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Nasopharyngeal Carcinoma: 11-Year Retrospective Study and Moleculer Characterization of Ebna1 Gene of EBV LMP1 Detected in Tissue Samples Obtained in National Ear Care Centre, Kaduna (2013-2023)

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Abstract
Nasopharyngeal carcinoma is a rare tumor of the head and neck which originates in the epithelial cells that are squamous in nature [Nyall *et al*., 2024] and NPC is the most common malignant tumor of the nasopharynx located at the postero-lateral recess referred to as the Fossa of Rosen Müller. This study aims to retrospectively study the prevalence of NPC patients using the histopathology unit register to collate their demographic studies over 11-year period (2013-2023), A total of 42 patients were diagnosed with NPC out of 228 cancer cases recorded in NECC and of the 42 patients diagnosed, thirteen,13 (31%) had UDNPC, seven,7 (17%) reported to have NKNPC making up 17% of the total, seventeen,17 had DNPC which is the highest occurring surmounting to 40%, with only 3 (7%) were unclassified with NPC recorded as their findings making up 7%, KNPC had only two,2 (5%) patients recorded, overall there is a male preponderance over the female patients reported in this study. LMP1 was detected in 29 patients with prevalent NPC diagnosed and subsequent molecular characterization of EBNA1 gene of the Epstein Barr Virus was carried out using a good representative of the samples amplification and DTCS sequencing. This showed resemblance to MT648647.1:96337-96506 Human gamma herpesvirus 4 isolate Necker-6, partial genome and GU475442.1:117-286 Human herpesvirus 4 isolate T5-biopsy_V-leu-vii EBNA-1 protein gene, partial cds with 160/171 identity. In conclusion, the overall prevalence of NPC cases among patients attending NECC Kaduna have been stipulated. Ad-infinitum, the histopathological features of NPC cases, providing detailed insights into the disease morphology, presentation and molecular analyses of the EBNA1 gene in NPC patients, identifying specific viral genetic markers associated with the disease were all achieved.

Keywords: Nasopharyngeal carcinoma, Epstein Barr Virus, Latent membrane protein 1, Epstein Barr Virus Nuclear Antigen 1, Epithelial linings.

Introduction

Nasopharyngeal carcinoma (also known as NPC) is a rare tumor of the head and neck which originates in the epithelial cells of the nasopharynx that are squamous in nature. The nasopharynx is located at the very back of the nose near the Eustachian tubes (Nyall *et al*., 2024) and NPC is the most common malignant tumor of the nasopharynx located at the postero-lateral or pharyngeal recess referred to as the Fossa of Rosenmuller. It has male preponderance and more common in certain regions of East Asia and Africa thereby giving rise to the Chinese and African subtypes. The major difference here is the

category of people it affects in which adults are more affected in Southern China where as children are more affected in Northern African population which is strange as in other parts of the world rhabdomyosarcoma affects children more in the nasopharynx followed by malignant lymphomas (Nyall *et al*., 2024). Though factors such as the EBV, that increase the risk of this cancer have been identified in about 95% of cases in China and Japan. The mutation take place in the squamous cell that line the surface of the nasopharynx and by the time a patient is diagnosed with NPC, the 3 years' survival rate is 60%. (Mayoclinic, 2024). NPC can be diagnosed and graded through clinical manifestations, histopathological analyses, physical exams and CT/PET scan for staging grouped from I-IV (He *et al*., 2022). Treatment involves radiotherapy, chemotherapy, resections of surgeries and biopsies. Types of NPC according to WHO include types 1,2,3 and 4 which include Keratinizing squamous cell carcinoma, Non keratinizing squamous cell carcinoma, Undifferentiated or poorly differentiated carcinoma and Differentiated squamous cell carcinoma respectively (Clevelandclinic.com). One of the most prevalent viruses in humans is the Epstein-Barr virus (EBV), also known as the Human gamma herpesvirus 4. It is one of the nine types of human herpesviruses that are currently known and the viral DNA is double-stranded. (Zanella *et al.,* 2020) Gamma herpesvirus 4 in humans was discovered by Michael Anthony Epstein and Yvonne Barr, who along with Bert Achong identified the virus, the Epstein-Barr virus was then renamed after its discoverers. (McGrath, 2014; Epstein *et al.,* 1964). EBV is an oncogenic virus that has been strongly linked to various epithelial malignancies such as nasopharyngeal cancer and gastric tumors. EBV affect both immune competent and immunocompromised patients. Globally it is believed that EBV is responsible for approximately 1.5% of all human cancer. EBV transmission primarily occurs through saliva, with increased level of viral DNA being found in salivary secretions after the initial infection (Oren *et al*., 2022). Traditionally, EBV strains have been classified into types 1 and type 2 (also known as type A and B respectively) based on the sequence of their EBV nuclear antigen (EBNA), specifically EBNA2 EBNA3 A/B/C latency genes. EBV1 and EBV2 differ in geographic distribution, commonly in America and south Asia. 85% of NPC Is commonly caused by EBV1 even though there are other newly discovered classes (Oren *et al*., 2022). EBV uses its viral protein in the action of which mimic several growth factors transcription factors and anti-apoptotic factors, to usurp control of the cellular pathways that regulate diverse homeostatic cellular function (Matthew and Razelle, 2004). Risk factors are sex, race, age, salt cured food. Common signs and symptoms, are swollen lymph nodes, blood in saliva, blood from the nose, nasal congestion, hearing loss, headache, sore throat, frequent ear infection among others.

The most important among the proteins listed for immunohistochemistry analysis is the LMP which seems to have important roles in EBV-induced cell transformation in vitro, and have been implicated as important effector molecules in EBV-associated lymphomagenesis and NPC (Ye *et al*., 2023). While studying monoclonal antibodies and immunohistochemically labelling of acetone-fixed cryostat sections, LMP was demonstrated in RS Cells of 40 cases and varied according to histological subtype of lymphomas, also demonstrated in nodular sclerosis and the most important thing to note here is that antibodies showed no cross-reactivity with negative control tissues indicating they are useful probes for the diagnosis of latent EBV infection in tissue sections and that LMP expression is related to histologically aggressive subtypes of malignancies (Ye *et al*., 2023). The null Hypothesis of this study include there is no significant difference in prevalence of NPC cases among patients referred to the Histopathology unit of NECC Kaduna, EBNA1 gene will not be detected in the NPC tissues referred for histopathological analyses and finally EBNA1 gene detected is not the same as the one in gene bank database.

Statement of the Problem

The prevalence of NPC, the presence of LMP1 of EBV has not yet been discovered in patients with this particular type of cancer which aids in the propagation and maintenance of tumour cells. Molecular characterization of the EBV's (Epstein-Barr Virus) EBNA1 gene in patients referred to the Histopathology unit of NECC Kaduna is extremely important as it has a significant concern, NPC is ranking as one of the deadliest cancers with survival rate of 3 years' at 60% [Mayoclinic, 2024] with very painful symptoms especially at the end stage of the disease. Understanding the extent of NPC cases and analyzing some of the molecular features of EBV, detecting novel mutation and diseases in affected individuals is crucial for effective diagnosis and treatment strategies (Meissner *et al*., 2008).

Aim:

The aim of the study is to determine the prevalence of Nasopharyngeal Carcinoma (NPC) in National ear care center during 11-year period, detect LMP1 of EBV and to characterize the EBNA1 gene of Epstein-Barr Virus in patients referred to the Histopathology unit of NECC Kaduna

Objectives:

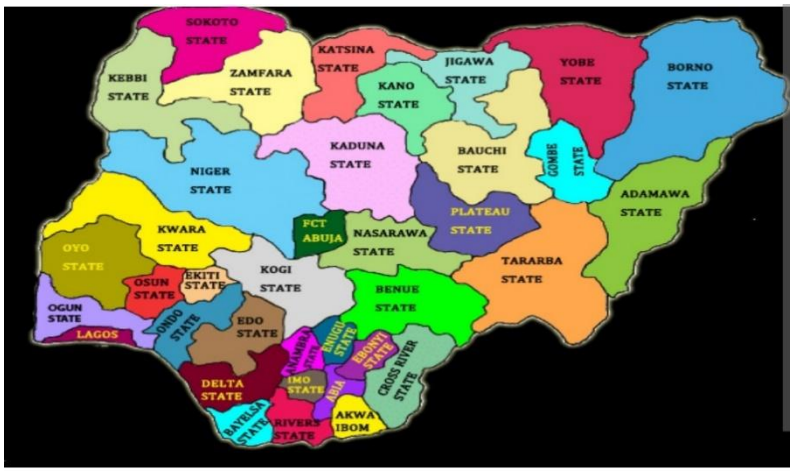
1- Determine the frequency and distribution of NPC cases among patients attending NECC Kaduna so as to determine the overall prevalence and demographic distribution.
2- Provide valuable insights the histopathological features of NPC cases, providing detailed insights into the disease morphology and presentation.
3- Determine the number of NPC cases that are positive for EBV through expression of latent membrane Protein (LMP1) using IHC techniques
4- To perform molecular analyses of the EBNA1 gene for homology searches

Methodology:

Description of the Study Area

The study took place in National Ear Care Center Kaduna, Kaduna State Nigeria. Kaduna is among the six states that constitute the northwest geopolitical zone. Other states include Jigawa, Kano, Kebbi, Sokoto, and Zamfara (Quadri, 2021). They together cover a total area of 42,752 sq. mi and an area rank of 4th of 36. They have a total population of 14,833,784 people going by 2022 census leaving them both the 3rd of 36 in rank and a density of 200.4/km^2 (340/sq. mi) population density with 2.5% annual population change (2006-2022). They are populated by about 59 to 63 different ethnic groups if not more (Hayab, 2014); where Hausa and Fulani are the dominant ethnic groups. Its water supply is sourced through damping of rivers and digging of wells and boreholes. Kaduna State consists of twenty-three (23) Local Government areas (National Bureau of Statistics,2022).

