcoma.

6.2 Recommendations for future research

There is need to embrace the use of PCR for identification of KSHV DNA in clinical molecular pathology laboratories. The identification of KSHV DNA by use of PCR is highly, if not optimally sensitive for KS and the absence of KSHV DNA in a well-prepared sample essentially excludes the diagnosis. If possible, an automated molecular diagnosis of KS could be performed at point of clinical care to replace histopathologic diagnosis. A similar approach has been adopted and implemented for molecular diagnosis of infectious diseases with GeneXpert platform.

Future research efforts in this filed should be focused on longitudinal study design to determine causal relationship between KSHV and the factors under investigation.

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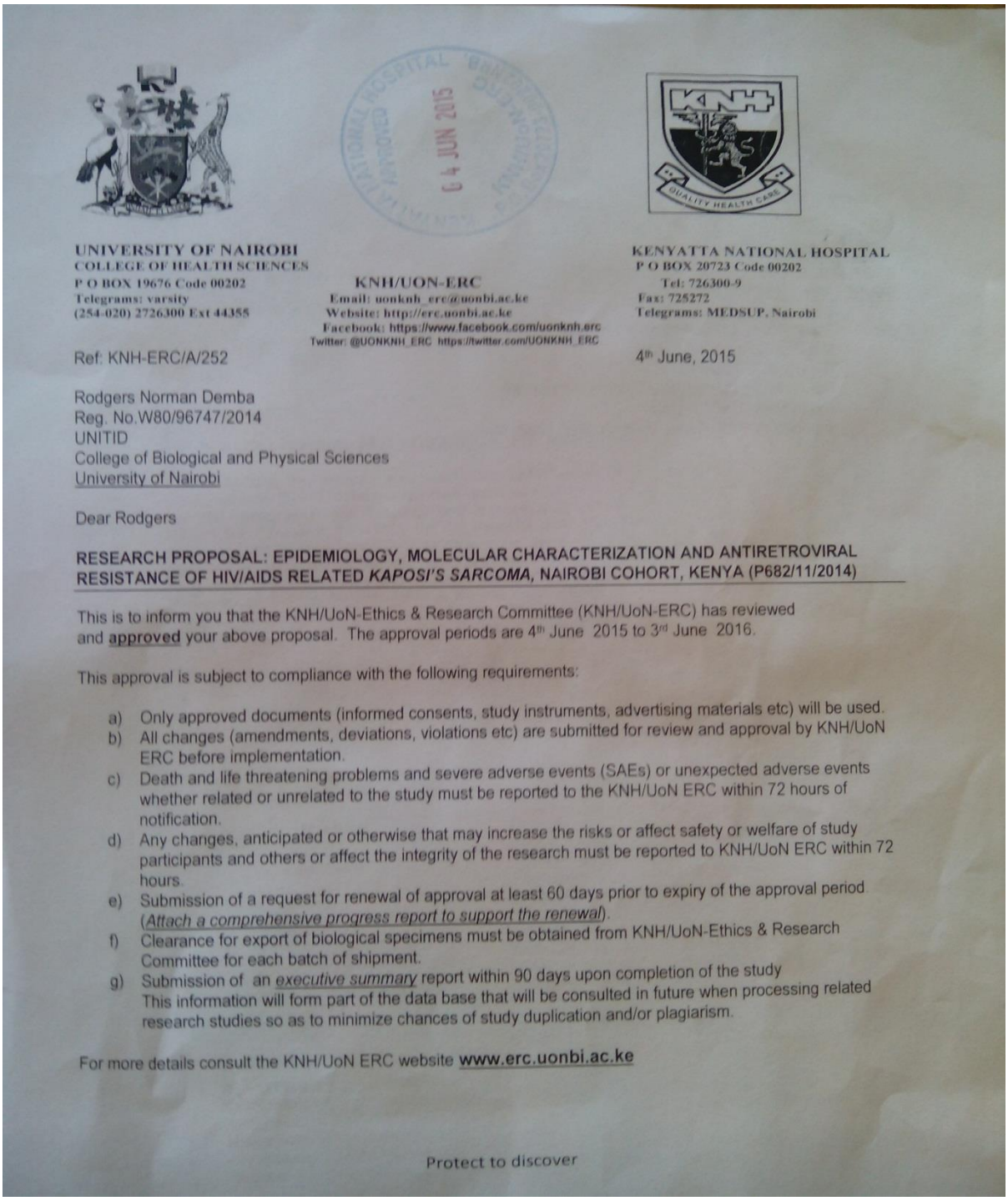
APPENDICES

