Original Article

The Possible Role of Diabetes in the Etiology of Laryngeal Cancer

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Abstract

Background: Laryngeal cancer and oral cancer are not always correlated with genetic mutations, HPV infection, smoking, and alcohol abuse. In the absence of these risk factors, there is an increase on these cancers with a parallel increase of diabetes. The aim of this study is to verify if diabetes could be a risk factor for the laryngeal cancer.

Methods: A questionnaire was given to a group of ninety laryngectomees to verify if these patients have presented diabetes and xerostomia before surgery. In two groups, diabetics and healthy persons, the values of the salivary mucins and the pH were evaluated. The results were statistically analysed using Fisher Exact Test and Chi–square Test

Results: Diabetes is a risk factor: $p \leq 0.0445$ for laryngectomees male vs control group. Xerostomia inlaryngectomees male is a risk factor: $p \leq 0.050$. The values of mucins and pH in diabetic group show significant difference: $p \leq 0.05$ vs control group

Discussion: In all autoimmune diseases, a decrease in the value of pH and salivary flow consequently decreases the value of spinnbarkeit which measures the capacity of the mucous layer to adhere to the epithelium and alter the protective oral mucin layer. We find that diabetes is epidemiologically correlated with laryngeal cancer. In fact, only diabetes increases the concentration of salivary mucins with a formation of mucin layer even more reduced, and so completely ineffective in protecting the mucosa.

Conclusions: The increase of mucin secretion in diabetes alters much the protective layer allowing the risk factors to promote cancer growth.

Keywords: Laryngeal cancer, epidemiology, mucous, spinnbarkeit, flow rate, pH, glycosylation, SIgA, oral cancer

Introduction

The most recent genetic research has identified some key genes that may be the main promoters of oral and laryngeal cancer. The critical examination of these data [(1)] allows us to say that they are interested in these anatomical sites for about eighty percent of cases, the genetic mutations of tumor suppressor genes, especially, T p53 and CDKN2A. These two genes are involved as "Tumor Suppressors" and this capacity is fundamental also in the promotion of many other cancers. It is therefore very clear that a particular and recurring family predisposition to suffer from certain types of cancer and the probability of inheriting one of these mutated genes in one allele prepares the risk of carcinogenesis due to the loss of heterozygosity (LOH). Only after LOH, the gene, for example T p53, loses fully its genetic expression that controls the cell cycle in the key role of restorative capacity. This cancer's promotion for the oral cavity and larynx is triggered by both endogenous and/or exogenous causes, that recent research has identified smoking and alcohol abuse as primary causes, and in cases of infection with HPV. This hypothesis contrasts with the epidemiological data that show (see Table 1) the main countries have the highest index (ASR) countries like Cuba and Iraq where the incidence of laryngeal cancer has increased but there is absolutely [(2)] no high consumption of alcohol and tobacco use.

The influence of the HPV virus, in the promotion of cancer of the larynx, cannot be considered very important, because this infection is primarily in other anatomical sites, as shown in Figure 1.

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COUNTRY	ASR INDEX	SMOKING	ALCOHOL
CUBA	7.6	+	+
HUNGARY	6.4	++	+++
IRAQ	5.6	+	-
KAZAKISTAN	5.5	++	++
UZBEKISTAN	5.5	++	++
MOLDAVIA	5.3	++	++
ROMANIA	5.0	++	+++
MONTENEGRO	5.0	++	+++
BULGARIA	4.9	++	+
CROATIA	4.7	++	+++
SYRIAN ARABIC REP.	4.6	+	+
PORTUGAL	4.6	++	+++
TURKMEKISTAN	4.5	+	+
VENEZUELA	4.4	+	+
POLAND	4.3	++	++
ITALY	3.2	++	++

Table 1. Laryngeal Cancer Epidemiology ASR Index in Correlation with Smoking and Alcohol Consumption

Legend:
ASR INDEX = Age Standardization Rate
NUMBER CASES /100000 People
+ Normal –moderate habits; ++ high; +++ excessive

This figure, derived from the data of the latest studies in that field, indicates the range of the HPV infection in various anatomical sites and the relative viral strains.