Fig. 1: Map of Nigeria



The map of Nigeria showing the location of Kaduna state color coded in Light Purple

Adopted from Research Gate

Ethical Approval

The Ethical clearance was approved and obtained from the Training, Ethics and Research Committee of National Ear Care Centre Kaduna (Check Appendix I) with the number **NECC/ADM/214/VI/269**.

Sample Population

This 11-year prevalence study that is 2013 to 2023 was performed between the period the proposal was approved and ethical clearance obtained to when samples were duly analyzed and results compiled and presented (from November, 2023 to May, 2024). The researcher adhered to the 1975 Helsinki

declaration and its next revisions. The inclusion criteria were defined as patients with confirmed histopathological (core/ fine needle biopsy) evidence of NPC and accessibility of fresh tissue samples for FFPE blocks processing. No limitations in age, type of cancer, tumor size, and stage were considered for the patients. Exclusion criteria on the other hand included different parameters such as people not diagnosed for NPC in the Centre. All tissue samples were formalin fixed paraffin embedded, furthermore, the tumors were diagnosed according to the types of nasopharyngeal carcinomas recorded in the WHO registry and carried out by consulting an expert cancer team consisting of a pathologist, oncologist, a radiologist, and a cancer surgeon.

Steps Taken to Obtain the Tissue Blocks

These were identified from the Unit and Departmental register of already diagnosed NPC tissues from the theatre or FNAC processed according to Standard Operating Procedure. They were traced back to the well labelled tissue block for slide preparation and staining using the H and E technique for histopathological subtype determination. Consequently, slides were prepared from all tissue blocks with NPC for EBV LMP1 detection using IHC techniques.

Immunohistochemistry Analyses for Detection of Latent Membrane Protein (LMP1)

1. Tissue sections were taken at 4microns from the tissue blocks and placed on hot plate for a minimum of one hour to melt away all the wax and incubate alongside a positive control
2. They were then brought to water and epitope retrieval carried out using heat at 100^{o}C with sections immersed in citrate for 30minutes.
3. Then enzymes blocking using peroxidase was carried out after washing with tap water and subsequent washing with phosphate buffer, liquid repellent marker (A-PAP pen) was used to demarcate the sections for effective procedure and reagent retention. The peroxidase was allowed to act for 20minutes
4. The sections were then thoroughly washed with phosphate buffer (PBS) and covered with LMP reconstituted antibody using 10ul LMP1 stock solution and 1000ul PBS as indicated in the leaflet and incubated at room temperature for 1hour to allow enough time for antigen antibody reaction.
5. They were then thoroughly washed with PBS, covered with secondary antibody using a Pasteur pipette and allowed to react for 20 minutes
6. They were then washed again with PBS and covered with Horse Radish Peroxidase (HRP) for 40minutes to allow for secondary antibody binding.
7. They were then thoroughly washed about 5times with PBS to ensure all free HRP has been taken out before finally adding DAB to stain sections for LMP1 detection for 5minutes.
8. Sections were then rinsed with water and stained with Hematoxylin for 15seconds to create a contrast, washed and rinsed in alcohol, inserted into the hot air oven to dry out and mounted using DPX.
9. Microscopy was then carried out and photomicrographs taken.

Sample Collection and Transportation

Tissue blocks and Processing of Clinical Samples

Specimen Collection and Processing: All tissue blocks were collected from the histopathology unit

Isolation and Purification of DNA from positive LMP1 tissue blocks of NPC patients

The EBNA1 gene was isolated from the processed tissue block collected from one NPC patient. All histopathological diagnoses were performed according to World Health Organisation (WHO) classifications and reviewed by two pathologists. The clinico-pathological characteristics of the study subjects were listed in the result section. DNA extraction were performed by Add Bio Corporation Fast DNA Tissue Kit according to the manufacturers' instructions as follows.

High molecular weight DNA were extracted from the tissue blocks of patients according to standard protocols as follows:

- The tissue block was sectioned and ribbons inserted into a labelled sterile tube
- They were then transferred to a glass flask for dewaxing as xylene dissolves plastic
- Briefly, sample was de-waxed twice with 1 mL of 2 changes xylene for 10 min each, vortexed, centrifuged and tissue fragment obtained after decanting
- It was then washed twice with 100% ethanol for 5 minutes each to remove xylene,

- It was then dried and re-suspended in 500 ll PK buffer that is the tissue lyses buffer [50 mmol/L KCl, 10mmol/L Tris–HCl (pH8.3), 2.5 mmol/L MgCl2, 100 lg/mL gelatine, 0.45% Tween20] and 100 lg/mL proteinase K. Samples were digested overnight at 60^0C
- 200ul of the lysed tissue was obtained into a 1.5ml micro centrifuge tube
- 350ul of lysis buffer was added to the sample tube, and then 3.5ul of Beta mercaptoethanol (14.2M) and mixed well by pulse-vortexing for 10~15seconds
- They were then incubated at room temperature for 10 minutes and centrifuged at 3000rpm for 5 seconds
- 150ul of isopropanol was added to lysate and mixed well by pulse-vortexing for 15seconds, tube was spun down briefly to get the drops clinging under the lid
- The lysate was carefully transferred into the upper reservoir of the spin column with 2ml collection tube without wetting the rim
- It was then centrifuged at 13,000rpm for 1minute, the flow through was poured off and assembled the spin column with the 2ml collection tube
- 500ul of wash 2 buffer was added to the spin columns with collection tubes and centrifuged at 13,000rpm for 1minute, the flow through was poured off and the spin columns assembled with the 2ml collection tubes.
- The spin column was then dried by additional centrifugation at 13,000rpm for 1minute to remove residual ethanol in spin columns
- The spin column was then transferred to the new 1.5ml micro-centrifuge tube, and waited for 1minute
- The viral nucleic acids were eluted by centrifugation at 13,000rpm for 1minute; purified DNAs were then stored at -20oC for nested PCR

Polymerase Chain Reaction Conditions (Nested)

Table 1: Amplification of the EBNA1 gene using nested PCR

Gene	Primer sequence	Expected amplicon size	Reference
EBNA DNA	1st set (EB3 5'-AAGGAGGGTGGTTTGGAAAG), (EB4 5'-AGACAATGGACTCCCTTAGC); 2nd set (EB1 5'-ATCGTGGTCAAGGAGGTTCC, EB2 5'-ACTCAATGGTGTAAGACGAC).	297bp for the first round, product of which were yield 209bp for the second PCR reaction	Cinque *et al*, 1993

The polymerase chain reaction (PCR) amplification of the Nucleic acid of EBV EBNA1 gene were conducted according to the method of Cinque *et al*, 1993.

Primer Reconstitution Stage

The primers were stated in Table 1 with each separate primer inserted in a green rubber tube after procurement from Inqaba Biotec West Africa Ltd Ibadan, surmounting to a total of 4 tubes. They were vortexed and 523.36ul, 600.5ul, 671.27ul and 509.96ul of PCR grade (Nuclease) water were added to EB3, EB4, EBV1 and EB2 and vortexed until homogenized respectively to activate the primers.

PCR reaction

Optimization step was carried out using annealing temperatures of 48ºC and 51ºC on the LMP1 positive sample as a good representative separately to know the better before commencing the test runs for characterization of the EBNA1 gene.

The 1st reaction mixture contained 2ul of each primer of EB3 and EB4, and a master mix of 200mol each of deoxyadenosine triphosphate, deoxyguanosine triphosphate, deoxycytidine triphosphate, and deoxyribosylthymine triphosphate in 1_amplification buffer (10 mmol Tris-HCl, Ph. 8.3; 50 mmol KCl; 1.5 mmol MgCl2; 0.01% w/v gelatin) and 2.5 U of Taq polymerase (PerkinElmer) in a total volume of 100_L. while the second reaction mixture contained everything but instead of the first set of primers it contained EB1 and EB2 using PCR products of the first reaction conditions as the test sample

1. First tube contained 10 ng of positive control DNA to the above mix
2. Other tubes contained test samples without the positive DNA control and the final tube has only the reaction mixture (hot start PCR master mix) and distilled water
3. The samples were treated for 5 minutes at 94°C for pre denaturing
4. They were then amplified by 35 cycles of PCR, each consisting of 30 seconds of denaturing at 94°C, 30 seconds of annealing at 51°C, and 30 seconds of extension at 72°C.
5. After treating for 10 minutes at 72°C, the DNA were concentrated by ethanol precipitation.
6. They were then electrophoresed on 1.8% agarose gel in TAE buffer (40 mmol Tris-HCl, pH 8.0; 5 mmol sodium acetate; 1 mmol EDTA)

Visualizations of EBNA1 genes Amplicon

- A total of 1.5g of QDLE Agarose were be dissolved in 100ml of distilled water and heated at 30seconds interval using the highest heat in the microwave until it is completely dissolves and 8ul of ethidium bromide added.
- It was then cooled down to a temperature of 65ºC
- 2 combs were then inserted properly into a cast and the cooled dissolved agarose poured while still molten and allowed to solidify to create wells where the PCR products were be carefully pipetted
- The cast was inserted into the electrophoretic tank containing buffer after removal of the combs
- Exactly 0.8µl of the PCR products of each sample were mixed with bromophenol blue stain included in the master mix and carefully pipetted into the wells and made to run for 30minutes for the amplicons to migrate alongside a ladder (DNA marker-1000MW)
- The positive amplicons were then finally optimized by repeating some reaction conditions like the annealing temperature to 60ºC from 45ºC to obtain a clearer band ready for sequencing

DTCS of EBNA1 gene

The dye terminator cycle sequencing was performed according to Big Dye Direct Cycle Sequencing Kit Protocol carried out at Inqaba, Benin State Nigeria as follows:

