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Original Research

Genetic Alterations of *C-MYC* Proto-Oncogene and Their Involvement in the Occurrence and Progression of Oral Cavity Cancers in Senegal

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Abstract

Background: Oral cancer is the 17th most common cancer worldwide, with a mortality rate of 1.8%. Their incidence varies considerably, with a clear prevalence in South Asian countries. In Africa, the mortality rate for cancers of the oral cavity is 1.3%. Senegal is a perfect illustration a perfect illustration of the seriousness and scale of this disease, with 177 new cases recorded in 2020, for a mortality rate of 1.4%. To add to the knowledge of the molecular mechanisms involved in the carcinogenesis of these pathologies in Senegal, mutations in the *C-MYC* proto-oncogene were examined in 22 patients with oral cavity cancers and compared with samples from 32 control individuals. **Methods**: Cancerous tissue (CT) and adjacent normal tissue (ANT) were sampled from diseased individuals, whereas whole blood was obtained from control individuals (C). A total of 67 samples were collected: 32 from controls, 22 from CTs, and 13 from ANTs of diseased individuals. Total DNA was extracted and polymerase chain reaction (PCR) amplification of exon 2 of the *C-MYC* gene was performed, followed by Sanger sequencing. Mutation analysis was performed using Mutation Surveyor Software v5.0.1. The effect of each non-synonymous mutation on the function of the encoded protein was determined using the POLYPHEN-2, PANTHER-PSEP, and PROVEAN algorithms. The probability of non-synonymous mutations causing diseases was predicted using Prediction of human Deleterious Single Nucleotide Polymorphism (PhD-SNP) and Predicting disease associated variations using GO terms (SNP&GO). The impact of non-synonymous variations on the stability of the encoded protein was determined using I-Mutant2 and In-silico analysis of Protein Stability (INPS). **Results**: Of the study participants, 63% were females. The mean age of patients was 46.43 $\pm$ 13 years, with extremes of 14 and 83 years and the age range of 40–70 years as the most representative age group. Only 5% of patients were alcohol drinkers and 15% were smokers. Most patients (80%) had stage III or IV tumors with lymph node invasion. A low polymorphism rate in exon 2 of the *C-MYC* proto-oncogene was identified, with one synonymous substitution (Q48Q) found in a diseased individual (CT and ANT). The non-synonymous substitutions (D31N, D31E, V33G, Y36N, and Y36D) found in the controls were predicted to be damaging and pathogenic, and might decrease the stability of the encoded protein. **Conclusions**: Our results indicate that the *C-MYC* protooncogene is not involved in the occurrence and progression of oral cavity cancers in Senegalese patients. However, the mutations found in controls could provide new markers for the early clinical diagnosis of oral cancer.

Keywords: *C-MYC*; oral cavity; cancer; mutation; pathogenicity

1. Introduction

Oral cavity cancers account for 3% of malignant tumors and 30% of upper digestive tract cancers [1]. They are head and neck squamous cell carcinomas, which are one of the main causes of death [2]. Malignant neoplasms occur in the structures or tissues of the mouth [3].

Oral cancer is the 17th most common cancer worldwide, with a mortality rate of 1.8% [4]. Large geographical variations in the incidence of these pathologies have been noted, with two-thirds of the cases identified in developing countries [5]. The highest incidence rates of oral cancer are observed in South and Southeast Asia (Sri Lanka, India, Pakistan, Bangladesh, and Taiwan), Western and Central Europe (France, Spain, Hungary, and Slovakia), Latin America, the Caribbean (Brazil and Uruguay), and parts of the Pacific (Melanesia) [6,7]. In Africa, the mortality rate of these pathologies is 1.3%. Senegal is an appropriate illustration of the seriousness of these diseases, with 177 new cases reported in 2020, and a mortality rate of 1.4% [4].

