



(RESEARCH ARTICLE)



ANALYSIS OF METHODOLOGICAL CHALLENGES IN DESIGNING RESEARCH PROTOCOLS FOR EPIGENETIC DIAGNOSTIC STUDIES OF OROFACIAL CANCER IN NIGERIA

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World Journal of Advanced Research and Reviews, 2026, 32(01), 1200–1214

Publication history: Received on 09 September 2026; revised on 15 October 2026; accepted on 17 October 2026

Article DOI: <https://doi.org/10.30574/wjarr.2026.32.1.2634>

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ABSTRACT

Cancer remains a disease burden with high morbidity and mortality rates worldwide, particularly in low- and middle-income countries. Orofacial cancer including oral squamous cell carcinoma (OSCC) makes up a large proportion of head and neck cancers which are often diagnosed late, are aggressive with high recurrence rate and cost of treatment and poor survival rate. Although clinical examination, histopathology, imaging, cytology, and immunohistochemistry remain conventional methods for cancer diagnosis, recent advances in molecular oncology have provided researchers a new interest in epigenetic biomarkers for early detection of cancer, risk assessment, prognosis and individualized therapy. Epigenetic alterations such as DNA methylation, histone modification, chromatin remodeling and deregulation of non-coding RNAs have been shown to play key roles in oral cancer development. Development of robust study designs for epigenetic diagnostic research on orofacial cancer remains limited by ethical, methodological, infrastructural, economic, regulatory and bioinformatics challenges in Nigeria. This review aims to look at the methodological considerations for epigenetic diagnostic research and explore challenges affecting study protocols in Nigeria. This review analysed the methodological framework necessary for epigenetic diagnostic studies of orofacial cancer and assessed the challenges impacting protocol development in Nigeria, utilising publicly accessible literature from sources including PubMed, Google Scholar, institutional reports, policy documents, and other pertinent scholarly materials. Suggestions on how to improve study quality, reproducibility, local relevance and translational research were also highlighted. Strategies to improve epigenetic diagnostic research in Nigeria may include the development of improved ethical review guidelines, investment in molecular diagnostic laboratory infrastructure, establishment of biospecimen collection and storage standard operating procedures, bioinformatics development, conduct of local validation studies and determination of sustainable funding avenues.

Keywords: Epigenetics; Orofacial Cancer; Oral Squamous Cell Carcinoma; DNA Methylation; Diagnostic Biomarkers; Nigeria; Molecular Diagnostics; Bioinformatics; Research Protocol.

1 INTRODUCTION

Despite advancements in prevention and treatment strategies, cancer continues to be one of the leading causes of morbidity and mortality worldwide. Globally, cancer caused nearly 10 million deaths in 2022, accounting for nearly one in six deaths across the world [1]. The cancer burden continues to grow worldwide, especially in low- and middle-income countries where late-stage presentation due to lack of screening services and poor treatment infrastructure, combined with weak healthcare systems contribute to low survival rates [1,2]. Cancer burden is also increased by behavioral risk factors such as tobacco use, excessive alcohol consumption, unhealthy diet, physical inactivity, obesity, and carcinogenic infections such as human papillomavirus and hepatitis B and C virus infections [1].

Head and neck cancers are a diverse group of cancers of which oral cancers are especially prevalent. Oral squamous cell carcinoma accounts for nearly 90% of oral cancers and is associated with aggressive tumor biology, loss of function, facial disfigurement, increased recurrence, and poor quality of life [3]. There were approximately 377,713 new cases of oral squamous cell carcinoma reported worldwide in 2020 and it is expected that incidence will dramatically increase by 2040 and mortality will continue to rise. The American Cancer Society estimates that there will be approximately 60,480 new cases of oral cavity and oropharyngeal cancers and 13,150 deaths in the United States in 2026 [4]. The average age at diagnosis is 64 years old but over 20% of cases are diagnosed in patients younger than 55 years [4].

Risk factors associated with cancer continue to increase the burden of cancer in Nigeria. Increasing population, ageing population, environmental factors, lifestyle changes, tobacco consumption, alcohol consumption, infectious causes of cancer, poor awareness, and late presentation to healthcare facilities all continue to add to the cancer burden in Nigeria. Nigeria had 269,109 new cancer cases in 2022 with breast cancer being the most commonly diagnosed cancer followed by prostate cancer. Nigeria has a small number of cancer treatment facilities compared to the population creating a large gap in early detection, diagnosis, treatment, and survivorship care [6]. Orofacial cancers account for a large proportion of head and neck cancers diagnosed in Nigerian tertiary centers. Many patients present late to healthcare facilities when disease is at an advanced stage which makes treatment more difficult, expensive, and less effective [7].

Diagnostic modalities for cancer have traditionally relied on physical exam, histopathology, imaging studies, cytology, and immunohistochemistry. Histopathologic evaluation of cancers remains the gold standard for confirmation of malignancy and guiding treatment [8]. Advances in molecular diagnostics have allowed for detection of cancers using molecular characteristics beyond morphologic analysis including genomics, transcriptomics, proteomics, and liquid biopsies [9]. While these methods have advanced our understanding of cancer, mutations alone are insufficient to diagnose oral cancer. Promoter hypermethylation of tumour suppressor genes APC, CDH1, PTEN, p16, MGMT, and DAPK have been connected to gene silencing, unrestrained proliferation, and tumour progression [12,13]. Modified histone modification landscapes and aberrant microRNA expression signatures have also been linked to oral cancer advancement, metastasis, prognosis, changes in the immune microenvironment, and drug resistance [14,15]. Epigenetic modifications can occur early during carcinogenesis and be detected in tissue saliva, blood, plasma, oral rinse, and exfoliated oral cells and have therefore received attention as potential diagnostic and prognostic biomarkers [13,16].

Despite recent advances in epigenetics and precision oncology research worldwide, applications of epigenetic diagnosis in Nigeria remain limited. Nigeria has taken steps toward improving cancer diagnosis and treatment through cancer control plans, oncology centers, cancer registries, and public-private partnerships; however, epigenetic technologies have not been fully integrated into routine clinical practice and research efforts [6,17]. Several molecular studies lack continuity or remain unfinished due to inadequate funding, poor laboratory infrastructure, lack of trained workforce, limited sequencing capacity, poor bioinformatics capabilities, and the need to send samples to overseas laboratories for specialty testing [18].