For the considerations and assessments set out above is therefore necessary to take into consideration other risk factors that could promote laryngeal cancer, for example, those that change the natural laryngeal defences, such as the mucin layer.

These causes may be present in many diseases and in connection with the latter investigation, there are few studies, mainly those related to diseases such as diabetes that alter the defences of the oral cavity and larynx. Research in the field of oncology oral cancer and larynx must study the mechanisms by which certain biochemical and physical factors alter the cellular defence structure consisting mainly of salivary mucin. Although laryngeal cancer in some respects is different from those of the oral cavity, it must be remembered that the mucin layer also protects this anatomical structure, and therefore it is conceivable that the processes of its alterations have in each case one and the same origin. From genetic point of view, the principal evidence in the genetic mutations of the p53 and CDKN2A is shown in almost seventy per cent of the cases.

The aim of this study begins with the hypothesis that each risk factor may cause alterations in the oral cavity and larynx protection that leads to the promotion of cancerogenesis. In this study it is very important to discuss the mechanism whereby the autoimmune diseases

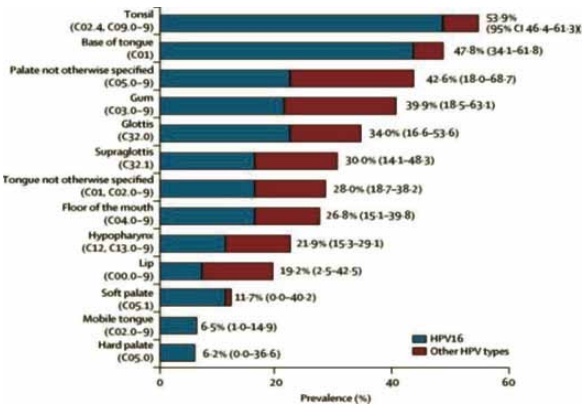

Figure 1. The anatomic sites for HPV cancers, and HPV STRAINS

induce these alterations, but mostly to understand why only in diabetes there are effective and safe epidemiological findings for its correlation, in the absence of other risk factors, that promote oral and larynx cancer. Our hypothesis is that diabetes involves modifications, extremely important and irreversible, in the salivary mucin layer which allows, for example smoking, HPV and other risk factors, to influence irreversibly the normal genetic structure of the cell thus, allowing the possibility to risk factors, acting on cell metabolism without finding an adequate protective barrier.

Materials and Methods

A medical questionnaire was administered to a group of ninety laryngectomees, comprising of sixty–seven men and thirteen women. Included in the laryngectomees group are men and women who have all been subjected to total laryngectomy after their fifty years with a maximum age for this surgery at seventy–two. This medical questionnaire was administrated to a laryngectomees group to see if these patients before surgery, have autoimmune diseases and/or symptoms of xerostomia. The same questions were posed to the healthy control group which comprised of sixty men and thirty women. This group have the same age range as thelaryngectomees group. The results of the proposed questionnaire were statistically analysed with Fisher Exact Test.

This study was made under the observation and ethical control of theLaryngectomees Italian Association. Under the control of the same ethical group in this study, we examined the differences in the concentration of total salivary mucin and the pH value in diabetic patients composed of twenty–eight males, non–smokers and no–alcohol users compared to the healthy control group which is also composed of twenty–eight males.

Mucin concentration was determined using the Alcian blue method, as briefly described here: The samples

of saliva diluted (1:10) were incubated for 30 min in a 1%solution of Alcian blue in 50 mm sodium acetate buffer with25 mm Cl2Mg, pH 5.8 under constant agitation at room temperature.After following the dissociation of mucin–dye complexes are stripped, with the addition of a 1: 2 dilution of Aerosol OT (Sigma Chemical Co, St Louis, MO, USA) in distilled water, mixing short and ultrasound the extracted samples ethyl ether and after centrifugation the concentration of dye was determined spectrophotometrically at 605 nm in the aqueous phase.

The pH wasdetermined by the use of sticks for urinalysis, type Uriscan Roche, reading the values with automatic analyser URIYSIS2400 ROCHE. These data were statistically analysed with Chi–square Test.