The main risk factors for the occurrence of oral cavity cancers are alcohol and tobacco consumption whose effects are synergistic [8] without additives [9]. Tobacco and betel nut consumption are considered as causes of oral cancers in the Pacific and South Asia [10]. In Europe and the United States, >74% of oral cancers are caused by tobacco and alcohol consumption [2]. Although the epidemiological profile of patients with oral cavity cancer reflects that of individuals who smoke and drink alcohol in Africa [11], stud-

ies have reported opposite trends in Senegal, where only few smokers and drinkers have been reported [3,12]. Gender and sex are also considered as risk factors of oral cancers. The peak incidence of oral cavity cancer occurs during the sixth and seventh decades of life, and is predominantly observed in men [13,14]. Nevertheless, rejuvenation and feminization of the affected population have recently been noted. Other risk factors of oral cavity cancer include poor oral hygiene [2], Human Papillomvirus (HPV) infections [15], poor socioeconomic status [16], genetic predisposing factors [17], and precancerous lesions [18].

Although the oral cavity is easily accessible for direct visual examination, most cancers in the oral cavity are diagnosed at an advanced stage (stage III or IV) and are associated with a poor prognosis (less than 5 years of survival) [19]. Genetically, many independent studies have suggested the involvement of some genes, including proto-oncogenes, in the development and progression of numerous cancers often associated with poor prognosis [20]. *C-MYC*, which belongs to the *MYC* family and is located on chromosome 8q24.21, is one of the most frequent proto-oncogenes involved in human carcinogenesis [21]. This study aimed to determine the mutational profile of *C-MYC* proto-oncogene in oral cavity cancer in Senegal.

2. Materials and Methods

2.1 Study Population

A total of 22 elderly patients with primary oral cancer and 32 control individuals were enrolled in this study. Patients were recruited after informed consent was obtained from the Department of Stomatology and Maxillofacial Surgery at Aristide Le Dantec Hospital. Cancerous tissues (CT) and adjacent normal tissues (ANT) were collected from patients during surgery, whereas whole blood samples were obtained from control individuals (C). A total of 67 samples were collected, including 32 from the controls (C), and 22 CTs and 13 ANTs from patients. In this study, the inclusion criteria for patients were as follows: Senegalese nationality, diagnosis of primary cancer of the oral cavity and signature of the information and informed consent form. Patients whose cancer was a recurrence of a previous cancer and those who declined the informed consent form were excluded from the study. For controls, the inclusion criteria were: non-cancer patients, patient companions, medical staff or any other person volunteering to participate in the study. Individuals who had once been diagnosed with cancer in previous years, even if cured, and those with benign tumours in the mouth were excluded from the control cases.

2.2 DNA Extraction, Amplification, and Sequencing of Exon 2 of the C-MYC Protooncogene

DNA was extracted using a Zymo Research kit (ZymoBIOMICS DNA & RNA kits, Zymo Research, Orange County, CA, USA) according to the manufacturer's protocol. Exon 2 was amplified using the One Taq 2X Master Mix (New England Biolabs, Ipswich, MA, USA) kit in a reaction volume of 25 µL. The reaction mixture comprised 2 µL of cDNA, 10.375 µL of water, 5 µL of 5X buffer, 0.5 µL of each primer (5′-TTTGTGTGCCCCGCTCCAGCA-3′ and 5′-TGCCGCTGTCTTTGCGCGCAG-3′), 0.5 µL of dNTPs, 0.125 µL of Taq polymerase, 2.5 µL of One Taq G/C, 2.5 µL of Hight G/C, and 1 µL of $MgCl_2$. polymerase chain reaction (PCR) was carried out in an Eppendorf thermal cycler under the following conditions: initial denaturation at 95 °C for 2 min, following by 35 cycles of denaturation/hybridization at 93 °C (1 min) and elongation at 72 °C (2 min) and final elongation at 72 °C (8 min). The reaction was terminated by incubation at 10 °C. Sanger Sequencing was performed using forward primers to determine the nucleotide sequences of the amplified fragments.