APPENDIX 1: KNH/UON-ERC: ETHICAL APPROVAL LETTER



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04 JUN 2015

Ref: KNH-ERC/A/252

4th June, 2015

Rodgers Norman Demba
Reg. No.W80/96747/2014
UNITID
College of Biological and Physical Sciences
University of Nairobi

Dear Rodgers

RESEARCH PROPOSAL: EPIDEMIOLOGY, MOLECULAR CHARACTERIZATION AND ANTIRETROVIRAL RESISTANCE OF HIV/AIDS RELATED *KAPOSI'S SARCOMA*, NAIROBI COHORT, KENYA (P682/11/2014)

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and **approved** your above proposal. The approval periods are 4th June 2015 to 3rd June 2016.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH/UoN ERC website **www.erc.uonbi.ac.ke**

Protect to discover

Yours sincerely,

PROF. M. L. CHINDIA
SECRETARY, KNH/UON-ERC

c.c. The Principal, College of Health Sciences, UoN
The Deputy Director CS, KNH
The Chair, KNH/UoN-ERC
The Assistant Director, Health Information, KNH
The Director, UNITID, UoN
Supervisors: Prof. Walter Mwanda, Prof. Matilu Mwau

APPENDIX II: INFORMATION DOCUMENT FORM

TITLE OF STUDY: MOLECULAR EPIDEMIOLOGY AND CHARACTERIZATION OF K1, K15 (P) AND ORF75 GENES OF HHV-8 ASSOCIATED WITH HIV/AIDS KAPOSI'S SARCOMA IN PATIENTS AT KENYATTA NATIONAL HOSPITAL.

INTRODUCTION

The proposed study seeks to validate the Molecular epidemiology and characterization of K1, K15P and ORF75 genes of hhv-8 associated with HIV/AIDS Kaposi's sarcoma in patients at Kenyatta National Hospital. Through screening of archived tissue biopsies from patients who attended Kenyatta National Hospital, by Histological reporting on biopsies, nested Polymerase Chain Reaction, and sequencing testing.

PARTICIPATION

After obtaining ethical approval for this study and before participation, permission was sought from Thematic Unit of Anatomic pathology, Department of Human pathology, College of Health Sciences, University of Nairobi and Department of Laboratory Medicine, Histology section, Kenyatta National Hospital. Participation in this study was voluntary. Refusal to participate did not involve penalty or loss of benefits to which you are otherwise entitled.

PROCEDURE TO BE FOLLOWED

The demographics, HIV-1 status, CD_4 cell count, tumor location, number of tumors, and the antiretroviral treatment status whose tissue blocks had been collected and used were obtained from the registry records in Thematic Unit of Anatomic pathology, Department of Human

pathology, College of Health Sciences, University of Nairobi and Department of Laboratory Medicine, Histology section, Kenyatta National Hospital.

INCONVENIENCES AND BENEFITS

The tissue blocks targeted are those already determined by the principal investigator as those that have met inclusion criteria. Archived tissue biopsies were collected. There were no immediate benefits for the patient.

PARTICIPANT'S RIGHTS

Participation in this study was voluntary and any form of decline to participate; participants were not being denied any services that are normally available to you.

COMPENSATION

No direct compensation in the form of salary will be paid for participating in the study and no special incentive will be offered to persuade persons to participate. Patients will be at liberty to refuse consent with or without explanation, and without penalty or prejudicial action towards them.

DURATION OF PARTICIPTION

This study only requires archived biopsy. There will be follow-up or further information needed should arise in the course of the study.

WHO CAN PARTICIPATE IN THIS STUDY?