1. All reagents were kept on ice during the sequencing reactions;
2. the reaction was carried out in a 2.0 ml tube and reagents added according to the following order: 1. Deionised H2O 7 ul 2. DNA template 3 ul 3. Primers 2.0 ul 4. DTCS Quick start master mix 8.0 ul This gave a total of 20 ul.
3. The reaction was mixed and centrifuged briefly at 100 rpm.
4. The sequencing reaction was set in the PCR machine as below; Thermal cycling program: • 95ºC for 30 sec to denature the DNA • 51ºC for 30 sec to anneal the DNA strands with both the labelled and normal dNTPs • 72ºC for 5 min to elongate the DNA strands This was set for 35 cycles followed by holding at 4ºC Ethanol Precipitation
5. A labelled sterile 0.5 ml tube was prepared for each sample.
6. A fresh stop solution/glycogen mixture was prepared as follows per sequencing reaction: 2 ul of 3 M Sodium acetate, 2 ul of 100 mM Na2-EDTA and 1 ul of 20 mg/ml of glycogen (provided in the kit). To each of the labelled tubes; 5 ul of the stop solution/glycogen mixture was added.
7. This sequencing reaction was transferred to each of the appropriately labelled tube and mixed thoroughly.
8. 60ul cold 95% (v/v) ethanol from -20º freezer was added and mixed thoroughly and Immediately centrifuged at 14,000 rpm at 4ºC for 15 min. the supernatant was carefully removed with a micropipette. (the pellet was visible at these stage).
9. The pellet was rinsed with 200 ul 70% (v/v) ethanol from -20º freezer, centrifuged at 14,000 rpm at 4ºC for 2 minutes. The supernatant was then carefully removed with a micropipette.

10. It was then vacuum dried for 10 minutes 7. The sample was re suspended in 40 ul of the sample loading solution (provided in the kit)

These were immediately followed by bioinformatics analyses of the sequence obtained in the FASTA format for homology searches utilizing the NCBI website Blast tool.

Results

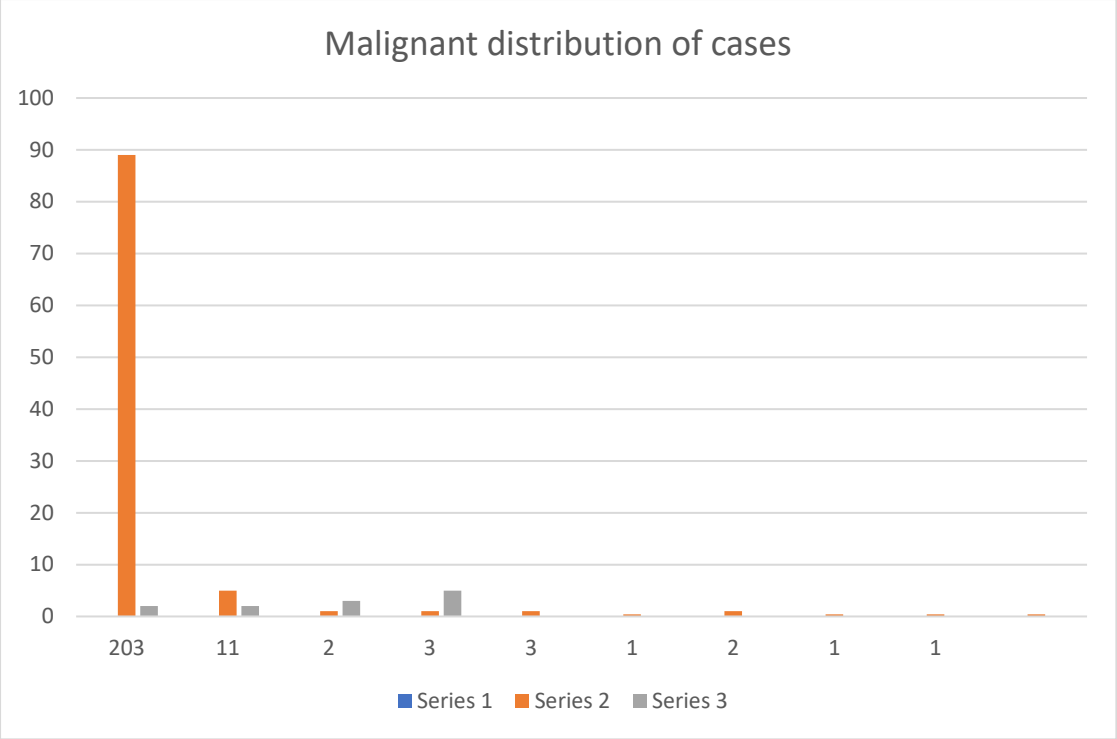
Symptoms Recorded in Study Participants

The summary of the clinico-pathological conditions recorded depending on the stage of malignancy and other factors in the patients include:

1. Painless lymph node in the cervical chain
2. Growth of the tumour which caused nasal obstruction, discharge and epistaxis
3. Invasion of the skull base and the 5th cranial nerve (trigeminal) to cause corneal reflex (neurokeratitis)-V1, loss of facial sensation-V2 and weakness of jaw muscles-V3
4. Weakness of the pterygoids which is the deviation of the jaw opening toward the side referred to as the ipsilateral weakness
5. 6th Cranial nerve (abducens) which is the loss of abduction and the eye moves medially to cause diplopia that is adduction
6. Cavernous sinus syndrome through metastasis to cause painless lymphadenopathy in some certain cases solely because it's a carcinoma and follows lymph unlike the sarcomas that follow blood. This involves thrombosis which abrupt onset unilateral periorbital oedema, headache, photophobia, orbital swelling also called proptosis that is bulging of the eye in layman's terms. Albeit, there is chemosis which is swelling of the conjunctiva
7. Neurological symptoms involving cranial nerve palsies of the III, IV, V1, V2 and VI causing 3rd nerve oculomotor leading patients to have ptosis, mydriasis, down and out movement (blown pupil).
8. The Last but not the list is the sensory loss of the periorbital, impaired corneal reflex that is neurokeratitis, loss of facial sensation, Cushing reflex to cause papilledema, retinal haemorrhage and of course spread of infection to even the contralateral cavernous sinus.

Table 1: Malignant Distribution of Lesions recorded over the 11-year period

Serial number	Diagnosis	Number of Cases	Percentage Distribution
1	Carcinoma	203	89%
2	Rhabdomyosarcoma	11	5%
3	Mature cystic teratoma	2	1%
4	Neuroblastoma	3	1%
5	Lymphoma	3	1%
6	Fibrosarcoma	1	0.4%
7	Bowen's disease	2	1%
8	High grade glioma	1	0.4%
9	Plasmacytoma	1	0.4%
10	Malignant endothelioma	1	0.4%
Grand total		228	100%

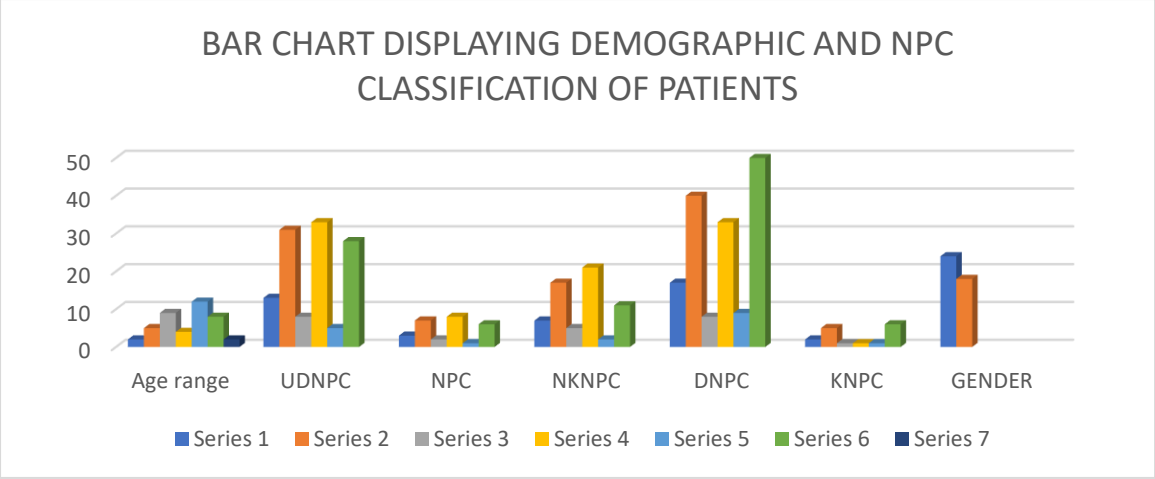


A total number of two hundred and twenty-eight, 228 malignancies were recorded over the eleven, 11-year period from 2013-2023 out of which two hundred and three, 203 (89%) were carcinomas, eleven, 11 (5%) were rhabdomyosarcoma, of the 228 cases, three,3 (1%) each were recorded for neuroblastoma and lymphoma as well two, 2 (1%) each of mature cystic teratomas and Bowen's disease and finally a case each of fibrosarcoma, high grade glioma, plastocytoma and malignant endothelioma.