2.3 Pathogenicity Tests

The obtained sequences were checked, corrected, and aligned using BioEdit v7.0.5.3 software (Rutherford house, Manchester, UK) [22] and ClustalW multiple alignment algorithm [23]. To determine the mutations in *C-MYC*, the chromatograms obtained after sequencing were input into Mutation Surveyor v5.0.1 software (Softgenetics, State College, PA, USA) [24] and compared with a reference sequence available on NCBI (NG_007161.2). The effect of each non-synonymous mutation on the function of the encoded protein was determined using Polymorphism Phenotyping v2 (POLYPHEN-2) [25], Position-Specific Evolutionary Preservation of Protein Analysis THrough Evolutionary (PANTHER-PSEP) [26], and Protein Variation Effect Analyzer (PROVEAN) [27] algorithms. The probability that non-synonymous substitutions cause disease was determined using PhD-SNP [28] and SNP&GO [29], which predicted whether the mutations were neutral (score <0.5) or pathogenic (score ≥ 0.5). The impact of non-synonymous variations on the stability of the encoded protein was determined using I-Mutant2 [28] and INPS [30], which predicted whether the variations increase ($\Delta\Delta G > 0$) or decrease ($\Delta\Delta G < 0$) the stability of the encoded protein.

2.4 Molecular Analysis

To estimate diversity and genetic variability, we determined the total number of sequences without gaps (N), sample size (n), number of invariable sites (c), number of variable non-informative and informative sites (Pi), the total number of mutations (*Eta*), nature of mutations (*s*) and (*v*), mutation rates (R), and types of substitution (Ks) and (Kns). These parameters were determined using DNA sequence polymorphism (DNAsp) v5.10.01 (Barcelona University, Spain) [31] and Molecular Evolutionary Genetics Analysis (Mega) v7.0.14 [32] software. The haplotypic diversity (*hd*) and nucleotide diversity (*Pi*) were estimated using DNAsp v5.10.01 [31].

Table 1. Heterozygous SNP of the *C-MYC* protooncogene.

Individuals	Mutations	Position	Nature	p. AA	Status	Score
CT17	G2793GA	245	Transition	Q48QQ	Heterozygous	33.68
ANT17	G2793GA	245	Transition	Q48QQ	Heterozygous	23.06

AA, amino acid.

To soften their frequencies, nucleotide sequences were converted into amino acid sequences using Mega v7.0.14 [32] software.

Genetic differentiation was estimated using Nei intra and interpopulation genetic distances obtained using Mega v7.0.14 [32] software. The genetic differentiation factor (F*st*) that gives the values for the degree of differentiation between controls, ANTs, and CTs, as well as the degree of differentiation based on the epidemiological factors were obtained using Arlequin v3.5.1.3 (Zoological Institute, University of Berne, Switzerland) [33]. This software was also used to determine the distribution of genetic variability at intra- and interpopulation levels using the Analysis of Molecular Variation (AMOVA) test.

The Z-test of selection was evaluated by selecting the starting hypothesis that exon 2 of *C-MYC* was under positive selection using the Tumura 3-parameter model and the complete deletion method on Mega v7.0.14 [32].

3. Results

The study population comprised 54 patients (63% women and 37% men). The mean age of patients was 46.43 $\pm$ 13 years, with extremes of 14 and 83 years. The most representative age group was 40–70 years. Our study population comprised a small number of male smokers (15%) and alcoholics (5%). Most patients (80%) had stage III or IV tumors with lymph node invasion.

3.1 Mutation Research

Analysis of the chromatograms using the reference sequence revealed four mutation spots in our population study, including one spot with a score >20 in CT17 and ANT17 cells (Table 1 and Fig. 1). However, the mutation score was higher in the CT group (score of 33.68) than in the ANT group (score of 23.06). This mutation was a synonymous heterozygous SNP (Q48Q).

The assessment of the possible modifications using BioEdit revealed the existence of substitutions at T15G and T23G in individual T5; G8A, T15G, and T23A in individual T8; and C10A in individual T9. The analysis performed using the pathogenic test software showed that these non-synonymous substitutions were damaging, pathogenic, and might decrease the stability of the gene encoded in these individuals (Table 2).