Inclusion Criteria:

1. Cases from which the tissue blocks were retrieved had to be of 18 years and above
2. Tissue blocks that were previously proven as KS or KS like.
3. A comprehensive bio-medical data linking the blocks and registry records.
4. Multiple tissue blocks of the same KS or KS-like case

Exclusion criteria:

1. Tissue blocks that were previously proven as Adenocarcinoma, Burkitt lymphoma, Carcinoma-insitu/severe dysplasia
2. Incomplete bio-medical data linking the blocks and registry records.
3. Fragmented tissue blocks

ASSURANCE OF CONFIDENTIALITY OF VOLUNTEER'S IDENTITY

Records relating to your participation in the study will remain confidential. Your name will not be used in any report resulting from this study. All computerized records and laboratory specimens will contain only a unique study number, not your name. You will receive a signed copy of this consent form.

USE OF ARCHIVE TISSUE BLOCKS

The archived tissue blocks obtained in this study will not be used for any other purpose other than the ones stated in the protocol and consent form. The results of all testing performed will be shared with the medical or clinical officer caring for you.

REVIEW OF RESEARCH RECORDS

It should be noted that consent forms will be kept in a locked file at University of Nairobi Institute of Tropical and Infectious Diseases or a designated storage facility for not less than 10 years following completion of the study. These data sheets will be made available only to the Principal Investigator and the Co-investigators, clinical personnel who require this information to treat the patient, or to members of the Ministry of Health who require this information for legal reasons.

DATA MANAGEMENT:

Data Storage

Most data entry will be performed at University of Nairobi Institute of Tropical and Infectious Diseases on a computer provided by this study. The only identifier used in this computerized database will be the subject's study number. Clinical data sheets will be kept on file at University of Nairobi Institute of Tropical and Infectious Diseases or a designated storage facility for not less than 5 years following completion of the study. These data sheets will be made available only to the Principal Investigator and the Co-investigators, clinical personnel who require this information to treat the patient or to members of the Ministry of Health who require this information for legal reasons or to investigate an outbreak. Data will also be stored in compact disks and flash disks.

Data Management

As this was primarily a crossectional descriptive study, data analysis planned was the determination of Epidemiology, molecular characterization of HIV/AIDS related *Kaposi's*

sarcoma Nairobi cohort, Kenya. Through screening of biopsy samples collected from patients from the Kenyatta National Hospital. At conclusion of the study, the biopsy collected will be discarded in accordance with the bio-safety measures and no other study besides the above mentioned will be done using these samples.

Dissemination of Data

Publication or presentation of any data resulting from this study will be a joint collaboration between Universityof Nairobi and Kenyan researchers. Nothing will be published or presented without a review from Kenyatta National Hospital/ University of Nairobi Ethics and Research Review Committee (KNH/UON ERRC). Manuscripts submitted for review to either organization will be completed and approval or recommendations for changes given.

APPENDIX III: INFORMATION CONSENT FORM

INTRODUCTION

The details about the study and the reasons for participation will be explained to the study participants by the principal investigator (Rodgers Norman Demba).

The study seeks to evaluate molecular epidemiology and characterization of K1, K15 (P) and ORF75 genes of HHV8 associated with HIV/AIDS Kaposi's sarcoma in patients at Kenyatta National Hospital, Kenya has not been done in this area. The information the study will gather will be useful to the government and other policy makers as it will makea significant contribution in patient management in the future. The study will summarize findings and disseminate it to various stakeholders including Ministry of Health, Kenya Expanded Programme for Immunization, University of Nairobi Institute of Tropical and Infectious Diseases, Kenya Medical Research Institute, and others. The Kenyatta National Hospital/University of Nairobi Ethical Review Committee, who is responsible for conducting such reviews at national level, has approved this study.

Research Procedures: Before participation, subjects will be required to fill informed consent forms with the help of a principle investigator. At conclusion of the study, the tissue blocks collected will be discarded in accordance with the bio-safety measures and no other study besides the above mentioned will be done using these samples.

You are here to have archived tissue biopsy from cytology laboratory store for the purpose of the study. The biopsy taken from cytology laboratory will be transported to the University of Nairobi Institute of Tropical and Infectious Diseases laboratories in for molecular analysis of *Kaposi's sarcoma*. In order to ensure complete confidentiality of the test results, no names will be attached to the blood samples, but an identification number assigned to you will be used to label the sample.