Robust research protocols are necessary to ensure the generation of high-quality, reproducible, and clinically relevant results from epigenetic diagnostic studies. However, there are several methodological, ethical, financial, infrastructural, regulatory, and logistical challenges to protocol development in Nigeria. This article provides a critical review of the methodological challenges encountered when developing protocols for epigenetic diagnostic research studies on orofacial cancer in Nigeria and solutions, focusing on study design, ethical approval, recruitment of study participants, biospecimen collection, laboratory methods, bioinformatics analysis, data governance, funding limitations, and translational considerations for clinical implementation.

2 EPIGENETICS IN CANCER: BRIEF OVERVIEW

In 1942, Conrad Waddington coined the term epigenetics to explain how genes and their products interact to determine phenotype. Since then, the definition of epigenetics has evolved to include the study of heritable and reversible changes in gene activity that do not involve alterations to the underlying DNA sequence [10]. Epigenetic regulation is vital for normal cellular function; it controls when, where, and how much genes are turned on and off.

Epigenetic processes, including DNA methylation, post-translational modifications of histones, and expression of noncoding RNAs, regulate numerous physiological processes, such as cell differentiation, embryonic development, genomic imprinting, X chromosome inactivation, immune surveillance, DNA damage response, and tissue-specific gene expression [10]. While most cells within the body contain the same genetic information, epigenetic regulation allows for cells to express distinct gene programs, maintaining specialized function.

Aberrant epigenetic phenomena have been established as drivers of oncogenesis and cancer progression. Unlike genetic changes, epigenetic changes are reversible in theory; this makes them attractive candidates for use as diagnostics, prognostics, and therapeutics [10,11]. Cancer cells often exhibit epigenetic alterations, including promoter hypermethylation of tumour suppressor genes, global DNA hypomethylation, abnormal histone modifications, changes in chromatin structure, and dysregulation of noncoding RNAs [10–12]. These changes can lead to oncogene activation, silencing of tumour suppressor genes, chromosomal instability, evasion of apoptosis, promotion of angiogenesis, and increased invasion and metastasis [11,14].

Epigenetic alterations have been found to play crucial roles in oral and oropharyngeal cancers as they can occur early during malignant transformation. Therefore, epigenetic alterations serve as excellent biomarkers for early detection especially among those with oral potentially malignant disorders. Epigenetic biomarkers are another advantage as they can be measured in saliva, oral rinse, blood, plasma, serum, and even exfoliated oral cells; biofluids that can be sampled using non-invasive or minimally invasive methods [16,19]. This can be helpful in Nigeria where most patients present late to health facilities and lack access to these diagnostic modalities.

2.1 Major Epigenetic Mechanisms Relevant to Orofacial Cancer

2.1.1 DNA methylation

DNA methylation is the most studied epigenetic mechanism in cancer. DNA methylation refers to the addition of a methyl group to the cytosine at the fifth carbon position (C5 position). DNA methylation occurs predominantly at cytosine-phosphate-guanine (CPG) dinucleotides and is mediated by DNA methyltransferase enzymes. DNA methylation plays a role in regulating gene expression, genomic imprinting, X-chromosome inactivation, chromosomal stability, and silencing of repetitive elements [10,20].

In normal cells, CpG islands located in gene promoters are generally unmethylated allowing genes to be transcribed. Cancer cells demonstrate promoter hypermethylation, which can silence tumour suppressor genes and global hypomethylation which can activate oncogenes and cause chromosomal instability [10,20]. Hypermethylation of genes involved in cell-cycle control, apoptosis, DNA repair and cell adhesion has been observed in oral squamous cell carcinoma [12,13]. Therefore, DNA methylation markers are some of the most attractive candidates for epigenetic diagnostic studies in OFC.

2.1.2 Histone modifications

Histones are proteins around which DNA winds to form nucleosomes. Histones undergo numerous post-translational modifications including acetylation, methylation, phosphorylation, ubiquitination, and SUMOylation. Post-translational modifications lead to changes in chromatin structure and regulate gene availability [11,14]. Histone acetylation is generally associated with transcriptional activation whereas histone deacetylation is usually associated with chromatin condensation and transcriptional repression.

Altered histone modification patterns have been implicated in oral carcinogenesis by promoting cellular proliferation, inhibiting apoptosis, modulating immune response and increasing metastatic potential [14]. Histone modifications are reversible, which makes them relevant targets for epigenetic therapeutics.

2.1.3 Non-coding RNAs

Non-coding RNAs refer to functional RNA transcripts that regulate gene expression but do not code for proteins. These include microRNAs, long non-coding RNAs, and circular RNAs [15]. MicroRNAs are small RNA molecules that bind to messenger RNA and prevent translation or target it for destruction. MicroRNAs can function as oncogenes or tumour suppressors in cancer.

Aberrant expression of microRNAs has been implicated in OSCC growth, invasion, metastasis, prognosis, and treatment response. MicroRNAs dysregulated in OSCC include miR-21, miR-31, miR-155, and miR-375 among others [15]. MicroRNAs can be detected in tissue, saliva and blood and are being widely explored as diagnostic and prognostic biomarkers.

2.1.4 Chromatin remodelling

Chromatin remodelling refers to ATP-dependent changes in chromatin structure that result in changes to DNA accessibility to transcription factors and other proteins involved in gene regulation. Chromatin remodelling complexes such as SWI/sucrose nonfermentable (SNF), imitation protein silencer wild (ISWI), Chromodomain helicase DNA-binding (CHD), and INO80 shift or remodel nucleosomes to regulate transcriptional output [11,14]. Alterations in chromatin remodelling complex activity can lead to activation of oncogenes and silencing of tumour suppressor genes in head and neck cancers.

2.1.5 Histone variants and nucleosome positioning

Histone variants refer to the variant versions of normal histone proteins. Histone variants influence chromatin structure, transcriptional regulation, DNA damage response, and genomic stability. Dysregulation of expression or incorporation of histone variants has been shown to contribute to cellular transformation and cancer development [11]. The positioning of nucleosomes along the DNA influences DNA accessibility and transcriptional activity. Altered nucleosome occupancy and chromatin structure may provide additional diagnostic and prognostic information in cancer cells.

2.1.6 RNA epigenetics and epitranscriptomics

RNA epigenetics, also known as epitranscriptomics, refers to chemical modifications of RNA that influence RNA stability, processing, localization, and translation. N⁶-methyladenosine (m⁶A) is the most well-studied RNA modification that has been linked to cancer initiation, progression, metastasis, and drug resistance [14]. While still in its early stages, RNA modifications may provide novel biomarkers and therapeutic targets for oral cancer.