Results

Results of Fisher Exact Test for xerostomia hypogeusia in internal laryngectomees group:

- only in males: XEROSTOMIAp–value = 0.04950 ; HYPOGEUSIA p–value =0.356
- only in females: XEROSTOMIA p–value = 0.183 ; HYPOGEUSIA p–value = 0.183

The result of the incidence of diabetes in men from the laryngectomees group (25%) is statistically significant if compared with the value of the incidence (1.8%) in the males of Italian people: p–value = 0.041, with a proportional test. For an explanation of these results, it is important to analyse the incidence of diabetes for males in Italy.

The percentage of people with diabetes in Italy is currently 4.3%, with an incidence between males and females almost identical, and includes all ages. Our sample of laryngectomy patients is a population aged between fifty and seventy–five years, for which the value of comparison of the incidence is related to a percentage of about 1.8% of the Italian population.

The Xerostomia in laryngectomees male has a risk factor of p≤ 0.05 while the autoimmune diseases has a risk factor of p≤0.0491 for both sexes. The diabetes incidence is 25.6% in laryngectomees male versus 1.8% incidence in Italian female population.With proportional Test the diabetes isa risk factor in laryngectomees male: p≤ 0.05.

Results of the Chi–square Test are:

- Mucin Concentrations: diabetes group vs control group: p =0.032
- pH Values: diabetes group vscontrol group; p= 0.047

Discussion

The results of our work expressed in Table 2 are similar to those of a pilot study conducted at the University of Naples, where a retrospective survey demonstrated that in about one hundred persons undergone an operation for laryngeal cancer, thirty–six were suffering from diabetes (lengo M, 2014 data not published). Diabetes, in fact, can play an important role in the development of

CONTROL GROUP 60 MEN 30 WOMEN				LARYNX GROUP 72 MEN 18 WOMEN		
RISK FACTOR	MEN	WOMEN	TOTAL M+W	MEN	WOMEN	FISHER TEST P-VALUE BETWEEN CONTROL AND LARYNX GROUP
AGE men ≥65 years women ≥60 years	45	18	63	54	12	1.000
SMOKING	42	15	57	51	9	1.000
ALCOHOL	3	0	3	6	0	1.000
CANCER FAMILY	6	9	15	12	9	0.748
XEROSTOMIA	3	0	3	22	6	0.012
HYPOGEUSIA	3	0	3	12	6	0.103
LIFE STYLE	21	9	10	8	1	1.000
AB GROUPS	12	9	21	12	9	1.000
AUTOIMMUNE DISEASES (DIABETES)	1(2)	4(1)	5(3)	23(18)	6(5)	0.9 (0.0440)

Table 2. Results of the Questionnaire Responses by the Two Groups

laryngeal cancer; this assumption becomes evident from data in countries like Cuba and Iraq (where there is a low alcoholic and tobacco consumption) with a high incidence of laryngeal cancer and a high incidence of diabetes as well.

More certain, even for recent studies, is the link between diabetes and oral cancer.Epidemiological data (3) carried out mainly in Hungary where the incidence of oral cancer is very high (ASR index = 9.0.)show that in this country there is a correlationbetween the incidence of diabetes mellitus,that affects 7% of the Hungarian population, and in generally glucose metabolic disorders among oral cancer patients and the frequency of different localizations of the same cancer.This study uses data spanning 14 years with two datasets of 1998 to 1999 and from 2012 to 2013 collected first hand by the authors. These datasets have led to examine the blood glucose level in 267 patients with histological confirmed malignant tumours in the oral cavity. Diabetes mellitus was found in 59 of them (22.1%) while our result is 21.5%. A comparative epidemiological study also demonstrates that there was an increased incidence of diabetes mellitus of 17.6% to 22.1% in the dataset 2012–2013.