Diagnosed NPC IN NECC FROM 2013 – 2023

Table 2: Showing Demographic Data of Patients and Diagnoses

	Age Range		**NPC Classification**		**Male**	**Female**
		Freq		Frequency	Freq	Freq
1	10-19	2(1%)	UDNPC	13(31%)	8 (33%)	5 (28%)
2	20-29	5(12%)	NPC	3(7%)	2 (8%)	1(6%)
3	30-39	9(21%)	NKNPC	7(17%)	5 (21%)	2(11%)
4	40-49	4(10%)	DNPC	17(40%)	8 (33%)	9(50%)
5	50-59	12(29%)	KNPC	2(5%)	1 (1%)	1(6%)
6	60-69	8(19%)	-	-	-	-
7	Above 70	2(1%)	Total	42(100%)	24(57%)	18(43%)



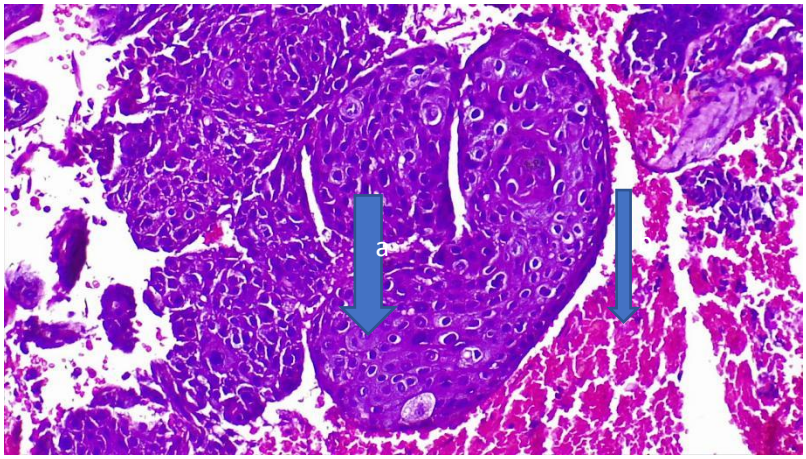
Key: DNPC- Differentiated Nasopharyngeal Carcinoma, **NPC**- Nasopharyngeal Carcinoma, **KNPC**- Keratinizing Nasopharyngeal Carcinoma, **NKNPC**- Non Keratinizing Nasopharyngeal Carcinoma, **UDNPC**- Undifferentiated Nasopharyngeal Carcinoma.

There are a total of forty-two (42) samples recorded in National Ear Care Centre from the year 2013-2023, of the total, twenty (24) were males while thirteen (18) were females making up 57% and 43% respectively.

Of the 203 (89%) carcinomas recorded, forty-two, 42 (18%) of the reported carcinomas were nasopharyngeal carcinoma with five, 5 different WHO classification seen. Two,2 (1%) belonged to the age range of 10-19, five, 5 (12%) patients belonged to the age range of twenty to twenty nine (20-29) while nine,9 (21%) belonged to the age range of thirty to thirty nine (30-39), there are a total of four,4 (10%) in the age range of forty-forty nine (40-49), of the forty two (42) patients, only twelve,12 (29%) were in the age range of fifty to fifty-nine (50-59) and mode among the age groups, while eight,8 (19%) belonged to the age range of sixty to sixty-nine (60-69) whereas only two,2 (1%) belonged to the age range seventy to seventy-nine (70-79).

Of the 42 patients diagnosed with NPC, thirteen,13 (31%) of them had UDNPC with eight,8 males and five,5 females recorded. seven,7 (17%) reported to have NKNPC making up 17% of the total with five,5 males and two,2 females. Seventeen,17 had DNPC which is the highest occurring surmounting to 40%, the mode had up to eight,8 males and only nine,9 females recorded while only three,3 (7%) were unclassified with NPC recorded as their findings making up 7% with two,2 males and one,1 female, KNPC had only two,2 (5%) patients recorded with a single male and female.

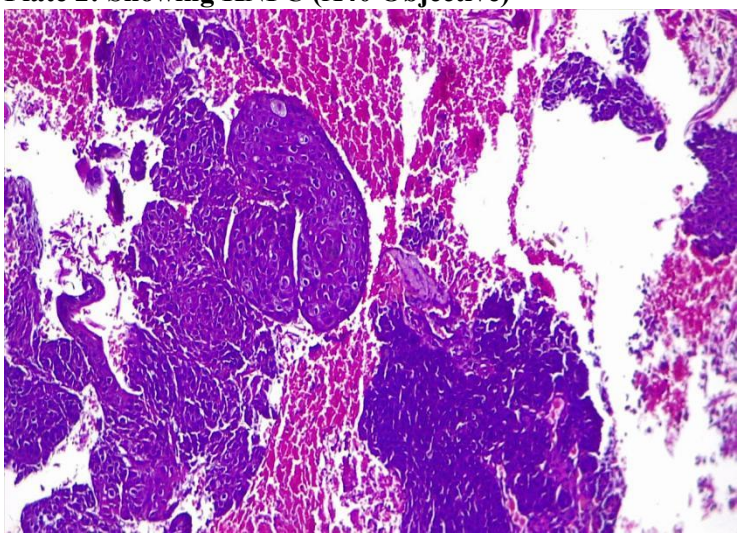
Some of the Photomicrographs of NPC Tumors
Plate 1: Showing KNPC (X100 Objective)



Microscopy: Section shows

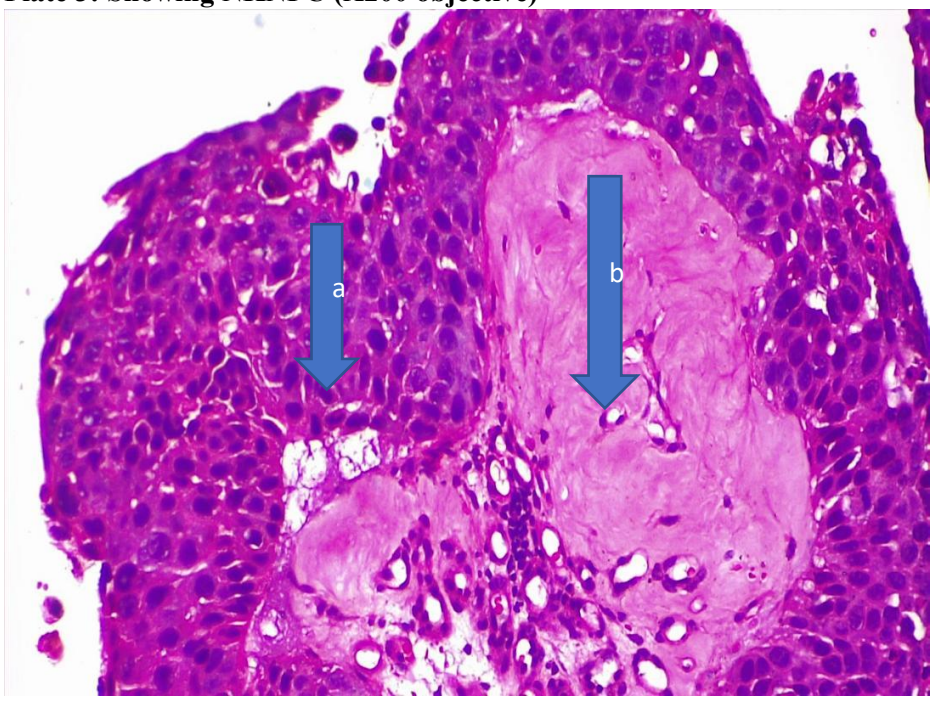
a) Cells disposed in small nests markedly pleomorphic, had large round nuclei, prominent nucleoli and moderate cytoplasm, they also showed individual cells dyskeratosis.
b) These were disposed within a background of extensive necrosis and inflammation typical of keratinizing nasopharyngeal carcinoma.

Plate 2: Showing KNPC (X40 Objective)



Microscopy: same as plate 1 with different magnification

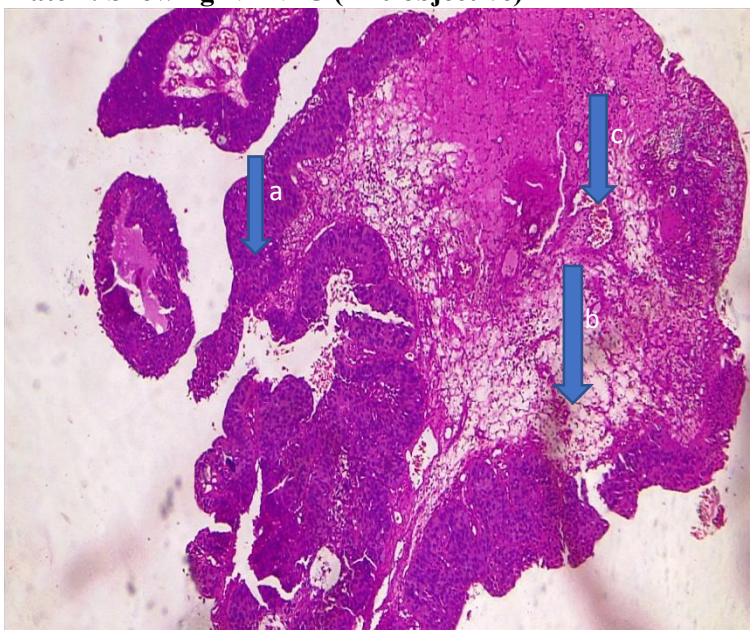
Plate 3: Showing NKNPC (X200 objective)



Microscopic findings: Section showed

a) Infiltrating nests and sheets of atypical squamous epithelial cells. The cells were markedly pleomorphic, having round to oval hyperchromatic nuclei, multiple nucleoli and scanty cytoplasm.
b) These were disposed within a scanty desmoplastic stroma with sprinkles of mononuclear cell infiltrates. These features were suggestive of Moderately Differentiated squamous cell carcinoma

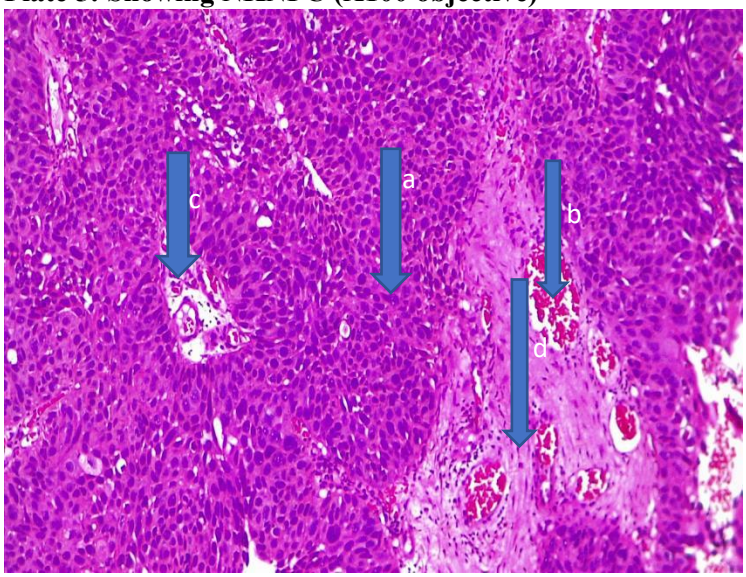
Plate 4: Showing NKNPC (X40 objective)



Photomicrograph showing

a) pseudostratified columnar epithelium. The stroma showed sheets of spindle shaped cells, round to oval cells having bland nuclei with scanty cytoplasm and
b) few medium sized vascular channels. Tumour was disposed as irregular nests and anastomosing trabeculae. The comprising cells have hyperchromatic to vesicular nuclei, prominent nucleoli and indistinct cytoplasm.
c) The stroma is desmoplastic with extensive lymphocytic infiltrates.