3 METHODOLOGY

3.1 Study design and scope

This paper provides a narrative literature analysis that explores the methodological difficulties encountered in formulating research protocols for epigenetic diagnostic investigations of orofacial cancer in Nigeria. To tackle the limitations of establishing epigenetic diagnostic protocols in Nigeria, a narrative review was constructed that considers the difficulties encountered by researchers in analyzing study outcomes. The assessment addresses ethical quandaries alongside practical issues, such as infrastructure, technology, and legislation, prevalent in low- and middle-income nations throughout Africa and worldwide. It additionally examines methodological challenges faced in research undertaken in this nation, as well as those experienced in other locations.

3.2 Information sources and search strategy

This involved thoroughly examining existing literature using publicly available databases such as PubMed and Google Scholar; reviewing institutional reports, policy documents along with other academic sources. The method of searching was based on different combinations of keywords, such as "epigenetics," "orofacial cancer," "oral squamous cell carcinoma," "DNA methylation," "diagnostic biomarkers," "Nigeria, molecular diagnostics, bioinformatics and biometrics. Such an approach ensures meticulous analysis of the subject matter while acknowledging that the assessment mirrors actual knowledge as formulated by experts and may not always include recent data.

3.3 Selection criteria and data synthesis

Literature was deemed relevant if it addressed: epigenetic mechanisms and biomarkers associated with orofacial or oral cancer; methodological considerations for executing epigenetic investigations; cancer diagnostic and research institutions in Nigeria or other low- and middle-income nations; ethical, regulatory, or infrastructural challenges in molecular research throughout Africa; protocols for the collection, preservation, and management of biospecimens; bioinformatics and data analysis in epigenetic research; or the utilisation of molecular diagnostics in clinical environments and research application. Literature was excluded if it failed to address research methodologies, was not composed in English, or lacked relevance to the research context in Nigeria. Data from the selected literature were meticulously organised into principal thematic categories: epigenetic mechanisms associated with orofacial cancer; the present state of cancer diagnosis and the significance of epigenetics in Nigeria; the methodology employed for conducting epigenetic diagnostic investigations; and the specific challenges and barriers in developing such protocols. The obstacles were categorised into seven primary domains: acquiring ethical approval and encountering regulatory

holdups, managing costly analyses and the necessity for outsourcing, lacking adequate research and laboratory resources, challenges in participant recruitment and sample collection, a shortage of skilled personnel, hindrances in bioinformatics and data interpretation, restricted access to population-specific reference

4 RESULT AND DISCUSSION

4.1 Government Efforts in Cancer Diagnosis and the State of Epigenetic Diagnostic Capacity in Nigeria

The Nigerian government has made efforts to improve cancer diagnosis through policy-making and establishment of institutions to scale up cancer diagnosis using the national cancer control plan, creating coordinating agencies, public-private partnerships and investing in diagnostic facilities. Improved access to quality, affordable and equitable cancer diagnostic services is one of the goals outlined by the National Strategic Cancer Control Plan 2023–2027 [6]. This policy statement is strategic as late presentation is one of the major factors responsible for poor cancer outcomes in Nigeria.

The establishment of coordinating agencies such as the National Institute for Cancer Research and Treatment will help coordinate cancer research/diagnostics/treatment activities, surveillance, and national cancer control efforts. Standardization of diagnostic protocols, cancer registries, optimization of referral systems, and research into molecular oncology can be achieved through this partnership. Reliable cancer registries allow for determination of cancer burden, establishment of appropriate study populations, development of sampling techniques, and analysis of diagnostic studies [21].

The Nigeria Sovereign Investment Authority invested in diagnostic facilities as part of efforts to improve cancer care. NSIA–Lagos University Teaching Hospital Cancer Treatment Centre was founded in 2019. The Center has seen over 10,064 patients since inception and has administered thousands of sessions of radiotherapy and chemotherapy treatments improving cancer care capacity. These facilities will play key roles in future studies as epigenetic biomarkers can be validated on the patient populations seen at the centers and access to tissue samples, pathology services, expert clinicians, imaging facilities and archived clinical notes will be made available to researchers.

Notwithstanding the aforementioned initiatives, sophisticated diagnostic services are predominantly situated in urban tertiary hospitals, resulting in a lack of access to cancer screening, biopsy, imaging, pathology, and molecular diagnostics for patients in rural and underserved regions. Government-initiated national screening programs have primarily concentrated on breast, cervical, and prostate cancers, whereas the screening of oral cavity and orofacial cancers remains inadequately integrated into the national cancer control strategies of most nations. Insufficient oral cancer screening techniques could significantly hinder the detection of early-stage patients, premalignant conditions, and suitable control subjects for epigenetic diagnostic research.

Epigenetic diagnostics research is in its infancy in Nigeria. Diagnosis of cancer in most Nigerian health facilities is mainly done by clinical evaluation, radiologic/imaging studies, cytology, histopathology, and immunohistochemistry. While these diagnostic tests are needed, they do not provide molecular-level information required for epigenetic diagnostic studies such as DNA methylation analysis, histone modification analysis, chromatin accessibility assays or ncRNA profiling. Cancer research in Nigeria faces major challenges including lack of funding, lack of infrastructure, lack of oncology research capacity, lack of access to modern diagnostic modalities, and lack of trained workforce.

Epigenetic diagnostic research requires standardized biospecimen collection techniques, nucleic acid stabilization methods, biobanking facilities, molecular laboratories with quality control measures, bioinformatics support, trained personnel, and reliable clinical information. These facilities are currently lacking in Nigeria. Therefore, even though Nigeria has made tremendous progress in cancer care, epigenetic diagnostics remain in its early stages. This directly impacts on how research plans are laid out for epigenetic diagnostic studies involving orofacial cancer.

4.2 Methodological Approach to Epigenetic Diagnostic Studies of Orofacial Cancer in Nigeria

Epigenetic diagnostic studies including human participants, clinical assessments, biospecimen collection, molecular examination, bioinformatics evaluation, diagnostic review, and data management require a careful methodological approach. All research involving human participants must comply with national and institutional ethical guidelines for health studies before enlisting individuals and collecting biospecimens. This section presents a structured approach to coordinating epigenetic diagnostic studies involving human participants in Nigeria.