3.2 Variability and Genetic Diversity

Based on the results, the sequences had the same number of sites (433 pb), more than 99% of which were conserved in controls (429), ANTs (432), and CTs (432). In the controls, the percentage of transversions (81.28) was higher than that of transitions (18.72), which correlated with the R-value (0.221). However, in the ANTs and CTs, the percentage of transitions (99.6%) was higher than that of transversions (0.4%). The non-synonymous substitution Kns (0.01) was higher than the synonymous substitution, Ks (0.02), in controls. In the ANTs and CTs, the non-synonymous substitution, Kns (0), was equal to the synonymous substitution Ks (0) (Table 3).

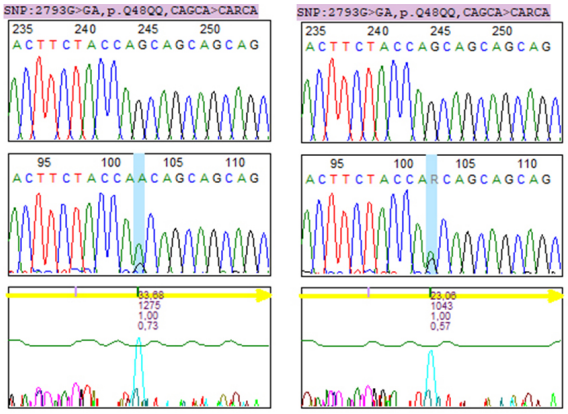

Fig. 1. Heterozygous SNP identified. SNP, Single Nucleotide Polymorphism.

The *C-MYC* proto-oncogene displayed low haplotype diversity (0.184) and low nucleotide diversity (0.00070). Low haplotype and nucleotide diversity were also noted in the controls, ANT and CT, with the highest values found in the controls (0.298 and 0.00142) (Table 4).

Transcription performed with the second frame reading revealed a significant increase in aspartic acid (8.99), valine (4.09), and tyrosine (4.09) levels in CTs and ANTs. However, significant decreases in glutamic acid (6.98), glycine (6.32), and asparagine (2.85) levels were observed in these populations (Table 5).

Estimation of the genetic distance within each population revealed that the controls had a low genetic distance (0.001). This genetic distance was zero in ANTs and CTs (0 $\pm$ 0). Furthermore, a low interpopulation genetic distance was noted between the control samples and CTs and between the control samples and ANTs (0.001) (Table 6).

Based on the genetic differentiation factor (F*st*), no statistically significant genetic difference in *C-MYC* ($p \geq 0.05$) was found between the controls and other populations. Furthermore, genetic variability was observed within

Table 2. Prediction of the effect of nonsynonymous substitutions on the structure, function, and stability of the encoded protein.

Mutations	G8A	C10A	T15G	T23A	T23G
p.AA	D31N	D31E	V33G	Y36N	Y36D
Prediction of the structure and function of the encoded protein					
Protein Variation Effect Analyzer (PROVEAN) prediction	Deleterious	Deleterious	Deleterious	Deleterious	Deleterious
Polymorphism Phenotyping v2 (POLYPHEN-2) prediction	Benign	Potentially damaging	Potentially damaging	Potentially damaging	Potentially damaging
Position-Specific Evolutionary Preservation of Protein Analysis THrough Evolutionary (PANTHER-PSEP) prediction	Potentially damaging	Potentially damaging	Potentially damaging	Potentially damaging	Potentially damaging
Conclusion	Damaging	Damaging	Damaging	Damaging	Damaging
Probability that mutations cause the disease					
Prediction of human Deleterious Single Nucleotide Polymorphism (PhD-SNP) prediction	Pathogenic	Pathogenic	Pathogenic	Pathogenic	Pathogenic
Predicting disease associated variations using GO terms (SNP&GO) prediction	Pathogenic	Pathogenic	Pathogenic	Pathogenic	Pathogenic
Conclusion	Pathogenic	Pathogenic	Pathogenic	Pathogenic	Pathogenic
Prediction of the stability of the encoded protein					
I-MUTANT prediction	Decreasing	Decreasing	Decreasing	Decreasing	Decreasing
In-silico analysis of Protein Stability (INPS) prediction	Decreasing	Decreasing	Decreasing	Decreasing	Decreasing
Conclusion	Decreasing	Decreasing	Decreasing	Decreasing	Decreasing

Table 3. Basic parameters of genetic variability.