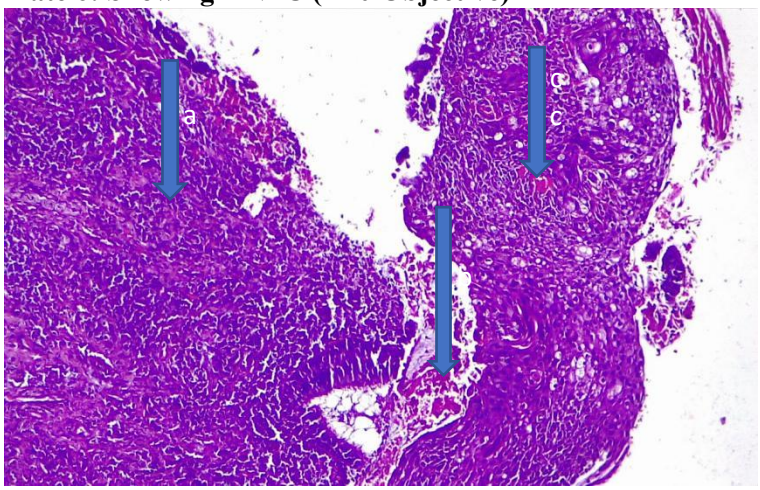
Plate 5: Showing NKNPC (X100 objective)



Photomicrograph

a) showing pseudostratified columnar epithelium.
b) The stroma showed sheets of spindle shaped cells, round to oval cells having bland nuclei with scanty cytoplasm and few medium sized vascular channels.
c) Tumour was disposed as irregular nests and anastomosing trabeculae. The comprising cells have hyperchromatic to vesicular nuclei, prominent nucleoli and indistinct cytoplasm.
d) The stroma is desmoplastic with extensive lymphocytic infiltrates.

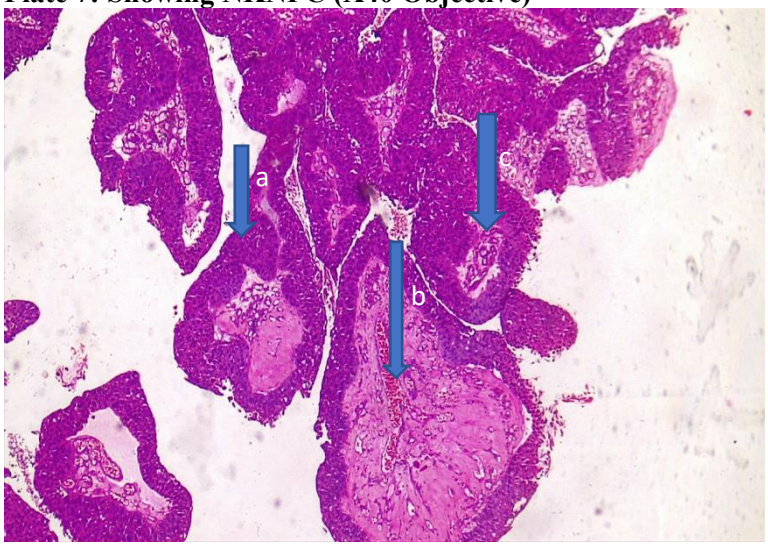
Plate 6: Showing KNPC (X40 Objective)



Microscopy: Section from this wholly embedded biopsy showed

a) Infiltrating nests and sheets of atypical squamous epithelial cells which were moderately pleomorphic with multiple prominent nucleoli, hyperchromasia and moderate to scanty amount of cytoplasm.
b) They showed individual cell dyskeratosis. Mitosis is brisk.
c) These were disposed within a desmoplastic stroma with mononuclear inflammatory cell infiltrates suggestive of squamous cell carcinoma.

Plate 7: Showing NKNPC (X40 Objective)



Photomicrograph showing

a) Pseudostratified columnar epithelium.
b) The stroma showed sheets of spindle shaped cells, round to oval cells having bland nuclei with scanty cytoplasm and few medium sized vascular channels. Tumour was disposed as irregular nests and anastomosing trabeculae.
c) The comprising cells have hyperchromatic to vesicular nuclei, prominent nucleoli and indistinct cytoplasm. The stroma is desmoplastic with extensive lymphocytic infiltrates.

Immunohistochemistry results of the positive EBV (LMP1) blocks
Plates 1-5: Showing positive Latent membrane protein (LMP1)