4.2.1 Writing the study protocol

The initial step involves writing out a detailed study plan also known as a study protocol. The protocol should include background information, statement of problem, study aims, study questions, study design, study population, sample size, inclusion and exclusion criteria, recruitment plan, types of biospecimens to be collected, molecular laboratory

techniques to be employed, data analysis plan, ethical considerations and expected diagnostic outcomes. The protocol must delineate whether the research intends to identify novel biomarkers or to authenticate previously identified biomarkers. The study must clarify whether it will be contrasting the epigenetic fingerprints of cancer patients with those of controls or whether it intends to create a diagnostic prediction model [20].

4.2.2 Approval by a health research ethics committee or institutional review board

The next step is to obtain authorisation from a recognised Health Research Ethics Committee or Institutional Review Board (IREB). Any research involving human subjects, tissue specimens, saliva, blood specimens, clinical documentation, genomic information, or epigenomic information in Nigeria is required to obtain ethical permission prior to the recruiting of participants and the collection of samples. This aligns with the Declaration of Helsinki, which asserts that “All research involving human subjects must receive review and approval from a properly established Ethics Committee prior to the commencement of the study.” [23]

4.2.3 Recruitment of study participants and obtaining informed consent

Upon receiving permission, qualified volunteers may then be recruited to partake in the study. Participants must receive a comprehensive explanation of the study's objectives, methodologies, possible risks and advantages, sample acquisition, nature of molecular analyses to be performed, data and sample storage protocols, access rights to the samples, potential future applications of the samples, and the possibility of publishing study findings. Informed consent in writing must be acquired from all participants prior to the collection of any biospecimens. If the research involves enrolling minors, secure agreement from their parents or guardians and, if appropriate, approval from the youngsters. [23,29].

4.2.4 Determining the study design

Epigenetic diagnostic studies can be designed as case-control studies, cross-sectional diagnostic accuracy studies or prospective validation studies. Case-control studies are ideal for biomarker discovery studies. Diagnostic accuracy studies and prospective validation studies are ideal when determining clinical utility of a diagnostic test. Diagnostic accuracy studies should follow reporting guidelines such as Standards for Reporting Diagnostic Accuracy studies (STARD 2015). STARD 2015 emphasizes transparent reporting of study details, including how participants were selected, which index tests were performed, which reference standard was used, and how the authors measured diagnostic accuracy [24].

4.2.5 Defining the study population and eligibility criteria

The protocol should clearly define what constitutes a case and what constitutes a control. Cases should include individuals with suspected clinical and histopathologically confirmed orofacial cancer. Ideally cases should be patients with oral squamous cell carcinoma. Controls should include healthy individuals, patients with oral benign disease or patients with oral indemandulatory disorders. Important variables such as age, gender, ethnicity, cancer subsite, histological type, tumor stage, tobacco and alcohol use, HPV status, oral hygiene practices, prior treatment and comorbidities should be documented since these variables may influence epigenetic signatures [25,26].

4.2.6 Clinical evaluation and histopathological confirmation

Patients with suspected orofacial cancer should be clinically evaluated and have lesion site, size, shape, duration, symptoms, risk factors, and regional lymphadenopathy assessed. However, clinical diagnosis alone is insufficient for study classification purposes. Biopsy-proven histopathological confirmation is the gold standard for diagnosing OSCC and other cancers [8,26]. In epigenetic testing studies, histopathology serves as the reference standard against which the epigenetic test is compared.

4.2.7 Biospecimen type and collection

Epigenetic studies can use tumor tissue, adjacent normal tissue, saliva, mouth rinse, oral exfoliated cells, blood, serum, or plasma. Tissue biospecimens provide direct access to tumor-specific epigenetic alterations. In contrast, saliva or oral rinse samples enable a non-invasive diagnostic approach because they can provide tumor DNA, RNA, proteins, and exfoliated cells from the oral cavity [16,19]. Biospecimen type should be determined by the study aims. Tissue would likely be preferred for confirmatory molecular diagnosis purposes. Still, saliva may be more appropriate for screening and early detection.

4.2.8 Biospecimen processing, storage, and transportation

Epigenetic data are only as good as the biospecimen from which it is derived. Improper collection techniques, delayed processing, improper storage temperatures, microbial growth, and multiple freeze-thaw cycles can degrade DNA, RNA, and chromatin integrity. Therefore, standard operating procedures should be implemented for sample labeling, coding,

transportation, processing, storage, and chain-of-custody tracking. Collection and processing of biospecimens should occur in an environment that ensures nucleic acid preservation and minimizes pre-analytical variability [27,37,38].

4.2.9 Epigenetic biomarkers and laboratory assays selection

DNA methylation is the most common assay used in epigenetic studies for oral and HNC diagnosis. Laboratory assays include methylation-specific polymerase chain reaction (MSPCR), quantitative methylation-specific PCR (qMSP), pyrosequencing, methylation-sensitive high-resolution melting (MS-HRM), methylation microarrays, reduced representation bisulfite sequencing (RRBS), and whole-genome bisulfite sequencing (WGBS) [20,33]. ncRNAs can be quantified using quantitative reverse transcription polymerase chain reaction (RT-qPCR), microarrays, or RNA sequencing.

4.2.10 DNA or RNA extraction and quality control

DNA or RNA is extracted from biospecimens using validated methods after collection and processing. Concentration, purity, and integrity should be assessed before proceeding with downstream applications. For DNA methylation analyses, purified DNA is treated with bisulfite, which converts unmethylated cytosines to uracil. This chemical change allows methylated cytosines to be distinguished from unmethylated cytosines. Poor bisulfite conversion can lead to inaccurate methylation measurements; therefore, bisulfite conversion rate and laboratory QC should be considered [20,33].

4.2.11 Epigenetic testing and laboratory quality control

Epigenetic assays should be performed under controlled laboratory conditions with appropriate positive controls, negative controls, technical replicates, and batch effects. Potential sources of technical variation include DNA extraction, bisulfite conversion, PCR amplification, sequencing depth, microarray performance, sample cross-contamination, and operator variability [20,34]. In multicenter studies, assay-specific standard operating procedures should be harmonized across collaborating laboratories to improve assay reproducibility.

4.2.12 Bioinformatic and statistical considerations

Epigenetic data require bioinformatic analysis. Sequencing-based methylation analyses may include read quality scores, adapter truncation, alignment to a reference genome, methylation calls, and identification of DMPs or DMRs. Array-based approaches may include background correction, probe filtering, normalization, batch effects, and identification of DMPs or DMRs. Statistical analyses should adjust for relevant confounders when possible (age, sex, smoking status, alcohol consumption, HPV status, tumor stage, tumor subsite, oral hygiene practices, oral inflammation, and cell-type estimates) [25,33,41,42].