Parameters	Populations		
	Controls	Cancerous Tissue (CT)	Adjacent Normal Tissue (ANT)
Number of sites	433	433	433
Sample size	19	21	12
Monomorphic sites (c)	429	432	432
Polymorphic sites (v)	4	1	1
Non-informative polymorphic sites (s)	3	1	1
Informative polymorphic sites (Pi)	1	0	0
%transition (s)	18.72	99.6	99.59
%transversion (t)	81.28	0.4	0.41
Mutation rates (R)	0.221	236.855	236.884
Synonymous substitutions (Ks)	0.002	0	0
Nonsynonymous substitutions (Kns)	0.01	0	0

these populations but not between them. The same phenomenon was observed when different epidemiological factors, such as sex, tobacco consumption, and alcohol consumption were considered (Tables 7,8).

The non-significant *p*-value of the Z-test for the selection indicated that exon 2 of *C-MYC* proto-oncogene followed neutral evolution in CTs and ANTs. In contrast, the significant *p*-value under the hypothesis of positive selec-

Table 4. Genetic diversity of the populations (C, ANT, and CT).

Populations	Parameters	
	Hd	*Pi*
Controls	0.298	0.00142
CT	0.095	0.00022
ANT	0.167	0.00038
Overall	0.184	0.00070

C, control; CT, cancerous tissue; ANT, adjacent normal tissue.

tion (0.048) and non-significant *p*-value under the neutrality hypothesis (1) and negative selection (1) indicate that exon 2 of *C-MYC* protooncogene is under positive selection in the controls (Table 9).

4. Discussion

The study population predominantly consisted of female patients (63%) who were non-alcoholics or nonsmokers, with a sex ratio of 0.59. This characteristic is comparable to that of the study by Toure *et al.* [34] in Senegal, as 60% of their study population was female patients who did not smoke or drink alcohol. This characteristic is similar to that reported by Bambara *et al.* [35], who reported a similar predominance (sex ratio of 0.55) in their Ouaguadougou cohort with a small number of alcoholics and smokers. Mbaye *et al.* [36] reported similar values (62%) in their study without the involvement of alcohol and tobacco consumption. The frequent feminization of this disease matches the increased risk of nontoxin-induced carcinoma in these patients. However, Varenne *et al.* [37] suggested that poor oral hygiene could play an important role in the occurrence of oral cavity cancers in women, as they revealed that the average number of carious teeth, missing teeth, and filled teeth was 6.1, with higher values in women than in men (8 *vs* 4.4; $p < 0.001$), and greater changes in periodontal status in women than in men.

In the present study, the mean age of occurrence of oral cancers was 46.43 $\pm$ 13 years in Senegalese patients, which is comparable to that found by other African authors, including Bissa *et al.* [38] in Togo (46.1 years). However, our mean age was lower than those reported by Toure *et al.* [34], Dieng *et al.* [12], Diakite [39], Bambara *et al.* [35], and Mbaye *et al.* [36]. Western authors have reported a higher mean age, which could be explained by the higher life expectancy in these countries, with an estimated mean age of 55–60 years [40]. In our study, the most representative age group was the 40–70 years group. A similar result was obtained by Bougar [14] in Morocco. This finding could be explained by the fact that the peak frequency of oral cavity cancer has been reported in both men and women aged 60–79 years [41]. The concept of aging has been evaluated as its effects affect cancer development [42].

Based on our findings, more than half of the oral cavity cancer cases (80%) were diagnosed as stage III or IV disease in Senegal. Advanced histoprognostic stages have also been reported by some Western authors, including Liu *et al.* [43] and Lin *et al.* [13]. The low socioeconomic level that compromises access to oral healthcare has often been implicated [44]. In a study conducted in Ouagadougou in 2015, Bambara *et al.* [35] found that the long delay in consultation (18 months on average), which is partially related to the low attendance to oral health care, could explain the high proportion of patients with advanced-stage disease, while prognosis is strongly related to the delay in diagnosis. Many reasons could explain this delay, including the trivialization of some symptoms (otalgia and discomfort of chewing) despite their persistence, absence of initial pain, use of traditional medicine first, and difficulty in accessing specialized services [11].

