Salivary Leptin as a Potential Early Diagnostic Tumor Marker in Suspected Oral Cancer Patients

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Abstract

Leptin a 16-kDa peptide hormone plays an important regulatory role in basal metabolism and food intake. It is considered as a linkage between metabolism and the immune system. It plays a key role in different types of cancer due to its angiogenic, mitogenic, pro-inflammatory, and anti-apoptotic properties.

The fact that leptin is associated with tumors and is produced and secreted by salivary glands prompted us to investigate the role of salivary leptin in the human salivary gland and evaluate salivary leptin hormone in suspected oral cancer patients and investigate the possible diagnostic relevance of salivary leptin hormone as a potential tumor marker.

36 individuals were included in this study, 20 controls and 16 suspected oral cancer patients. The salivary samples were obtained from both groups using a standardized method and the leptin levels were measured in the salivary samples by using Human LEP (Leptin) ELISA kit. Data analysis was performed using the Mann-Whitney U test for Social Sciences (SPSS version 24).

The median leptin level for healthy control is 21.60 (20.26,24.08) pg/ml while it is 32.03 (27.38, 35.09) pg/ml for suspected oral cancer patients. A statistically significant difference in leptin levels is shown in suspected oral cancer patients compared to control.

Therefore, this study suggests a promising diagnostic role of leptin hormone as a tumor marker in suspected oral cancer patients.

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Introduction

Oral cancer cases that arise have sacrificed many lives all over the world. Many countries are aggressively involved in the oral cancer crisis including India, Pakistan, Bangladesh, Sri Lanka, and Taiwan.[1] Other than the Asian countries, the areas of Western and Eastern Europe, and the Caribbean are also involved in the incidence of this cancer.[2] The malignancy of oral cancer can occur at any oral cavity's subsites such as the tongue, lip, floor of the mouth, buccal mucosa, retromolar trigone, and oropharynx. Many causes of oral cancer are often ignored or neglected such as smoking, tobacco, betel quid chewing habit, poor oral hygiene as well as nutritional deficiencies.[3] In addition, a study found that betel quid consumption was a significant risk factor for oral leukoplakia and submucosal fibrosis. Betel quid chewers will be facing 10 years earlier of oral cancer with shorter 5-year survival rates and have oral mucosal lesions 8.21 times larger than non-chewer.[4]

Leptin a hormone that was discovered in 1994 is the product of an obese gene.[5] It is

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mainly secreted by adipocytes [6,7,8] and at another tissue or organ which are the ovary, stomach, and skeletal muscle but only in a small amount.[6] Leptin circulatory levels are mostly controlled by insulin, glucocorticoids, and catecholamines. There are several factors associated with the decline of leptin expression, which is fasting, β-adrenergic agonists, and thiazolidinediones.[9] Leptin in general, regulates energy homeostasis by altering the central anorexigenic pathway, where it will reduce food or energy intake and increase metabolism or energy expenditure.[7,10]

Previous study shows that leptin has a link with cancer, immunity, and autoimmune disease. The rising leptin level can promote cancer cell survival and proliferation by expanding the articulation of anti-apoptotic proteins and angiogenesis. So, leptin activates angiogenesis by leading the formation of capillary tubes and vascular endothelial growth factor (VEGF) that support tumor growth.[11] Other than that, the most type of cancer involving the neoplastic process of leptin are breast and colorectal cancer.[12] Leptin is over-expressed and higher in colorectal cancer and breast cancer patients. It plays a significant role in the development of breast cancer by regulating estrogen receptor signaling and aromatase activity, transactivating HER2 via epidermal growth factor receptor and Janus Kinase 2 (JAK2) activation, and increasing the production of VEGF.[9] However, there were several studies have been conducted to find the relationship between leptin and oral cancer. Leptin might probably be associated with oral squamous cell carcinoma. [13]

Materials and methods

Study Protocol

This is a cross-sectional study and was conducted at Sultan Ahmad Shah Medical Centre (SASMEC@IIUM). Ethical issues were considered since medical records were explored to obtain information about the patient. Permission to look into patients' medical records in the medical record unit was obtained from SASMEC. Privacy and confidentially of patients were maintained. Ethical clearance was obtained from the IIUM Research Ethics Committee (IREC) of IIUM, Kuantan, and SASMEC with the reference IIUM/504/14/11/2/IREC 2021-124.