4.2.13 Assessment of diagnostic accuracy

Diagnostic performance of candidate biomarkers should be determined by their ability to distinguish disease states. Sensitivity, specificity, positive predictive value, negative predictive value, receiver-operator curve analysis, and area under the curve should be calculated to assess diagnostic accuracy. If multiple markers are combined, diagnostic classifiers can be created using logistic regression or machine learning algorithms. However, these classifiers should be validated to avoid overfitting the data which can inflate test performance [24,36].

4.2.14 Validation and clinical implementation

Candidate biomarkers discovered during the discovery phase should be validated in an independent cohort before clinical implementation is proposed. Ideally, local validation will be important in Nigeria due to ethnicity-driven differences in genetics, diet, environmental exposures, tobacco and alcohol use, infectious disease prevalence, oral hygiene practices, and health-seeking behavior that may impact epigenetic marks. Strategy for clinical implementation should consider test cost-effectiveness, turn-around time, laboratory capacity, regulatory approvals, acceptability, and integration into existing oral cancer care continuum [36,44].

4.2.15 Data security, reporting, and ethical sharing

Epigenetic-based diagnostic tests can produce clinically actionable molecular and clinical data. Data should be coded, stored on a secure server, and only accessible to approved study team members. Participant identifiers and study data should be kept in separate files. Data used for publication or external sharing should be stripped of any participant identifiers, and participant consent should be obtained before sharing any study data. Ethics committee approval is necessary for any external data sharing agreements. Results from the study should be reported regardless of the outcome (including limitations such as sample size, study design, test performance, confounding variables, and ability to generalize findings) [29,35,43].

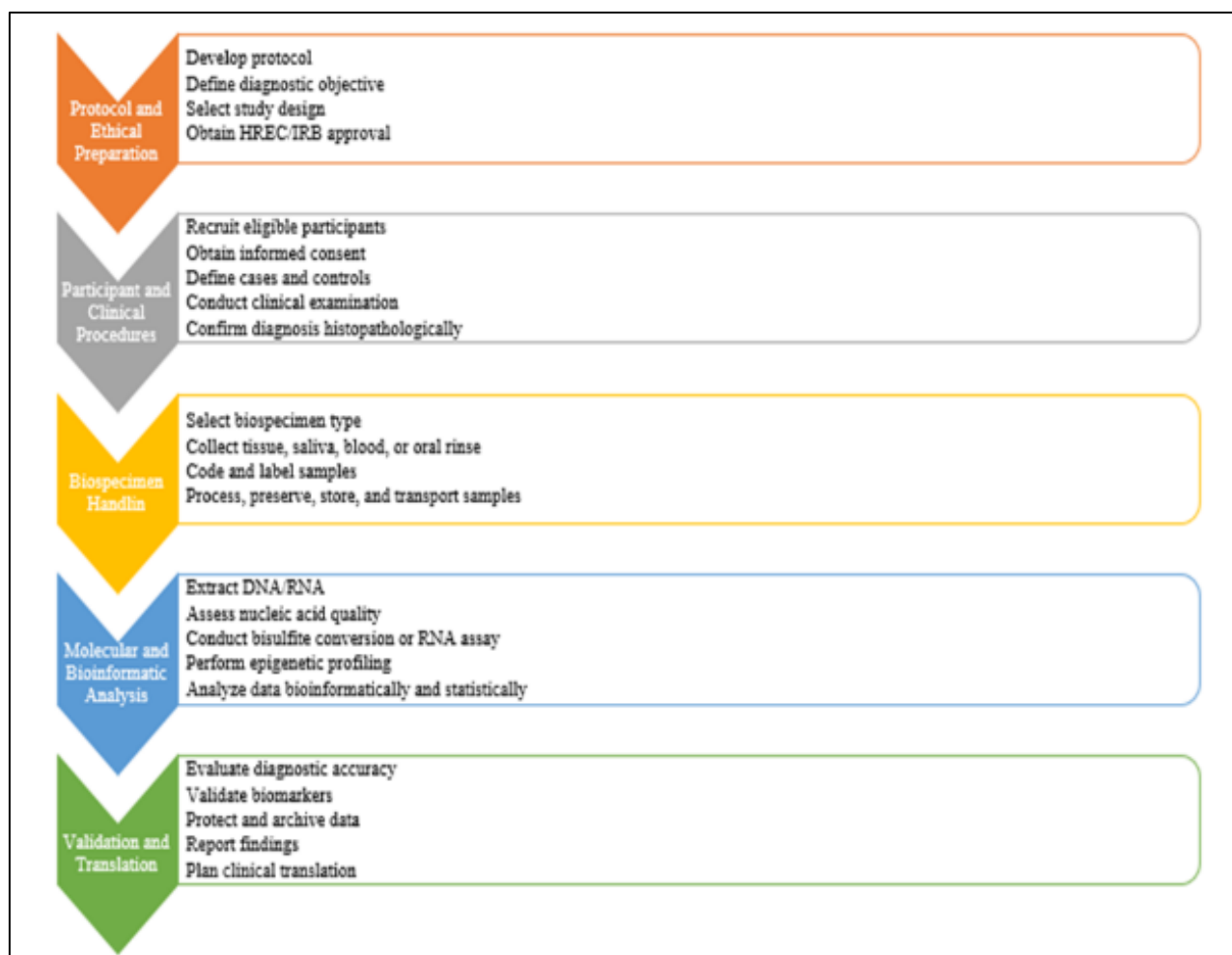


Figure 1: Describes each step of the proposed phased methodological framework for conducting epigenetic diagnostic testing studies of orofacial cancer in Nigeria.

5 METHODOLOGICAL CHALLENGES IN DESIGNING EPIGENETIC DIAGNOSTIC PROTOCOLS IN NIGERIA

5.1 Ethical approval and regulatory bottlenecks

Ethical approval is required for research involving human participants, biological samples, clinical records and molecular information. Epigenetic diagnostic studies raise additional considerations because they typically involve human biospecimens, genomic or epigenomic information, future use of samples/data, data sharing and sometimes international transfer of biospecimens. According to international ethical guidelines, researchers should not begin recruiting or sample collection until an accredited ethics committee has reviewed and approved the protocol [23,29]. In addition, ethical guidelines for health-related research involving humans recommend that consent language specifically outline the purpose of collection, potential risks, future use of samples/data, data sharing, privacy measures, and withdrawal from the study.