A low rate of polymorphism in the *C-MYC* proto-oncogene was found in our study population. Such finding suggests that the *C-MYC* proto-oncogene is not involved in the occurrence of oral cavity cancers in Senegalese patients. However, other authors have revealed the involvement of *C-MYC* protooncogene mutations in some pathologies, including Johnson [45], who showed that nearly 50% of Burkitt's lymphomas had *C-MYC* mutations, and Xu-Monette *et al.* [46], who found *C-MYC* mutations in 33% of Diffuse Large B-cell Lymphoma samples. Our results highlight the existence of a single mutation (G2793GA) corresponding to the synonymous substitution, Q48Q. This variation is described as a somatic polymorphism, because it is present in both ANTs and CTs of the same individual. This result shows that it is not possible to have normal tissue in the oral cavity once cancer occurs because the oral cavity is a restricted area.

The substitutions found in the controls were predicted to be damaging and pathogenic (could cause the disease) and might decrease the stability of the encoded protein in these individuals. The functional importance of these substitutions and their involvement in carcinogenesis remain to be elucidated.

The analysis of basic parameters revealed a strong presence of conserved sites (432/433) and missing informative polymorphic sites (0) in CTs and ANTs. Such finding confirmed the hypothesis that the *C-MYC* proto-oncogene is not subject to mutations in oral cavity cancer in Senegal. Genetic diversity analysis revealed a low haplotype diversity (*Hd*) and low nucleotide diversity (*Pi*) in the controls compared with ANTs and CTs but with clear superiority in the controls. Overall, the synonymous substitution rate was equal to the non-synonymous substitution rate, and *C-MYC* mutations can be deduced to impose few constraints on the encoded proteins. This hypothesis was confirmed by the Z-test of selection, which was statistically non-significant in our study population, indicating the existence of negative selection tending to eliminate nonsynonymous mutations.

Table 5. Amino acid frequencies between the controls, adjacent normal tissues, and cancerous tissues.

Amino acid	Controls	CT	ANT	*p*-value
Ala	6.3	6.3	6.3	
Cys	2.777777778	2.777777778	2.777777778	
Asp	8.99122807	9.027777778	9.027777778	2.81×10^{-5}
Glu	6.980994152	6.944444444	6.944444444	9.243×10^{-5}
Phe	5.555555556	5.555555556	5.555555556	
Gly	6.323099415	6.3	6.3	2.272×10^{-5}
His	0	0	0	
Ile	4.166666667	4.166666667	4.166666667	
Lys	4.166666667	4.166666667	4.166666667	
Leu	7.638888889	7.638888889	7.638888889	
Met	2.083333333	2.083333333	2.083333333	
Asn	2.850877193	2.777777778	2.777777778	9.243×10^{-5}
Pro	7.638888889	7.638888889	7.638888889	
Gln	7.638888889	7.638888889	7.638888889	
Arg	2.777777778	2.777777778	2.777777778	
Ser	11.11111111	11.11111111	11.11111111	
Thr	3.472222222	3.472222222	3.472222222	
Val	4.093567251	4.166666667	4.166666667	1.432×10^{-5}
Trp	1.388888889	1.388888889	1.388888889	
Tyr	4.093567251	4.166666667	4.166666667	1.43×10^{-5}

Table 6. Nei intra and interpopulation genetic distances.

Distance		Populations					
	Intrapopulation	Controls	0.001	Interpopulation	Controls	CT	ANT
		CT	0	Controls	-	0.01	0.001
		ANT	0	CT	0.001	-	0
				ANT	0.001	0	-

Table 7. Genetic differentiation and structuration factor between controls, adjacent normal tissues, and cancerous tissues.

AMOVA				Fst			
Intrapopulation		Interpopulation		Populations	Controls/CT	Controls/ANT	CT/ANT
Controls	2.78	Controls/ANT	97.12				
ANT	0.37	Controls/CT	99.63	Fst	0.028	0.003	–0.058
CT	5.81	ANT/CT	105.81	*p*-value	0.145	0.386	1

AMOVA, Analysis of Molecular Variation.