MUCINS mg/dL		pH	
CONTROL	DIABETES	CONTROL	DIABETES
31	39.7	7.4	6
32	37	7.7	6.4
31	38.9	6.9	6
30	39.6	7.7	6
29.9	39	7.6	6.1
29.8	39	7.3	6.0
28.9	39.6	7..5	6.0
32.1	39.7	7.1	6.4
30.9	39.8	7.4	7
33	38.8	7.4	6.0
33.4	36.1	7.2	6.0
31	38.9	7.6	6.1
31.9	38	7.6	5.9
32.1	39.6	7.6	6.6
31	38.1	7.7	6.3
33.3	37.2	7.9	5.9
31	37.4	7.9	6
31	39.8	6.9	6.1
31.3	39.7	7.4	6.2
32.1	40.1	7	6.4
32.1	39.3	7	5.9
30.9	39.2	7	6
30.4	39	7	5.9
31.1	39	6.9	5.8
30.5	38.9	7	6.2
31.1	38.9	7.9	6.2

Table 3: The salivary mucins concentration and the pH values

Our study also achieved very similar results especially considering the comparison between the incidences of diabetes in Laryngectomees group compared to that of the Italian population. Equally significant is the incidence of xerostomia. This symptom is independent of the type of autoimmune disease and the gender. If we consider xerostomia as a symptom that indicates less mucosal oral and laryngeal protection, we should consider all autoimmune diseases a real risk factor. However, in practice this does not happen and there is no evidence in literature of epidemiological correlation, for example, between Sjogren's syndrome and cancers of the oral cavity and larynx in general however, it must be acknowledged that autoimmune diseases, more than for their genotoxic intervention within cells, induces very substantial alterations in mucin layer allowing other risk factors such as smoking, viruses, alcohol, free radicals, to bring the onset of the cancer process. Such changes may take place mainly according to these possible mechanisms:

a. decrease in salivary flow;
b. defect in glycosylation process of mucins;
c. change in concentration of SIgA;
d. decrease in pH;

The factors and/or processes outlined above led to the "spinnbarkeit" modification of the mucosal layer that lines the epithelium, which is the clear slippery elastic consistency characteristic of the same. The spinnbarkeit of saliva reflects the ability of saliva to adhere to surfaces within the mouth, thereby serving as a protective role in adherence and lubrication. Therefore, alterations in the spinnbarkeit of saliva may result in the loss in adhesiveness or the ability to bind onto surfaces which may be related to the oral dryness. Experimentally, the minimum value (cut–off) must not be less than 11–12 mm and the optimal value is about 14 mm [(5)]. The same study has demonstrated the negative correlation between contact angle (this value is directly proportional to the wettability) and spinnbarkeit for the sub–mandibular and labial glands and test saliva samples, where an increase in spinnbarkeit leads to a decrease in the contact angle (greater wetting ability and little adhesion of oral epithelium). The spinnbarkeit of saliva is expected to arise from the presence of high molecular weight glycoproteins (mucins) that aggregate end–to–end. Mucin glycoproteins and their structures are known to be important factors for the extensional

rheological properties of saliva [6]. The conformation of mucin depends on factors such as pH and ionic strength, but different rheological properties have been identified in saliva produced by the different salivary glands with mucin–rich submandibular or sublingual secretions being most viscous and viscoelastic, and parotid saliva secretions being the least viscous and viscoelastic saliva. These various salivary secretions contribute to rheology of the mucosal layer and contribute to its viscoelasticity and extensional rheology, aiding in the maintenance of a normal mouth feel. In addition, submandibular/sublingual saliva have varying concentrations of proteins when stimulated by smell, chewing or taste in comparison to unstimulated saliva.

A. Reduction In–flow Saliva

It is known that the salivary flow may decrease even if not always, in autoimmune diseases [7], and in diabetes [8,9,10], especially in the latter when there is a non–effective glycemic control [11]. A recent study [12] showed that the decrease of the salivary flow is directly proportional to the decrease of the spinnbarkeit (Figure 2), and this implies the low value of flow index; in addition to the occurrence of xerostomia, and it also presents an alteration of the mucin layer unable to adhere sufficiently to protect the oral epithelium.