In Nigeria, ethical review for advanced molecular research could be delayed by lengthy administrative processes, multiple levels of review, limited institutional capacity, and uncertainty around which regulatory body is appropriate for genomes and epigenomics research. Some institutional Health Research Ethics Committees may lack expertise in reviewing protocols that involve molecular diagnostics, biobanking, genetic information, exportation of samples, cloud-based bioinformatics or collaborative analysis abroad. Similar challenges have been described in genomic research across Africa. These include challenges around informed consent, community engagement, data sharing, exportation of samples, benefit sharing and governance of biospecimens in repositories [30,31]. Review committees may therefore refer investigators to another regulatory body that supersedes them for approval. This may lead to multiple submissions/revisions of study protocols, prolonged timelines, increased administrative costs and disruption to study

timelines. Lengthy ethical approval can impact sample quality, study participant availability, funding timelines and collaborative agreements.

A second ethical challenge involves broad consent and future use of samples. Epigenetic research often generates findings that could be applicable to contexts beyond the original study question. Future use of biospecimens should be compatible with participants' consent and approved by the ethics committee [29,32]. Vague consent language can limit future validation studies, secondary analysis or data sharing efforts which ultimately limits the long-term utility of collected samples for scientific research. Overcoming this challenge will require clearer ethical review guidelines, increased capacity of Health Research Ethics Committees, online submission platforms, templated reviews and established policies on genomics, epigenomics, biobanking and data sharing at research institutions.

5.2 Costly analysis and outsourcing to foreign laboratories

Epigenetic diagnostic research requires complex laboratory procedures, specialized equipment, trained personnel and extensive bioinformatics support. Technologies such as DNA methylation analysis, microRNA profiling, chromatin immunoprecipitation sequencing, next-generation sequencing, reduced representation bisulfite sequencing, whole-genome bisulfite sequencing and genome-wide methylation arrays are expensive and labour-intensive [20,33]. These technologies require high-quality nucleic acids, validated controls/reagents, trained laboratory personnel and robust laboratory quality assurance measures to ensure low technical variability and repeatability [20,34].

In Nigeria, several institutions do not have regular access to next-generation sequencing platforms, methylation arrays, automated nucleic acid extraction systems, high-performance computing infrastructure or epigenomics laboratories. Some studies may have to ship samples to Europe, North America, Asia or neighboring countries in South Africa for biomarker analysis. Shipping samples internationally increases research cost (cold-chain shipping, export fees, special packaging materials, shipping cost, customs fees, insurance, foreign exchange rates). This may limit sample numbers, prevent replication and limit inclusion of independent validation cohorts. Outsourcing samples also introduces scientific and ethical challenges. Principal investigators may have limited control over sample processing, choice of assays, laboratory quality control, data capture and turn-around times. Collaborating with international teams can create confusion around ownership of samples, access to data, intellectual property, authorship and benefit sharing if materials transfer agreements and data use agreements are absent/non-existent [30,31,35]. These issues can decrease local investigator ownership and limit capacity building in the long-term.

Fluctuating foreign exchange rates can also impact development of realistic study budgets. Since most sequencing platforms, commercial methylation arrays, reagents and bioinformatics software are denominated in foreign currencies, the cost of conducting a study may increase between the time of protocol development and sample analysis. Researchers may be forced to reduce sample size or alter studies to accommodate less robust experiments. In biomarker discovery studies, reduced sample size and lack of validation cohorts limit statistical power, increase risk of false positives and reduce the ability to generalize findings [24,36].

5.3 Inadequate research and laboratory capacity

Many Nigerian research centers lack the infrastructure for advanced molecular diagnostics. Epigenetic studies require real-time polymerase chain reaction machines, sequencing platforms, automated nucleic acid extraction machines, methylation platforms, biosafety level II cabinets, cold chain storage, stable electrical power, temperature logs and bioinformatics machines. Many laboratories across institutions are equipped for conventional histology/pathology, cytology, haematology, microbiology or basic molecular diagnostics. Laboratories lack the infrastructure for high-volume epigenomic analyses. Sample storage limitations can affect RNA or DNA integrity. Delayed processing can introduce pre-analytical variability. Unstable power supply can affect freezers, centrifuges, sequencing machines, and computational analyses. Poor calibration and maintenance of laboratory equipment can produce inaccurate results. Standards and guidelines for biospecimens highlight the importance of sample quality, pre-analytical factors, storage temperature, processing time, record keeping, and standard operating procedures (SOPs) for reliable molecular data [27,37,38].

The capacity for biobanking is likewise constrained. Uniform biospecimen acquisition, handling, preservation, recovery, and documentation are essential for high-caliber epigenetic investigation. The International Society for Biological and Environmental Repositories offers protocols for biobanks that encompass comprehensive SOPs, quality assurance frameworks, temperature documentation, chain-of-custody logs, sample identification, and inventory oversight. In the absence of adequate systems, samples may become compromised, misclassified, damaged, or unfit for subsequent epigenetic evaluations.

Infrastructure challenges in Nigeria are often related to inadequate funding, lack of technical support, high costs for equipment maintenance, importing reagents, and slow ordering processes. Each of these obstacles can cause delays in

research, extended project timelines, and poor reproducibility. Protocols should be tailored based on current laboratory capacity, equipment functionality, sample storage conditions, and back-up plans for loss of power or lack of reagents.

5.4 Recruitment and sample collection issues

Recruitment of individuals can also be a methodological challenge when conducting epigenetic diagnostic discovery for orofacial cancer. Diagnostic biomarker studies require well-characterized cases and appropriate control groups. Orofacial cancer patients may present late to clinics in Nigeria [7], which limits opportunities to recruit early-stage cases needed for early detection purposes. Late presentation limits opportunities to identify epigenetic changes associated with early cancer development as opposed to changes associated with late-stage disease, treatment effects, inflammation, or tissue necrosis. Selection of control groups is also a major consideration. Healthy controls, patients with oral benign lesions, patients with oral potentially malignant disorders, and disease controls may differ in age, sex, tobacco use, alcohol use, oral hygiene practices, inflammatory conditions, HPV exposure, socioeconomic status, and medical comorbidities. These confounders can impact epigenetic changes and introduce bias [25,26]. If cases and controls are not well matched or adjusted for in statistical analyses, observed methylation or microRNA changes may reflect exposure differences rather than cancer-specific mechanisms.