Table 8. Genetic differentiation and structuration factor considering epidemiological factors.

Factors		AMOVA		Fst	*p*-value
		Intrapopulation	Interpopulation		
Sex	Male Female	2.15	97.85	0.02145	0.15287
Gender	Young (<40 years) Intermediate (40–70) Old (>70 years)	4.86	95.14	0.04856	0.14366
Tobacco cons.	Yes No	5.22	94.78	0.05217	0.10683
Alcohol cons.	Yes No	–13.22	113.22	–0.13220	1.00000

Table 9. Z-test of selection for each population.

Population	Z-Test of selection		
	Neutrality	Positive selection	Negative selection
Controls	0.095	0.048	1
CT	0.336	1	0.163
ANT	0.325	1	0.152

In contrast, in the controls, the non-synonymous substitutions (Kns) were higher than the synonymous substitutions (0.01) (0.002). Accordingly, we can deduce that somatic mutations involve amino acid changes. These mutations are no longer eliminated by natural elimination processes (splicing, excision, and ligation), which prevent correction of the mutated genes and consequently eliminate the truncated protein in these individuals. These results align with the significant *p*-value (0.048) under the hypothesis of positive selection in the controls. In this group of controls, some individuals (T5, T8, and T9) should undergo medical follow-up.

A significant increase in the levels of aspartic acid (Asp), valine (Val), and tyrosine (Tyr) was observed in the CTs and ANTs. This increase was associated with a significant decrease in glycine (Gly), asparagine (Asn), and glutamic acid (Glu). Notably, Glu and Gly, which were significantly decreased, are non-essential amino acids (can be synthesized by the body). Recent studies have shown that several non-essential amino acids (e.g., GLU, Ser, and Gly) play important roles in cancer metabolism [47]. Moreover, an increase in valine, an essential amino acid, suggests that the nutrition of tumor cells is influenced by the nutrition of their host [3].

Of all the epidemiological factors considered in this study, no genetic differentiation was found. Thus, there was no genetic differentiation between tumors due to epidemiological factors. Instead, each case was found to be unique at the molecular level.

5. Conclusions

Oral cavity cancers remain one of the most common and widespread cancers worldwide and constitute a real public health problem. Our study has shown that Senegal is a perfect illustration of the seriousness and extent of this disease. In fact, the majority of patients presenting stage III and IV tumors with lymph node invasion testify the delay in the detection and early diagnosis of these pathologies. We attempted to use a population genetics approach to relate the *C-MYC* protooncogene mutations with the occurrence and progression of oral cavity cancers in Senegal. However, the results obtained invalidate our initial hypothesis that *C-MYC* mutations were involved in the onset and progression of these diseases in Senegalese patients. Mutation studies revealed a low rate of polymorphism of the *C-MYC* in our study population, with the existence of one mutation corresponding to the synonymous substitution, Q48Q which shows that it is not possible to have normal tissue in the oral cavity once cancer occurs because the oral cavity is a restricted area. Moreover, there was no association between the genetic structure and epidemiological factors such as age, sex, alcohol consumption, tobacco consumption, and tumor stage. Nevertheless, the nonsynonymous substitutions noted in the controls could provide new markers for the early clinical diagnosis of oral cavity cancers. The functional significance of these mutations found and their involvement in carcinogenesis need to be studied in more detail to determine how *C-MYC* has been altered. In addition, further study of other genetic alteration pathways including gene expression could provide useful information on the role of the *C-MYC* proto-oncogene in the development of oral cancers in Senegal.

Availability of Data and Materials

These data analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

DB, FM, HD and MS conceived, designed, analysed, wrote and edited the article. MDS, MMN and ST collected samples. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The patients and control subjects provided signed, informed consent and the study was approved by the University Cheikh Anta Diop Research Ethics committee (Reference: /2018/CER/UCAD).

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Conflict of Interest

The authors declare no conflict of interest.

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