Plate 1a: Magnification x100

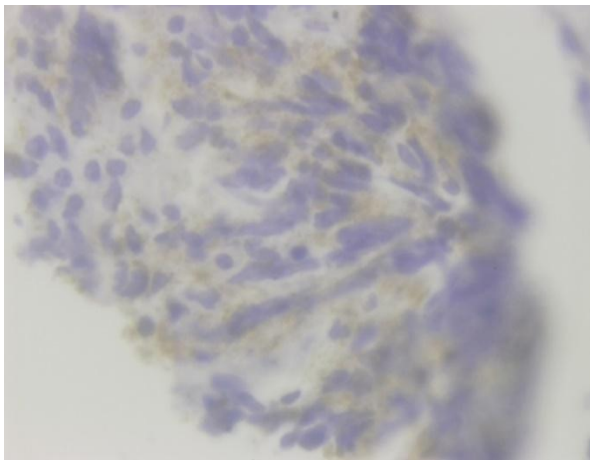


Plate 1b: Magnification x40

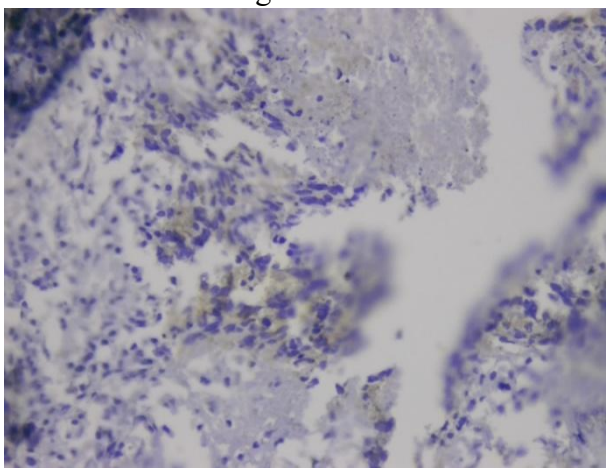


Plate 2a: Magnification x40

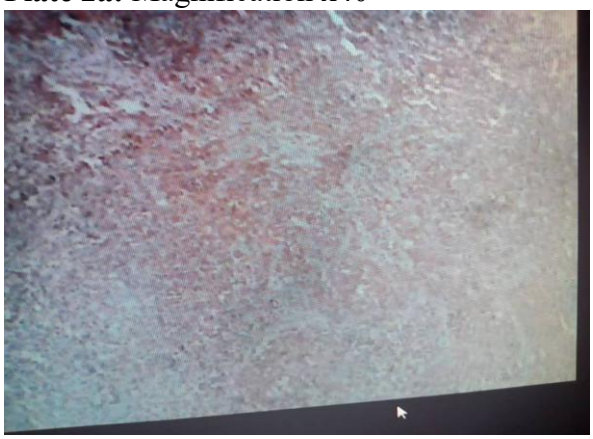


Plate 2b: Magnification x100

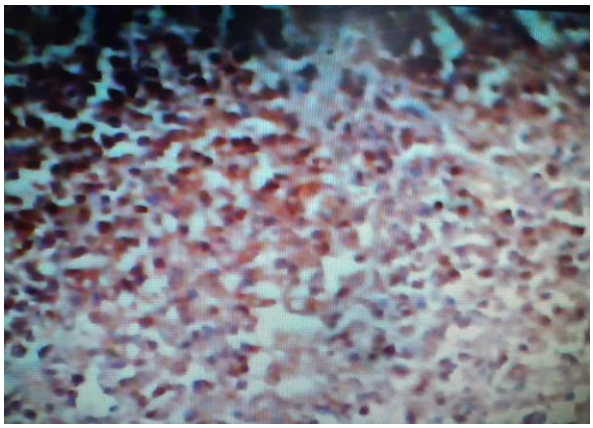


Plate 3: Magnification x100

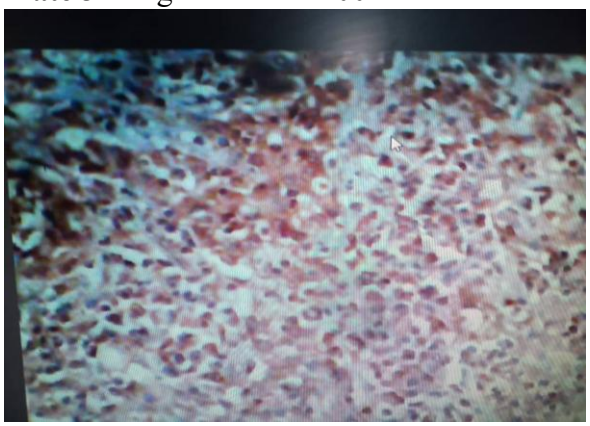


Plate 4: Magnification x100

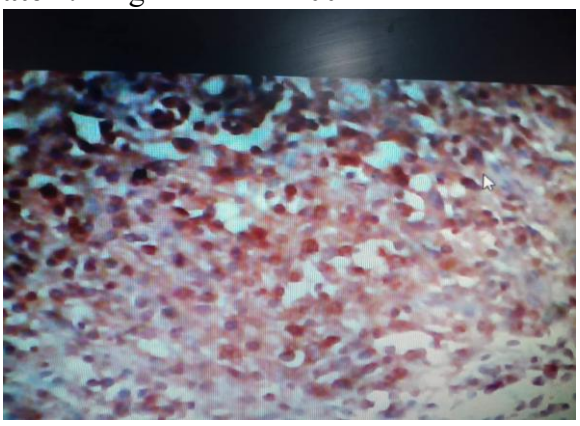
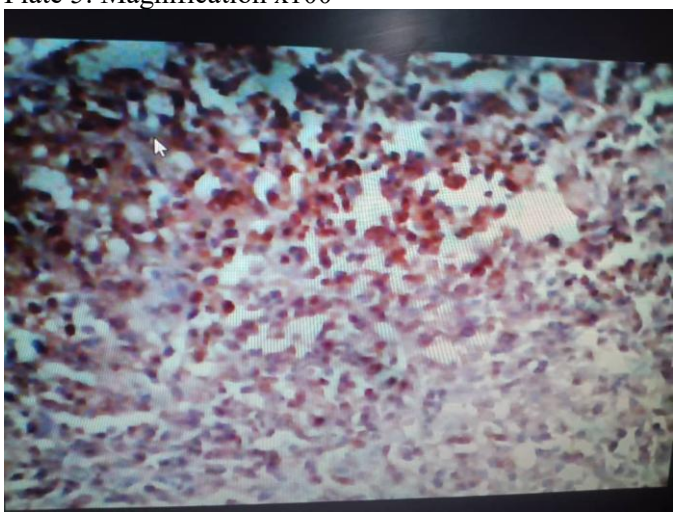


Plate 5: Magnification x100



Photomicrographs displaying expression of LMP1 in NPC biopsies against a Hematoxylin stained background. Almost all carcinoma cells exhibit nuclear positivity assessed with a remarkable staining intensity.

Immunohistochemistry Results:

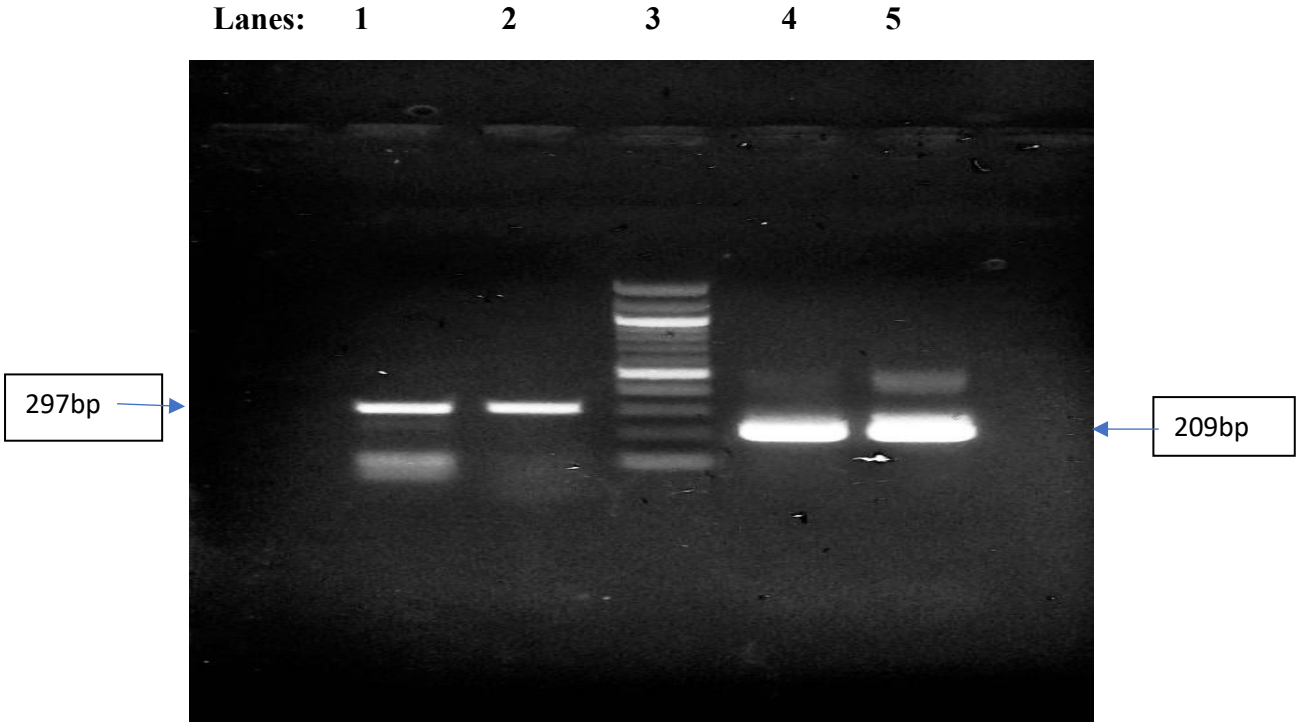
Frequency of EBV (LMP1) in Nasopharyngeal Carcinoma in the Histopathological samples

Histopathological type	**Frequency**	**Age**	**frequency**	**gender**	**Frequency**
NDNPC	5 (17%)	20-29	5 (17%)	Male	25 (86%)
DNPC	18 (62%)	30-39	6 (21%)	Female	4 (14%)
NKNPC	6 (21%)	50 and above	18 (62%)	-	-
Total	**29**		**29**		**29**

Twenty-nine,29 (69%) out of the forty-two (42) samples taken were positive for EBV (LMP1) protein. Of the 29 positive samples, Five,5 (17%) were positive for the Non Differentiated Nasopharyngeal Carcinoma, Eighteen,18 (62%) were of the differentiated NPC and remaining six,6 (21%) were Non Keratinizing NPC. Of the 29 samples, Five,5 (17%) and six,6 (21%) were within the age ranges of 20-29 and 30-39 respective whereas most of the positive samples were males above the age range of 50 and above. 86% of the positive EBV LMP1 were males and only 14% were females.

NB: A good representative from the 29 specimens positive for LMP1 were utilized for amplification and characterization of EBNA1 gene.

Plate 1: **Showing positive amplicons of EBNA1 gene**



Lane 1: positive amplicon at 48°C, **lane 2:** positive amplicon at 51°C for the first reaction conditions using DNA extracted from the tissue block. **Lane 3:** Molecular weight ladder, **lane 4:** positive amplicon at 48°C, **lane 5:** positive amplicon at 51°C using PCR product from the first reaction conditions utilizing nested PCR.

Sequencing Results of EBNA1 gene in Fasta Format

CGTTGCAGAAGGTTTAAGACTTCTCCTGGCTAGGTGTCACGTAGAAAGGACTACC
GAGGACGGAAATTGGGTCGCCGGTGTTCGTATATGGAGGTAGTAAGACCTCCCT
TTACAACCTCAGGCGAGGAATTGCCCTTGCTATTCCACAATGTCGTCTTACACCA
TTGAGTA

Blast Results

The sequence obtained from the positive PCR amplicon in the NPC tissue block matched MT648647.1:96337-96506 Human gamma herpesvirus 4 isolate Necker-6, partial genome and GU475442.1:117-286 Human herpesvirus 4 isolate T5-biopsy_V-leu-vii EBNA-1 protein gene, partial cds with 160/171 (94%) identity, a score of 244 bits (132), and E-value of 4e-60, 11/171(6%) gaps through a plus plus strand and the query sequence displayed in the fasta format as shown below downloaded from the NCBI website:

1. MT648647.1:96337-96506 Human gamma herpesvirus 4 isolate Necker-6, partial genome

TTGCAGAAGGTTTAAGACTTCTCCTGGCTAGGTGTCACGTAGAAAGGACTACCGA
GGACGGAAATTGGGTCGCCGGTGTG
TTCGTATATGGAGGTAGTAAGACCTCCCTTTACAACCTCAGGCGAGGAATTGCCC
TTGCTATTCCACAATGTCGTCTTAC
ACCATTGAGT

2. GU475442.1:117-286 Human herpesvirus 4 isolate T5-biopsy_V-leu-vii EBNA-1 protein gene, partial cds

TTGCAGAAGGTTTAAGACTTCTCCTGGCTAGGTGTCACGTAGAAAGGACTACCGA
GGACGGAAATTGGGTCGCCGGTGTG

TTCGTATATGGAGGTAGTAAGACCTCCCTTTACAACCTCAGGCGAGGAATTGCCC
TTGCTATTCCACAATGTCGTCTTAC
ACCATTGAGT

Query_7051583	MT648647.1	98.824	170	0	1	3	170	96337	96506	2.41e-77	302
Query_7051583	GU475442.1	98.824	170	0	1	3	170	117	286	2.41e-77	302

Human gamma herpesvirus 4 isolate Necker-6, partial genome
Sequence ID: MT648647.1Length: 160959Number of Matches: 1
Range 1: 96336 to 96506

Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
244 bits(132)	4e-60	160/171(94%)	11/171(6%)	Plus/Plus