Collection of samples also requires careful standardization. Saliva, oral rinse, tissue biopsies, blood samples, and exfoliated oral cells differ in cell composition, RNA/DNA yield, risk of contamination, and storage requirements. Saliva has been considered an attractive sample matrix for oral cancer detection because it is non-invasive and can contain tumor-derived DNA and RNA; however, saliva contains cellular material from the whole oral cavity and salivary biomarkers can be affected by oral hygiene, gum disease, tobacco use, alcohol intake, diet, medications, time of day, microbial contamination, and saliva collection technique [16,39]. Therefore, saliva collection methods (passive drool vs mouth rinse vs swab), restrictions on food/drink/smoking/toothbrushing prior to collection, and sample stabilization/transportation procedures should be defined in protocols.

Potential participants may also be reluctant to enroll in studies. Some individuals may fear that samples could be used for nefarious reasons, exported without permission, used for spiritual rituals, or discriminated against based on genetic information. In African genomic studies, community engagement, open informed consent discussions, culturally relevant education, and discussions on future use of samples can promote trust [30,31]. Epigenetic diagnostic studies should consider community education, participant training, culturally sensitive informed consent language, and clear explanations of sample storage, privacy limitations, and data sharing.

5.5 Human capacity

Epigenetic diagnostic research requires a multidisciplinary approach and collaboration between oral pathologists, maxillofacial surgeons, oncologists, molecular biologists, geneticists, bioinformaticians, statisticians, laboratory technicians, epidemiologists, and ethics and policy professionals. However, many Nigerian universities and research centers have limited numbers of professionals who are well trained in epigenomics, molecular oncology, bioinformatics, diagnostic biomarker development, and translational research [18].

Human capacity gaps can affect study protocols, research design, assay validation, biospecimen processing and tracking, quality control, bioinformatic analysis, statistical interpretation, and clinical translation. Lack of knowledge on limitations of methylation platforms can lead to improper assay selection, while lack of statistical knowledge can lead to underpowered studies and false biomarker claims. Studies evaluating diagnostic accuracy should be carefully designed to account for sample size, reference standards, index tests, blinding, and validation to reduce bias [24,36]. The exodus of talent to nations with superior research facilities also constrains capability. The emigration of proficient professionals restricts mentorship prospects, leadership roles in laboratories, and the advancement of enduring local research initiatives. Enhancing local capabilities via indigenous postgraduate education, brief courses, regional centers of excellence, visiting mentorship programs, and multi-institutional collaborative research will be crucial for advancing epigenetic research in Nigeria.

5.6 Bioinformatics/data analysis hurdles

Modern epigenetic studies yield large, complex datasets that require extensive computational resources and data analysis skills. DNA methylation microarrays and sequencing require quality control metrics, probe filtering, adapter trimming, alignment, normalization, batch effects, methylation calling, differential methylation comparisons, annotation, pathway analysis, and functional interpretation [20,33]. Without proper bioinformatics pipelines researchers may interpret technical artefacts as physiologically relevant findings.

Many Nigerian facilities lack access to high-performance computers, stable internet connections, licensed analysis software, secure servers and skilled bioinformaticians. Facilities may need to send samples to analysts or collaborate with overseas institutions. Open-source software and cloud-based interfaces (such as Galaxy) have made analysis more

accessible to researchers; however, researchers will still require education on how to conduct analyses within reproducible workflows. They will also require education on command-line analysis, best practices for data storage and security, and fundamentals of statistical analysis [40]. Popular methylation analysis packages like ChAMP and RnBeads provide great pipelines for analysing array-based methylation data; however, proper usage of these packages will require understanding normalisation, batch effects, cell- type heterogeneity, multiple-testing correction, and biological annotation [41,42].

Batch effects are a large concern in epigenomic studies. Differences in day of sample collection, DNA extraction methods, bisulfite conversion batch, array chip, sequencing lane, laboratory location, and even personnel could contribute to systemic technical differences. If batch effects are not properly controlled for, false-positive biomarkers could be identified [20,33]. Epigenetic signatures can also be influenced by tissue composition. Saliva and blood are both heterogeneous mixtures of cells. Differences in cell- type proportions could be identified as differences in methylation if properly accounted for [25,33].

Security of participant data is another concern. Epigenomic data can contain information about individuals and populations. Policies will need to address where the data is stored, who has access to the data, if cloud-based platforms will be used, how identifiers will be removed from the data, and how long data will be kept. Data governance should follow principles of open data sharing, controlled-data access, and protecting participant identity [29,35,43].

5.7 Limited population-specific reference data

Most epigenetic studies published to date have been conducted on individuals of European, North American, and East/ South Asian descent. Nigerian populations may differ in ancestry, environmental exposures, diet, prevalence of infectious diseases, oral hygiene, tobacco use, alcohol consumption, socioeconomic factors, and access to health care. Any of these factors can influence DNA methylation, miRNA expression, inflammatory status, and cancer risk [25,30].

As such, biomarkers discovered in other populations may not translate to similar performance in Nigerian patients. Differences in background methylation signatures can influence sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). This is particularly important for diagnostic biomarkers. A diagnostic marker that performs well in one population may perform poorly in Nigerian patients due to differences in background exposures, genetic ancestry, sample-type, or stage of disease distribution [36].

The lack of national biobanks, molecular databases, and population-specific epigenomic reference data will force local labs to validate findings. Without Nigerian reference data, it may be difficult to determine whether differentially methylated regions are unique to disease or if they vary within the normal Nigerian population. This limitation also affects machine learning algorithms which require training and validation datasets. Algorithms will be poorly equipped to make diagnostic predictions if they are trained on populations that are not representative of the local population [36,43].

5.8 Data management, privacy and regulatory concerns

Epigenetic studies collect sensitive biological samples, clinical information, and molecular data. Initial considerations include participant privacy, data ownership, open data access, future use of samples, international data sharing, export of samples, long-term data storage, and intellectual property. Some of these considerations become blurred in international collaboration if samples are leaving Nigeria to be stored and analysed at a partner facility.