Methodology

Method of saliva collection

Saliva samples were collected in the following manner, each unstimulated whole saliva sample was collected during patient admission Consequently, donors have fasted as well as refrained from smoking or using oral hygiene products (brushing with toothpaste or mouthwash) at least 1 hour prior to collection which will be at 8:00 am , routine clinical review sessions, or during the oral mucosal examination. Consequently, donors were food and water fast as well as refrained from smoking or using oral hygiene products (brushing with toothpaste or mouthwash) at least 1 hour prior to collection,5 mL into 15 mL of passive-drooled saliva samples from donors were collected into sterile centrifuge tubes. Patients who are excluded from this study are: Patients on radiotherapy or chemotherapy, patients on immunosuppressant therapy, patients with Sjogren syndrome, dry mouth disorder, patients with a history of degenerative disease, immunity-mediated disorder, patients with a history of cancer in another extra oral region currently/ in the past, patients already underwent surgical excision of lesion/tumor, and patients with body mass index 25kg/m2 and above.

Leptin hormone analysis

Saliva samples were obtained from 36 individuals. Participants were asked to expectorate into disposable tubes every 30 seconds over a period of 5 minutes. After centrifugation (4000x g; 10min), the samples were stored at -25°C and tested using Leptin Abnova LEP Human ELISA kit (KA3080) and female/male saliva test. For the purification of salivary leptin, the clear solution were extracted using N-hydroxysuccinimide (NHS) activated columns (HiTrap, 1 ml; Amersham Pharmacia) coated with leptin-specific antibodies. Ligand coupling will be performed using a standard coupling buffer (0.2 M NaHC03, 0.5 M NaCl; pH 8.3). After equilibration of the column with ten volumes of PBS buffer, pH 7.4, saliva was applied in 1 ml portions to the column (flow rate 0.2—1 ml/min). During this step of the procedure, leptin binds to the antibodies. After repeated washing steps (five column volumes PBS; pH 7.4) salivary leptin was eluted by alteration of the pH value (three column volumes 100 mM glycine/0.5 M HCI; pH 2.7). The leptin content of the elution buffer was determined by RIA. The material was lyophilized and stored at -80°C until

use. Determined salivary leptin concentrations by using a highly specific ELISA (Leptin-Duo-Set, R&D-Systems) with a sample volume of 100 L. The sensitivity of the system was 7.8 ng/L, with a CV of 3% in intraassay and 3.5%–5.4% in interassay analyses. Saliva standards were diluted in a low-protein matrix to adjust the system to the protein content of saliva (R&D-Systems). Figure 1 illustrates the flowchart of the research methodology.

Figure 1. Flowchart of research methodology.

Statistical analysis

Data analysis was made by using IBM SPSS for Windows, Version 28. Mann-Whitney U- Test was used to determine the difference in leptin concentration between the two groups. Descriptive statistics were presented as the median and interquartile range (IQR); meanwhile, frequency and percentage (%) were used for categorical variables (gender). The data was analyzed using an independent t-test.

Results

In this study, we have 2 groups:16 patients were involved in the test group and 20 individuals as the control. Table 1 shows the demographic distribution based on gender. Figure 2 shows the percentage of males and females in the group of control and suspected patients.

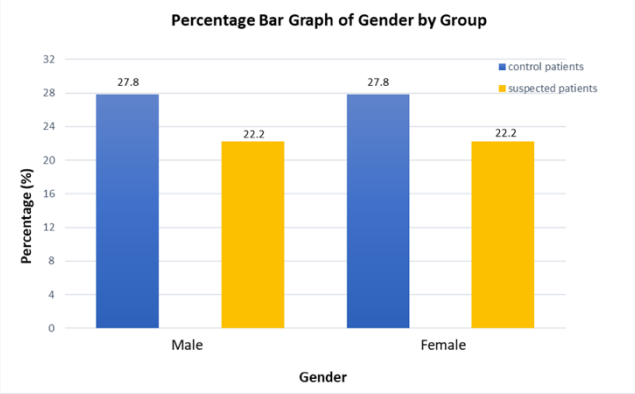

Figure 2. Bar graph that represents the percentage of gender by the group.

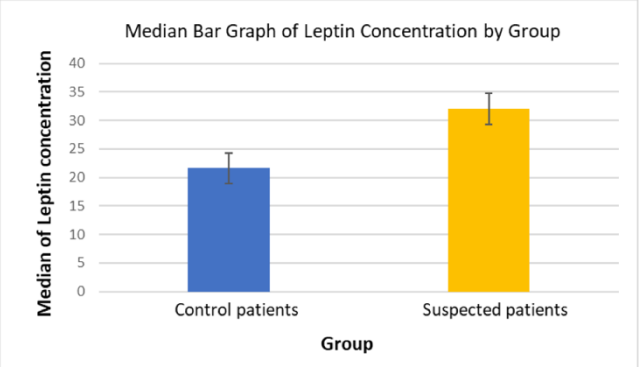

Figure 3. Median bar graph of leptin concentration in controls and suspected patients.