Query 5 ATTGCAG-AGGTTT-AGACTTCTCCT-G-TA--
TGTCACGTAGAAAGGACTACC--GGAC 56

||||||| |||||| |||||||||| | || |||||||||||||||||| ||||

Sbjct 96336
ATTGCAGAAGGTTTAAGACTTCTCCTGGCTAGGTGTCACGTAGAAAGGACTACCG
AGGAC 96395

Query 57 GGAAATTGGGTCGCCGGTGTGTTC-TATATGGAGGTAGTAAGACCT--
CTTTACAACCTC 113

|||||||||||||||||||| |||||||||||||||||| ||||||||||

Sbjct 96396
GGAAATTGGGTCGCCGGTGTGTTCGTATATGGAGGTAGTAAGACCTCCCTTTACA
ACCTC 96455

Query 114
AGGCGAGGAATTGCCCTTGCTATTCCACAATGTCGTCTTACACCATTGAGT 164

|||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct 96456
AGGCGAGGAATTGCCCTTGCTATTCCACAATGTCGTCTTACACCATTGAGT 96506

Human herpesvirus 4 isolate T5-biopsy_V-leu-vii EBNA-1 protein gene, partial cds
Sequence ID: GU475442.1Length: 306Number of Matches: 1
Range 1: 116 to 286

Alignment statistics for match #2

Score	Expect	Identities	Gaps	Strand
244 bits(132)	4e-60	160/171(94%)	11/171(6%)	Plus/Plus

Query 5 ATTGCAG-AGGTTT-AGACTTCTCCT-G-TA--
TGTCACGTAGAAAGGACTACC--GGAC 56

||||||| |||||| |||||||||| | || |||||||||||||||||| ||||

Sbjct 116
ATTGCAGAAGGTTTAAGACTTCTCCTGGCTAGGTGTCACGTAGAAAGGACTACCG
AGGAC 175

Query 57 GGAAATTGGGTCGCCGGTGTGTTC-TATATGGAGGTAGTAAGACCT--CTTTACAACCTC 113

Sbjct 176 GGAAATTGGGTCGCCGGTGTGTTCGTATATGGAGGTAGTAAGACCTCCCTTTACAACCTC 235

Query 114 AGGCGAGGAATTGCCCTTGCTATTCCACAATGTCGTCTTACACCATTGAGT 164

Sbjct 236 AGGCGAGGAATTGCCCTTGCTATTCCACAATGTCGTCTTACACCATTGAGT 286

Discussion of Results

Except in endemic places, NPC is uncommon over the world (Ferlay *et al*., 2012; Torre *et al*., 2012). Albeit, it accounts for 21% of all malignancies seen at the Histopathology unit during the course of this study. This study population is considered to be in a low-risk region (Torre *et al*., 2012); however, some reports from different parts of Nigeria have shown that NPC is not so uncommon in our environment (daLilly *et al*., 2003, Ahmad and Pindiga; 2004, Iseh and Malami; 2006, Nwawolo *et al*., 2001, Onotai and Nwogbo; 2012, Nwaorgu and Ogunbiyi; 2004, Ologe *et al*., 2005, Iseh *et al*., 2009, Mandong *et al*., 2011). In comparison to the global report of 0.6% of all cancers, NPC accounted for 2% of all cancers recorded in Ibadan and Ilorin (Nwaorgu and Ogunbiyi; 2004 and Alabi *et al*., 2010).

There are a total of thirty-two (42) samples recorded in the National Ear Care Centre from the year 2013-2023, and patients were of different diversity and culture ranging from Hausa Fulani, Yoruba, Igbo, and quite a number from the southern part of the country, while a very similar study conducted for 22 years' retrospectively on all cases of NPCs diagnosed in the Department of Pathology, Ahmadu Bello University Teaching Hospital (ABUTH) Shika-Zaria, from January 1st, 1992 to December 31st, 2013 recorded a total number of 112patients (Yates *et al*., 2018). Consequently; another study included 47 NPC patients who were diagnosed at the Department of Otolaryngology, Head and Neck Surgery, Kanazawa University Hospital between 1997 and 2020. It is important to note that diagnoses of NPC were done histologically too. Of the total in our findings, twenty,24(57%) were males while eighteen, 18 (43%) were females where as ABUTH were 87 males (77.7%) and 25 females (22.3%) with a male-to-female ratio of 3.5:1. Consistent with reports in the literature of 2–3:1, irrespective of the incidence rate or geographical location (Parkin *et al*., 2015 and Bernard and Stewart; 2003). It is similar to most local reports, which are approximately 2:1 (daLilly *et al*., 2003, Nwaorgu and Ogunbiyi; 2004, Ologe *et al*., 2005, Mandong *et al*., 2011, Garandawa *et al*., 2009).

The ages varied from 6 to 91 years, while the NECC recorded ages ranging from 20 to 79 years. While ABU had an overall mean age of 42.4 years and a median of 40 years, NECC's range was 50-59 years. Females had an average age of 41.8 years (range 16-91 years), while males had an average age of 42.6 years (range 6-83 years). Similar to our findings, the 40-49year age group had the highest incidence, with 21 cases (18.8%). Unlike what was recorded in our results, the lowest Incidence was seen in the 0-9 years and 90-99years age groups, both of which had one case each (0.9%), our lowest incidence was reported in the age range of 70-79 years, and nothing was documented at ages less than 20 years in our findings.

This is consistent with findings from other sections of Nigeria, where the average age ranged from 41.1 to 44.4 years (de Lilly *et al*., 2003; Nwaorgu and Ogunbiyi, 2004). In Ilorin, the mean age was slightly higher at 48.7 years (Alabi *et al*., 2010). In patients from North America, Western Europe, and China, however, the mean age of incidence is about a decade higher (Parkin *et al*., 2015). The cause of this disparity is unknown, however it may be linked to Nigeria's lower life expectancy when compared to these countries (Paul; 2014). The average age of females is 41.8 years (age range 16-91 years), while

males average 42.6 years (age range 6-83 years). According to the literature, males and females have similar age distributions (Barnes, 2005).

There was a noteworthy increase in the number of patients contracting NPC. Nwaorgu and Ogunbiyi, who analyzed the trajectory of NPC in Ibadan over two decades (1981-2000), also noticed a consistent increase in incidence. Otoh *et al*. Similarly found an increase in the prevalence of NPC between the pre- and post-advent of HIV/AIDS in Nigeria in 1986 (Otoh *et al*., 2009). The explanation for this recent increase is unknown, however it could be attributed to increased public health awareness, improved diagnostic tools, the HIV/AIDS pandemic, or environmental, nutritional, and lifestyle changes. In Hong Kong, however, the incidence of NPC has steadily decreased since the 1970s, In Taiwan since the 1980s, and in Singapore Chinese since the late 1990s.

A tendency that might be attributed to the commencement of rapid economic development and the resulting change in lifestyle and environmental risk factors in these areas (Chang and Adami, 2006; Luo *et al*., 1997). The highest age of incidence in this study is 40-49 years (5^{th} decade), with 40 as the modal age. Similar findings have been reported in Maiduguri and Jos (Garandawa *et al*., 2009), while Ife, Sokoto, and Ilorin have seen a peak age of incidence in the fourth decade (Iseh and Malami, 2006, Ologe *et al*., 2005, Amusa *et al*., 2004, Fatusi *et al*., 2006, and daLilly *et al*., 2009).

This unimodal age pattern contrasts with the universally bimodal age distribution observed in low-risk groups, regardless of gender or geographic location (Bray *et al*., 2008). In Nigeria, such bimodalities have been documented in research conducted in Ibadan and Lagos. Kitcher *et al*. (2004) observed a bimodal age distribution in Ghana. On the contrary, recent thorough assessments have found that NPC incidence increases monotonically with age in the majority of low-risk groups (Chang and Adami, 2006). This is more consistent with the findings of this study and the majority of previous Nigerian investigations, which do not show bimodality.

Despite the relatively low frequency of NPC in our study, the majority of patients were between the ages of 20 and 69, which corresponds to their productive years and has far-reaching repercussions for their socioeconomic output (Skirbekk, 2003). The lowest age at presentation in this study is 6 years, which is similar to what was reported in Maiduguri and Ibadan (Nwaorgu and Ogunbiyi; 2004 and Garandawa *et al*., 2009), but higher statistics were seen in Sokoto and Ilorin (Iseh *et al*., 2009 and Alabi *et al*., 2010). In Ghana, Kitcher *et al*. Observed a primarily young age of presentation, with 60.4% of the sample population falling below 40 years, and the highest age of incidence being 10-19 years (Kitcher *et al*., 2004).

Some investigations in Tunisia, Kenya, Uganda, and Sudan (intermediate risk regions) found that NPC was widespread among children and adolescents (0-19 years), albeit the explanation for this is unknown. This contrasts with the findings of this study, which found that 1% of participants were between the ages of 0 and 19.

Unlike the findings in ABUTH, DNPC was the most prevalent histological type, accounting for 40% of cases. In terms of gender, there were 82 males (77.4%) and 24 females (22.6%) with NKC where as NECC recorded 24 males and 18 males with each gender having prevalent frequency stated in table 1. Shika receives more referrals than NECC due to the hospital's age and popularity, but patients are now increasing significantly as a result of increased awareness and various campaigns. This is consistent with reports from across Nigeria and around the world, regardless of age, gender, or geographical location (Barnes; 2005, daLilly *et al*., 2003, Iseh and Malami; 2006, Kitcher *et al*., 2004, Nwaorgu and Ogunbiyi; 2004, Alabi *et al*., 2010, Bray *et al*., 2008, Gacani *et al*., 2001, and Wei *et al*., 2011). The proportion of NKC varies with population, with incidences of 95% and 75% in high- and low-risk locations, respectively (Wei *et al*., 2011). The high frequency seen in studies (94.6%) from ABU, Shika, it is paradoxically closer to that of high-risk locations than low-risk ones, as expected. Further sub-classification of NKC revealed that the undifferentiated subtype (previously WHO Type III) predominates (79.4%) over the Differentiated subtype (formerly WHO Type II), with a minor fraction having both categories. This is in keeping with current literature (Barnes; 2005, daLilly *et al*., 2003, Iseh and Malami; 2006, Kitcher *et al*., 2004, Nwaorgu and Ogunbiyi; 2004, Alabi *et al*., 2010, Bray *et*

al., 2008, Gacani *et al*., 2001 and Wei *et al*., 2011). However, distinguishing between differentiated and undifferentiated categories has no therapeutic or prognostic importance (Barnes, 2005).