Data governance will be important. Global policies state that genetic and health-related research data should be collected, stored, shared, and analysed in ways that respect individuals and populations, honour their permissions, and promote responsible scientific practices [29,35]. FAIR principles state that all research data should be findable, accessible, interoperable, and reusable — all while following ethical and legal policies [43]. Many resource-constrained institutions do not have genomic data governance policies in place, controlled-access repositories, cybersecurity infrastructure, and long-term data storage solutions.

Material transfer agreements (MTAs) and data use agreements (DUAs) are required when shipping samples or data to other institutions. MTA and DUAs should include terms describing ownership, authorised use, authorship, benefit sharing, intellectual property rights, return of samples or destruction procedures, rights to publish, and maintaining participant privacy [30,31,35]. If agreements are not established prior to sample collection, there can be disputes between partners on data-access, publishing, and future use of samples.

In Nigeria, epigenetic diagnostics will require clear plans for data-coding, de-identification, storage, access, backup, sharing, archiving, and destruction. Policies should define if data will be stored domestically, abroad, or on cloud-based servers. Policies should outline how researchers will protect the privacy of study participants.

5.9 Sustainability and translation of research findings

A key challenge with epigenetic diagnostic discovery is translating biomarker discovery into clinically usable assays. Many molecular studies end with publication. Studies rarely make it to independent validation, development of a diagnostic kit, regulatory approval, clinical implementation or cost-effectiveness analyses. This is a large issue within biomarker discovery, as many potential biomarkers falter due to small discovery cohorts, lack of external validation, poor study design, inconsistent assays, and poor reporting [24,36]. For epigenetic biomarkers to aid in the prevention and management of oral cancer in Nigeria, they will need to have analytical validity, clinical validity, and clinical utility. Analytical validity refers to the assay's ability to measure the biomarker accurately and precisely.

Clinical validity refers to how well the biomarker distinguishes between diseased versus non-diseased states. Clinical utility refers to how well a test improves patient outcomes, guides clinical decision-making, or improves health system efficiencies [36,44]. Without data informing all of these stages, epigenetic biomarkers will not be responsibly translated into the clinic. Translation also requires affordability and feasibility. If a novel diagnostic test requires sequencing that costs thousands of dollars and is done overseas, it will likely be infeasible to implement this test routinely at hospitals in Nigeria. Thus, techniques should evaluate early on if the proposed biomarker can eventually be measured using affordable platforms such as MSP, qPCR, or targeted sequencing. Implementation will also need to consider laboratory accreditation, quality control measures, physician education, turnaround time, reimbursement rates, regulatory approvals, and patient acceptability.

Finally, translation requires long-term dedication, multidisciplinary team science, local validation cohorts, public-private initiatives, and national cancer control planning. Without these supports in place, epigenetic diagnostics research may hold scientific value but will lack therapeutic impact.

Recommendations

The ethical review processes must be enhanced, streamlined, and expedited to facilitate prompt approval. Ethics committees based at university teaching hospitals and universities should receive training in genomics, epigenomics, biobanking, data governance, exportation of samples, and ethics of international collaboration. This will reduce unnecessary bottlenecks while ensuring the rights of participants are protected.

Nigeria should invest in building national capacity for molecular diagnostics. Although whole genome and high-throughput sequencing may be outsourced to regional or international laboratories initially, Nigerian laboratories can begin by offering targeted assays that are more affordable, such as MSP, qMSP, and pyrosequencing.

Researchers should secondly consider using biospecimens that will be practical and acceptable to study participants. Saliva, oral rinse, and buccal swabs are non-invasive samples ideal for early detection studies and may reduce participant reluctance. Whenever possible, researchers should use standardized collection kits with stabilizing buffers.

Fourth, partnerships between Nigerian entities and international genomic laboratories should be strengthened. Nigerian teaching hospitals, cancer centers, universities, and research institutes can collaborate with laboratories across Africa and globally to increase access to equipment, training, technical assistance, and external quality assurance. These partnerships should be established with clear material transfer agreements, data use agreements, authorship plans, and benefit-sharing agreements.

Bioinformatics capacity can be strengthened by training, mentorship, and participation in local and regional bioinformatics networks. Free or low-cost software such as Galaxy and cloud-based platforms can be used to get started with bioinformatics training and analysis. Additionally, plans for governing sensitive data should be considered. Seek funding from governmental and non-governmental organisations. Researchers can apply for grants from national agencies like TETFund and the Federal Ministry of Health, as well as international organisations such as NIH, EDCTP, Wellcome Trust, and other global health research agencies. Funding should be used to cover costs associated with the laboratory work, bioinformatics, validation studies, biobanking, and clinical translation.

Lastly, study designs should be tailored to what is feasible in-country. Instead of starting with epigenome-wide association studies, researchers can start with candidate epigenetic markers that have already been linked to oral cancer in other populations and validate these markers in Nigerian populations. This step-wise approach will be cheaper and more relevant to the Nigerian context.

The Nigerian government can begin developing national biobanks, oral cancer registries, and molecular databases. Ideally, these repositories could be used to accelerate discovery, repeatability, validation, and clinical translation of biomarkers that are specific to Nigerian populations.

6 CONCLUSION

Epigenetic research holds promise for improving early detection, risk prediction, prognosis, and personalized medicine for people with orofacial cancer. As patients in Nigeria face challenges with late presentation to care and limited diagnostic resources, epigenetic biomarkers have the opportunity to impact care for patients with oral cancer. Designing research studies for epigenetic discovery and validation is no simple task. Researchers face many ethical, infrastructural, financial, technical, bioinformatic, and regulatory challenges when conducting epigenetic research in Nigeria. A comprehensive approach to epigenetic diagnostics research begins with clearly defined research questions and continues through ethical approval, study enrollment, clinical/histopathological classification of samples, standardized biospecimen collection and storage, selection of appropriate assays, rigorous laboratory QC, bioinformatic analysis, evaluation of diagnostic accuracy, independent validation, and responsible data governance. Addressing these methodological challenges will require coordinated investment in ethical guidelines, molecular diagnostics laboratories, bioinformatics training, local discovery/validation cohorts, biobanking, cancer registries, and long-term research funding. Improving these will improve the overall quality and reproducibility of epigenetic diagnostics research and hopefully the lives of patients with orofacial cancer in Nigeria.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Acknowledgments

The authors of this manuscript acknowledge and sincerely appreciate TETFUND for the financial and moral support that enabled the publication of the first phase of this work while awaiting the second phase.

Funding Source

IBR TETFUND Research Grant.

Disclosure of conflict of interest

The authors declare no conflicts of interest regarding this manuscript.

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