Risk/benefits: The study will benefit the community since by helping us and the government to understand the problems your community is facing as a result of*Kaposi's sarcoma* and the circulating gene in Nairobi, Kenya.

Participant's Rights: Your participation in this study is voluntary and if you decline to participate, you will not be denied any services that are normally available to you.

Confidentiality: We will make every effort to protect your identity. You will not be identified in any report or publication of this study or its results.

Contact Information: If you have questions now or in the future regarding your rights or problem about how you are treated in this study, contact the Principal Investigator: Norman R. Demba, Contact: 0723875756, 0753926431; Lead Supervisor Prof. Walter Mwanda Contact: 0705849623 or Prof. Matilu Mwau Contact: 0728073633 or Secretary, Kenyatta National Hospital/University of Nairobi- Ethical Review Committee,Email: k.research@knh.or.ke P.O

BOX 20723-00202, Nairobi Kenya; Telephone number: +2542726300-19. Institution website address: http://www.uonbi.ac.ke/colleges/chs/knh/index.htm

Consent for the blocks/cases as sample:

May I now ask if you would allow me pick archived tissue blocks for the study?

The above details about the study and the reasons of giving consent has been explained to me by: Dr/Mr/Mrs/Miss..... and I allow the principal investigate to pick tissue blocks from the archive store. I give my consent for blocks to be collected and used for the study.

Please sign here or put your right hand thumb mark if you agree:

Signature/ Thumb mark----------

Date ----------

Witness (Phlebotomist):Dr/Mr/Mrs/Miss.....

Signature ----------

Date ----------

Kiini na sababu ya utafiti huu kuhuisisha mgonjwa atakae chaguliwa itafafanunuliwa na Mkuu wa utafiti huu (Rodgers Norman Demba).

Tunafanya utafiti juu ya Epidemiology na Molecular Characterization of *Kaposi's sarcoma* kwa watu walio ambukizwa na ukimwi Nairobi, Kenya. Matokeo ya utafiti huu yatasambazwa kwa serikali na wadau kama Wizara ya Afya, Kenya Expanded Programme for Immunization, University of Nairobi Institute of Tropical and Infectious Diseases, Kenya Medical Research Institute, na zengineo, ambao watatengeneza miradi mbalimbali itakayolenga kupunguza maambukizi na kuboresha huduma za walioathirika katika jamii hii, na nyingine hapo baadaye. Utafiti huu umepitishwa na kuruhusiwa na kamati inayohusika na utoaji wa vibali vya utafiti ya Kenyatta National Hospital/University of Nairobi Ethical Review Committee.

Utaratibu: Kabla ya kushiriki katika utafiti huu, mgonjwa atatakikiwa ajaze fom akisaidiwa na daktari. Sampuli itatolewa kwa mgonjwa na daktari aliyehetimu. Damu itakayo tolewa kwa mgonjwa itatumiwa kwa utafiti na baada ya utafiti damu hayo hayata tumiwa kwanjia ingine yeyote ile. Damu hayo yata mwangwa kwa njia ya usalama ili yasi dhuru mtu yeyote.

Daktari wako ameshakueleza kwamba anahitaji damu yako kwa najili ya utafiti. Ikiwa utakubali kujihusisha na utafiti huu, kwanza tutakuuliza maswali machache juu ya umri wako na unapoishi. Baadaye, damu tutakayotoa kwa huu utafiti itapelekwa maabara kwa upimaji wa*Kaposi's sarcoma*. Ili kuhakikisha usiri wa jina lako katika utafiti huu, jina na maelezo yako hayataandikwa kwenye sampuli ya damu, bali sampuli itatambulishwa na namba tu. Ni utaratibu wa utafiti huu kuwa watafiti hawatakujulisha majibu yako.