B. Defect in the Process of Mucins Glycosylation

The glycosylation modification is a widespread interesting event with about 70% of human proteins and principally characteristic of the cell surface and of those with a secreting function. Recent experimental study on salivary secretion[14] have demonstrated that only a correct glycosylation of mucins can allow in the oral cavity the smooth application of the phonation function of its normal lubrication, the formation of molecular recognition loci needed to protect the same against bacteria and viruses, and a proper hydrophilic process to create a mucin layer with a normal spinnbarkeit value.In many studies, over 90% of patients with dry mouth have a spinnbarkeit below the lower limit cut–off of 11 mm. Further analysis of mucins has revealed that the reduced glycosylation of mucins led to a sensation of dry mouth. The result of this study indicated that salivary mucin concentrations are not reduced in dry mouth but that the mucin structure (glycosylation) is altered. Incorrect glycosylation can occur due to many intra and extra cellular factors but the most common involves a flaw in the formation of terminal residue of the polymer chain, namely that of glucose and the level of sialic acid. In this regard there are few studies that have analyzed the relationship between the amount of sialic acid in the saliva and autoimmune diseases, and/or diabetes. One of them [15] notes that there is a decrease of concentration in saliva and the hypothesis of the authors is that the decrease of salivary sialic acid level in type 1diabetes may be due to changes in the activities of the enzymes taking part in the synthesis and catabolism of sialic and according to our opinion, it may also involve one linked to mucin.

C. Modification of SIgA Concentration

It is now clear that some protective salivary proteins including mucins and IgA are concentrated on oral mucosal surfaces in the bound pellicle through specific interactions. Concentration of mucins would contribute to lubricate and prevent abrasion damage to soft tissues whilean increase at IgA could create an 'immune reservoir' against the mucosal infection. In fact, the latest research highlights another key role of SIgA or one that allows the construction of the mucin layer in defence of oral epithelium; salivary mucins may initiate the formation of the mucosal pellicle through interactions with membrane–bound mucins

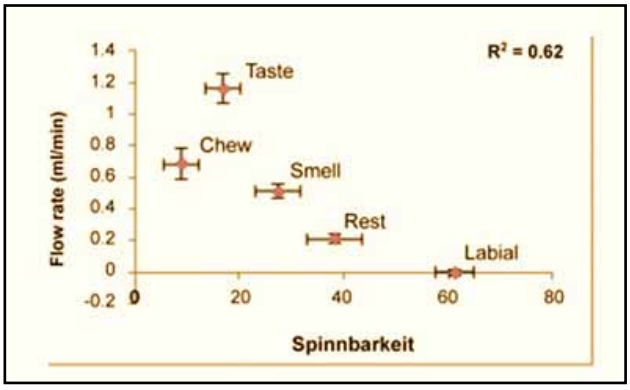

Figure 2. Influence of Flow Rate on Spinnbarkeit Values

on cells following the MUC5B MCU7, and form bonds between components of their oligosaccharide terminals with some of the immunoglobulin domains as described in the recent comment in "Nature" [16]. In this study, a possible interaction scheme and subsequent binding which agrees with the crystallographic examination is supposed.

The interaction process in the saliva between the SIgA and Mucins is very important because the stability of the mucin layer also depends on the concentration of the same immunoglobulin and in consequence the rheology of the mucosal system is susceptible to alterations when the amount of SIgA is low or in excess. In autoimmune diseases such as rheumatoid arthritis, the decrease of SIgA is frequent [17, 18] with the increase of xerostomia, and symptoms are even more accentuated if a secondary Sjögreen syndrome is present.

Diabetes is a disease with a difficult metabolic control, where the SIgA may be higher [19] or as in many other cases lower [19,20]; in each case the patients are suffering from xerostomia and show a salivary flow rate much lower than the standard [21] and this we know that it is directly proportional to a spinnbarkeit value lower than the normal cut–off.

D. Reduction of the Salivary pH Value

The pH value of the saliva depends on the bicarbonate buffer system and the concentration of calcium ions, K, and Na affecting the hydrophilic ability of the salivary mucins. This happens in autoimmune diseases and in diabetes in general leading to a lowering of the pH in the saliva [22] up to the values 5.5–6. As the pH of saliva decreases from its physiological value ofabout pH 7.1, the spinnbarkeit shows a gradual fall. However, the spinnbarkeit of saliva shows a steep fall when pH increased from its baseline as you can see in Figure 3. At these pH values, the related value of the spinnbarkeit is below the cut–off.