Table 2 below shows the median for control patients and suspected patients. It shows a significant difference in leptin concentration between the two groups. This is because of the median for control patients is 21.60 (20.26, 24.08) while 32.03 (27.38, 35.09) is for the suspected patients. The p-value is 0.001 which is less than 0.05 shows that it has a significant difference. The median leptin concentration can be seen as well in Figure 3, but it's displayed as a bar graph.

In addition, Table 3 shows the median leptin concentration of each group separated by gender. The result shows a significant difference

in leptin concentration for each gender but when comparing the median it was not significant (21.19 and 22.51), (31.67 and 32.03) respectively.

Gender	Group	n(%)
Male	Control patients	10 (27.8)
	Suspected patients	8 (22.2)
Female	Control patients	10 (27.8)
	Suspected patients	8 (22.2)

Table 1. Demographic distribution based on gender.

	Median (IQR)		Z statistics	*p-value*
	Control Patients	Suspected Patients		
Leptin Concentration (pg/ml)	21.60 (20.26,24.08)	32.03 (27.38, 35.09)	-4.584	0.001

Table 2. Median of leptin concentration for control and suspected patients.

Gender	Leptin Concentration (pg/ml)		Z statistics	*p-value*
	Median (IQR)			
	Control Patients	Suspected Patients		
Male	21.19 (20.29,23.34)	31.67 (25.07,36.21)	-3.021	0.003
Female	22.51 (20.21,25.11)	32.03 (29.21,35.09)	-3.5541	0.001

Table 3. Leptin concentration for controls and suspected patients based on gender.

Discussion

Leptin can impact the tumor microenvironment in several ways. Leptin is associated with a number of biological components that could lead to tumor cell invasion and distant metastasis. This includes interactions with carcinoma-associated fibroblasts, tumor-promoting effects of infiltrating macrophages, activation of matrix metalloproteinases, and transforming growth factor-β signalling. Recent studies also have shown that leptin plays a role in the epithelial-mesenchymal transition, an important phenomenon for cancer cell migration and metastasis. Furthermore, leptin's potentiating effects on insulin-like growth factor-I, and epidermal growth factor receptors have been reported. Regarding unfavorable prognosis, leptin has been shown to influence both adenocarcinomas and squamous cell carcinomas. Features of poor prognosis such as tumor invasion, lymph node involvement, and distant metastasis have been recorded in several cancer types with higher salivary leptin levels.[7]

Many studies investigate the relationship between leptin concentration and different types of cancer such as breast, lung, colorectal, pancreatic, and prostate cancer. [9,11,12] The results of this study show that there was a significant difference in the median leptin concentration between the control and suspected oral cancer cases. This agrees with a previous study by Schapher et al found that leptin has a high amount in salivary gland tumors compared to healthy and the analysis of leptin concentration in saliva might be used to diagnose salivary gland tumors and other tumors.[14] Another study on thyroid cancer documented that expression of leptin was associated with increased tumor size and lymph node metastasis.[15] In addition, leptin was spotted to be associated with the initial stage of oral squamous cell carcinoma. [13]

However, in a previous study by Hellström L *et al*, leptin concentration in women is two times higher than in men due to their higher proportion of adipose tissue.[16] This can be expected since salivary leptin concentration differs from total blood leptin level.[17] A thorough study should be done that considers many aspects of the human body that affect leptin concentration.

Leptin may be involved in the tumorigenic process. This hormone affects cell growth and appears to be related to both the risk of developing cancer and how invasive tumors spread. One potential explanation for the connection between these processes and tumors is the stimulation of vascular endothelial growth factor and endothelial growth factor expression, which increases angiogenesis.

Conclusions

Leptin could influence cancer cells through numerous phenomena, like inflammation and oxidative stress, cell proliferation, inhibition of apoptosis, angiogenesis and immune modulation. Obviously, the manipulation of this environment surrounding the neoplastic growth or tumor microenvironment is a challenging task. Leptin has been demonstrated to affect the prognosis of suspected oral cancer patients as a promising diagnostic tumor marker for early detection of oral cancer.

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Declaration of Interest

The authors report no conflict of interest.

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