Faida/Mapungufu: Utasikia maumivu kidogo ama woga wakati unachomwa sindano, lakini hutapata maumivu ya muda mrefu. Hutapata malipo yoyote ya kifedha, na pia hutatumia pesa zako mwenyewe katika utafiti huu. Utapewa neti ya kuzuia kuumwa na mbu. Utafiti huu utasaidia jamii yako kwa sababu tukifahamu matatizo ya jamii hii, tutaweza kushauri na kutengeneza miradi mbalimbali ya kupunguza athari za *Kaposi's sarcoma*.

Haki za mshirika: Ushiriki wako katika utafiti huu ni wa hiari kabisa. Ukikataa kushiriki, hutanyimwa huduma zinazotolewa kwa kawaida.

Usiri/Utunzaji wa taarifa: Katika utafiti huu, tutahakikisha kuwa maelezo yako, jina lako ni siri kabisa. Jina lako halitaandikwa au kuhusishwa kwa hii fomu, sampuli, na popote ndani ya ripoti nzima tutakayotoa baadaye.

Mawasiliano; Iwapo utakuwa na swali kuhusiana na haki zako ama utafiti huu, unaruhusiwa kuwasiliana na afisa yeyote wa utahini, au Mkuu wa utafiti huu;Norman R. Demba, Nambari ya rununu: 0723875756, 0753926431; Msimamizi Prof. Walter Mwanda, Nambari ya rununu: 0705849623 au Prof. Matilu Mwau Contact: 0728073633 auKatibu, Kenyatta National Hospital/University of Nairobi Ethical Review Committee,Barua pepe: k.research@knh.or.ke P.O BOX 20723-00202, Nairobi Kenya; Nambari ya simu: +2542726300-19.

Institution website address: http://www.uonbi.ac.ke/colleges/chs/knh/index.htm

Ridhaa kKutumia tissue blocks yawagonjwa kama sampuli kwautafiti huu:

Napenda kukuuliza ridhaa yako kunikubalia nichukue tissue blocks kama sampuli kwa utafiti huu.

Nimeelewa maelezo ya hapo juu yanayohusu utafiti huu, baada kufafanunuliwa na Daktari/Mr/Mrs/Miss.....na ninakubali kuwa anaweza kuyachukua tissue blocks zenye ziko kwa kabati iliaweze kuzifanyia utafiti. Natoa ridhaa kuwa hizi tissue blocks zitumike katika utafiti huu.

Sahihi/dole gumba……

Tarehe……..

Mshahidi (Mtoaji sampuli ya damu): Daktari/Mr/Mrs/Miss.....

Sahihi: …….

Tarehe: …….

APPENDIX IV: QUESTIONNAIRE

MOLECULAR EPIDEMIOLOGY AND CHARACTERIZATION OF K1, K15 (P) AND ORF75 GENES OF HHV-8 ASSOCIATED WITH HIV/AIDS KAPOSI'S SARCOMA IN PATIENTS AT KENYATTA NATIONAL HOSPITAL.

Date of hospital visit …….

Study Number ……..…

Village ……

Sub-location …….

Location …….

Division ……

District …….

Province ……

Demographic characteristic:

Sex:

Male ( ) Female( )

Age (years) ( ) Date of birth ( )

Social characteristic:

Marital status: Single ( ) Married ( ).

Have you ever been tested for HIV?

Yes ( ) No ( )

If Yes, when

Year ( ) Date ( ) Age ( )

Are you on antiretroviral therapy (ART)?

Yes ( ) No ( )

If Yes, when

Year ( ) Date ( ) Age ( )

Have you been consistent in taking your antiretroviral (ARV)?

Yes ( ) No ( )

If Yes, which ART are you currently on?

…….

CD_4 cell count

a) < 350cells/mm^3

b) >350 cells/mm^3

Anatomical site of the KS

a) Upper limbs b) Lower limbs c) Trunk d) Genitals d) Skin e) Lymphedema f) Others specify

…….

Number of KS lesions

a) > 10

b) < 10

Any other remarks:

……

……

……

Name of person completing form: ……... ……

Signature: …….. Date: …….. Time: ……

APPENDIX V: PUBLICATION

Reference in a peer reviewed medical journal I:

APPENDIX VI: PUBLICATION

Reference in a peer reviewed medical journal II:

APPENDIX VII: TURNITIN ORIGINALITY REPORT