Our study shows (Figure 3) a decrease of about one unit from a medium value of 7.3 pH in the control group, to a medium value of 6.4 pH in diabetes group vs control group but this does not necessary implies a reduction of the spinnbarkeit value to go below the normal cut–off. The possible hypothetical explanation for the presence of the symptoms of xerostomia reported in the questionnaire by the males in the laryngectomees group should be seen within the modification of other parameters such as an increase of the fraction of the mucins secreted in saliva. This fraction is the principal component of a structure formed by proteins, primarily mucins MUC5B, MUC7, as well as water and mineral salts, amylase, SIgA, and other components. Figure 3 shows a schematic representation of the main functions of these components, which form the protective layer, the oral mucosa and larynx.

E. The Over–Expression of Mucins:

Another fundamental parameter of the mucin layer involved for the presence of diabetes in the normal layer functions of the mucins, is also the concentration of salivary mucins. In diabetes, contrary to other autoimmune diseases, there is a considerable increase of this.

These results could be explained assuming that only in diabetes (with an increase of salivary mucins) there is a mechanism yet to verify with experimental studies that promotes alterations to the saliva layer (always composed by MUC5C and MUC7) with subsequent loss of its protective nature for the larynx. In fact, diabetes greatly increases the concentration in the saliva of the type of secreted mucins, MUC5B and MUC7, but not the

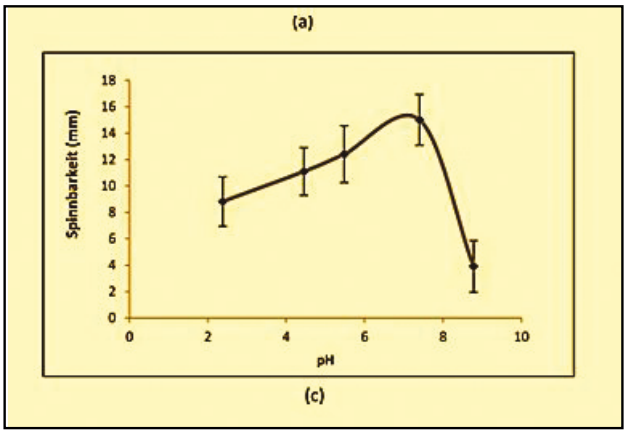

Figure 3: The Influence of pH on Spinnbarkeit Value

MUC1, this linked to epithelial cells and that ensures the anchoring of the protective layer to the oral mucosa.

One can reasonably assume that this increase in secreted fraction is excessive compared to that of MUC1 fraction, which entails an equally increasing mutual interactions between the terminal polysaccharide structures of MUC5B and MUC7, with formations of bonds between sialic acid saliva while that in diabetes it is much lower than normal concentrations, is easily seized in this mucin structure, making it even more compact and highly hydrated. Practically this situation of over–expression in the formation of mucins, especially MUC5B and MUC 7, contributes to a compaction of the type of a bubble. It can be assumed that the saliva present in diabetes is not as a relaxed layer and adhering to the oral mucosa and/or laryngeal, but, as a layer formed from many droplets. Figure 5 shows a didactic representation of the normal protective layer structure of the oral cavity and larynx, as can also be shown by electron microscope images. In diabetes, the mucin layer that should protect the epithelium, as we hypothesized, becomes a structural assembly, which doesn't look as a stratified epithelium, but for the high concentration of mucins and the high absorption effectof water becomes like a drop of water.

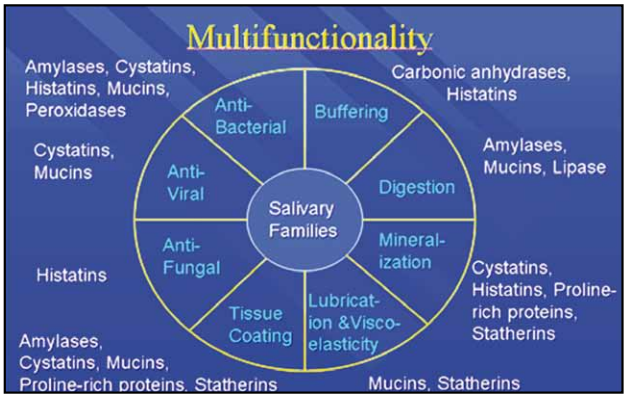

Figure 4. The schematic representation of the components of the Saliva with theirs correlated functions