NKNPC is the second most common histological type observed (17%) recorded in our findings, which is slightly different from consistency with global reports (Kitcher *et al*., 2004; Wei *et al*., 2011) and KNPC was the list occuring. Chang and Adami (2006), on the other hand, found that KSCC was the most common histological type in low-risk locations. Incidence varies by region, with high-risk areas having the lowest incidence (<2%), intermediate-risk areas having 5%-10%, and low-risk areas having 25% (Wei *et al*., 2011). KSCC has an unexpectedly low value (4.5%) for a low-risk location; such values are expected in intermediate-risk zones. Low numbers have also been observed in Ghana (5.8%) (Kitcher *et al*., 2004) and Portugal (6.3%) (Breda *et al*., 2007), both of which are considered low-risk areas. The reported 20% in Ilorin is consistent with the expectedly high frequency of KSCC in low-risk settings (Alabi *et al*., 2010). Furthermore, a research in Sudan, an intermediate risk area, found no KSCC (Abuidris *et al*. 2008). A solitary instance of BSCC was observed (0.9%), confirming the rarity previously recorded in the literature (Barnes, 2005). NPC is notoriously malignant, with substantial local and regional infiltration, early lymphatic spread, and a high rate of hematogenous migration to the bone, lung, liver, and distant nodes (Juan, 2010). This is exacerbated by the difficulty of reaching the nasopharynx and the presence of ambiguous symptoms; as a result, most patients come in advanced stages: 50%-65% with locally advanced disease and 5%-8% with disseminated disease (Kitcher *et al*., 2004, Iseh *et al*., 2009, and Muchiri, 2008). Although the majority of the specimens in this study were from the nasopharynx (57.1%), implying that they may be limited to the nasopharynx, the remaining specimens (42.9%) were found outside the nasopharynx, indicating advanced disease. Although the clinicopathological symptoms were summarized in this study, ABUTH recorded cervical lymph nodes in only 11.6% of the specimens, which is a relatively low figure when compared to the existing literature, which reports cervical lymph node enlargement as the most common presenting symptom accounting for 72.5%-96.7% of cases (Barnes; 2005, Iseh *et al*., 2009, Garandawa *et al*., 2009, Alabi *et al*., 2010, Wei *et al*., 2011, and Muchiri; 2008).

However, because this was a histological study rather than a comprehensive clinicopathological survey, this may not accurately reflect the patients' clinical presentation, and the site of biopsy does not exclude out cervical lymph node enlargement. The elevated frequency of advanced disease could be attributable to late presentation.

The 29 positive samples exhibited a positive reaction against a stained background, this is in consonance with the expression of BALF2 which was immunohistochemically examined in NPC biopsy specimens at the first medical examination. BALF2 expression was independently evaluated by two investigators (SK and HD) without information on the clinical data of the patients. According to our positive immunohistochemistry results, LMP-1 encodes the primary viral membrane protein and is transcriptionally downregulated by numerous viral microRNAs binding to its 3′-UTR region (Ye *et al*., 2023)

This research showed high prevalence and is in consistency with the known association between Epstein-Barr Virus (EBV) and nasopharyngeal carcinoma (NPC). (Wang et al., 2023).

Of the 29 positive samples: 5 (17%) were Non-Differentiated NPC, 18 (62%) were Differentiated NPC, and 6 (21%) were Non-Keratinizing NPC, nothwithstanding, no positive result was recorded in the keratinizing subtype. This distribution aligns with current understanding, as EBV is typically associated with Non-Keratinizing and Differentiated types of NPC, with Non-Differentiated NPC being less common but still significantly related to EBV infection (Li et al., 2023). Among the positive samples, 5 (17%) were in the 20-29 age range, and 6 (21%) were in the 30-39 age range. The majority of the EBV-positive samples were from individuals aged 50 and above. NPC has a bimodal age distribution, as discussed earlier with a higher incidence in young adults and a second peak in older adults. The predominance of cases in older age groups observed in your results aligns with the global trend, where NPC is more common in middle-aged and elderly males (Sharma et al., 2023). Eighty-six, 86% of the EBV LMP1-positive samples were from males, and only 14% were from females. This gender disparity is consistent with existing literature, which shows a higher prevalence of NPC in males compared to females. The male-to-female ratio varies by region but often shows a marked male predominance (Chua

et al., 2023). While the other 13 cases could be as a result of other factors like the nasopharyngeal carcinoma-associated gene 6 (NGX6) which if downregulated leads to the malignant condition and has also been reported to cause lung, gastric, liver and colorectal carcinomas and to a lesser extent those involved in metastasis. This is because it functions in protein regulation expression of related genes, involved in cell signal transduction pathways, negatively controlling cell cycle progression among others and as such impairment or mutations in the gene lead to the propagation of NPC (He *et al*., 2016).

This study recovered one positive amplicon of EBV EBNA1 gene using nested PCR. The output of the first PCR reaction was utilized as a template to conduct the second PCR reaction using a distinct set of primers, and bands appeared at 209bp and 297bp, respectively. As a result, EBV genotyping by Kotia and colleagues utilizing the EBNA-2 gene revealed 250 bp and 300bp segments, which correspond to genotypes 1 and 2, respectively. (Kotia *et al*., 2013).

Similar to what Kotia and associates did, the genotypes of EBV in both patients and controls were discovered using nested PCR with particular primers as previously reported, with minor changes to cycling conditions. They also used first round of the PCR involved amplifying a shared area of EBNA-2 using EBNA-2F and EBNA-2I as sense and antisense primers, respectively.

The sequencing of the positive PCR amplicon in the NPC tissue block matched MT648647.1:96337-96506. Human gamma herpesvirus 4 isolate Necker-6, partial genome, and GU475442.1:117-286 Human herpesvirus 4 isolate T5-biopsy_V-leu-vii EBNA-1 protein gene, partial cds with 160/171 (94%) identity, a similar study analyzed 178 EBV isolates of other EBV-associated diseases from Japan (European Genome-phenome Archive, accession no. EGAD00001004298), as well as 110 EBV genomes from NPC in Southern China/Hong Kong (randomly chosen from a total of 217 publicly available data in the NCBI SRA database, accessed on November 20, 2020).

EBV DNA was detected by EBNA-1 amplification from a female, Kotia and colleagues also detected the EBV DNA both in the control group and test group to be 53% (29/55) and 70% (37/53), respectively. They postulated EBV DNA positivity signifies EBV infection history and suggests that females were twice as likely to have EBV infection than males (Kotia *et al*., 2013). This is very much different from what was obtained in our study as males were slightly higher than females and at an advanced stage using IHC techniques of LMP1 detection just as reported in Japan (Maeda *et al.,* 2009).

While the Japanese classification used hierarchical clustering for EBV type ½, it matched the classification using SNVs in EBNA-2, EBNA-3A, EBNA-3B, and EBNA-3C. BALF2 subtype classification was solely based on the variant call. Following these classifications, EBV genomes were classified as type 2 (independent of the BALF2 subtype), the H-H-H subtype (in type 1 EBV genomes), the H-H-L subtype (in type 1 EBV genomes), or the other subtype (H-L-L and L-L-L subtypes in type 1 EBV genomes).

Consequently; Sequenced 47 EBV genomes recovered from Japanese NPC patients were compared to those from other Japanese EBV (n = 178) and endemic NPC genomes (n = 110) (Kondo *et al*., 2022). The SNVs were similar. The EBV genotypes in both patients and controls were determined using nested PCR with specific primers, as previously reported, with minor adjustments to cycling conditions. The first round of PCR included amplifying a common region of EBNA-2 with EBNA-2F and EBNA-2I as sense and antisense primers, respectively. Japanese NPC EBV genomes and other Japanese EBV genomes in terms of effects on amino acids and nucleotide alteration patterns, and synonymous variants were more frequent than expected from random simulation (dN/dS = 0.34 for NPC and 0.38 for other disorders), implying that the majority of the Variants were the result of highly conservative evolution (Kondo *et al*., 2022). Nucleotide alteration patterns were biased toward transitions (cytosine-to-thymine and adenine-to-guanine alterations) (Kondo *et al*., 2022).

Conclusion and Recommendations

In conclusion, the overall prevalence of NPC cases among patients attending NECC Kaduna have been stipulated successfully and the potential associations between specific EBV genetic profiles and the development or progression of NPC achieved. Valuable insights into the epidemiology and genetic aspects of NPC in the region, aiding in the development of targeted prevention and treatment strategies

known. Ad-infinitum, the histopathological features of NPC cases, providing detailed insights into the disease morphology, presentation and molecular analyses of the EBV EBNA1 gene in NPC patients, identifying specific viral genetic markers associated with the disease has been achieved. This study found that NPC is a subset of carcinomas diagnosed in NECC that has increased in recent years, has a slightly larger male preponderance, a peak age of incidence in the fifth decade, and a bimodal age distribution. It has also shown that DNPC predominates over NKPC and UDNPC in the referred patients. Prevalence of LMP1 has also been stipulated using immunohistochemistry. As a result, the findings of this study share numerous parallels and some differences with those of other studies conducted throughout the country, Africa, and around the world.

It is therefore suggested to screen for EBV. In all patients suffering from the aforementioned symptoms, particularly the earliest onsets, as a biomarker for the development of NPC, a search of the literature finds an insufficient number of local research on NPC. More local investigations, including multicenter surveys and, most importantly, population-based studies, are required. These investigations should also be conducted frequently to document any changes that may be noted, and because not all samples were positive for EBV LMP1, more researches are recommended to know and understand other causes of the condition.

Conflict of Interest

All authors declare no conflict of interest.

Acknowledgement

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