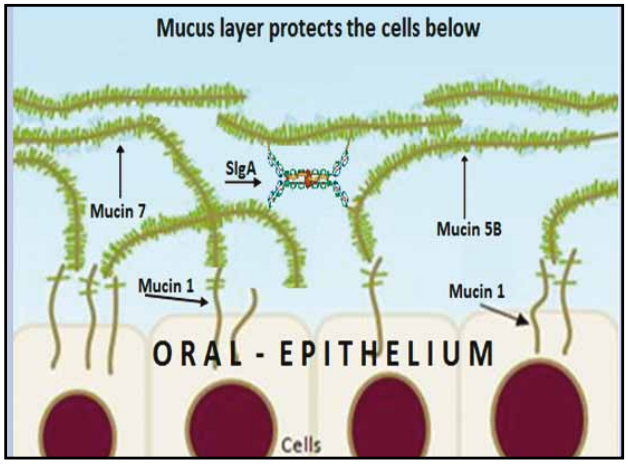

Figure 5: The Hypothetical Structure of Normal Salivary Mucus

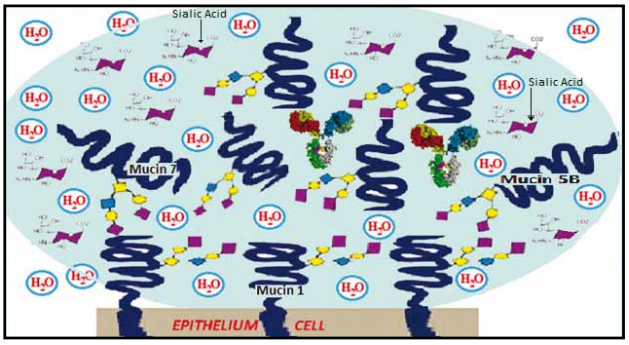

Figure 6: The Hypothetical Structure of Salivary Layer in Diabetes Conditions

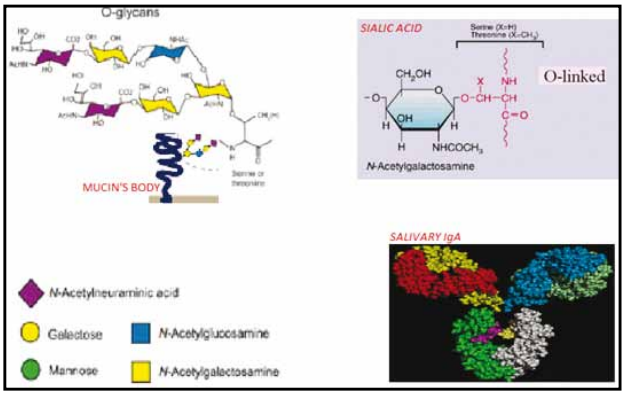

Figure 7. The most important parameters involved in Mucin Layer

A hypothetical representation is shown in Figure 6 where the most obvious difference is the formation of complex, and not more layered regularly on oral and laryngeal epithelium, and therefore less effective in its protection.

Conclusions

In this study we try to demonstrate with sufficient reliability that there could be four distinct reasons why autoimmune diseases and especially diabetes, can alter the mucin layer that protects the oral cavity. By decreasing the effectiveness of such a protective structure, other factors are able to come into direct contact with oral and laryngeal epithelium, spreading in its cells carcinogens contained in the smoke for example, and/or oncogenic viruses, that can promote the carcinogenic process.

These considerations may justify the hypothesis derived from epidemiological data and the relative studies, as there may be a correlation between diabetes, autoimmune diseases, and oral cancer or laryngeal cancer

The results of this study are very interesting, because they could explain the high incidence of laryngeal cancer in countries like Cuba and Iraq where in the presence of a low alcohol and tobacco consumption, there is a public health public on diabetes.

Ethical Statement

This study was undertaken under the observation and ethical control of the Laryngectomees Italian Association.

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