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CONGRESS PROCEEDING

Potential Biomarkers For Early Detection Of Oral Cancer: Leptin And Adiponectin

Nezaket Ezgi Özer [1]*, Aslı Tetik Vardarlı [2], Betül Karaca [1], Ali Veral [3] and Pelin Güneri [1]

[1]Department of Oral and Maxillofacial Radiology, Faculty of Dentistry, Ege University, Izmir, Turkey and [2]Department of Medical Biology, Faculty of Medicine, Ege University, Izmir, Turkey and [3]Department of Medical Pathology, Faculty of Medicine, Ege University, Izmir, Turkey

*Corresponding Author; nezgiozer@hotmail.com

Abstract

Purpose: To determine adiponectin and leptin expression levels in samples from healthy, dysplastic and cancerous oral lesions and to investigate the potential role of these biomarkers in oral cancer development.
Methods: Tissue samples from the archives which were histologically diagnosed as oral squamous cell carcinoma (OSCC) (n=24), oral dysplasia (n=12) and healthy tissue (n=16) were included. The concentration and purity of samples were determined after total RNA extraction. Thereafter, cDNA synthesis was performed. The differences between expression levels were evaluated with qPCR. Expression levels related to OSCC were performed according to normalizing gene ACTB. $2 - \delta\delta CT$ method was used for comparison of expression levels. Student t-test was used to compare the differences in gene expression levels between groups, using $2 - \delta\delta CT$ value for each gene.
Results: Adiponectin and leptin expression levels were decreased by 1.09 (p = 0.49) and 10.3 (p = 0.22), respectively, in OSCC group, however differences were not statistically significant. The level of adiponectin expression decreased by 1.65 times (p = 0.62) and leptin expression increased by 3 times (p = 0.30) in the dysplasia group. When OSCC and dysplasia groups were compared, it was found that the level of adiponectin expression increased by 1.52 times (p = 0.99) and the level of leptin expression decreased by 30.94 times (p = 0.16) in the OSCC group. The samples were re-grouped as increased risk (OSCC+dysplasia) (n=36) and healthy (n=16) and a decrease by 16.5 (p= 0.3) in leptin expression and an increase by 1.14 (p= 0.6) in adiponectin expression was observed in the increased risk group compared to the healthy group.
Conclusion: In tissue samples diagnosed as healthy, dysplastic and OSCC, expression levels for leptin and adiponectin have not been compared before. The results revealed a decrease in leptin expression and an increase in adiponectin expression in increased risk group.

Key words: Adinoceptin; Leptin; Oral Cancer

Introduction

More than 90 per cent of all oral and oropharyngeal cancers are histologically diagnosed as squamous cell carcinomas (SCC).[1] Oral SCCs cause severe damage to the oral and facial anatomical structures before and after treatment. However, most SCC cases are diagnosed at advanced stages.[2] The 5-year survival rate for oral SCC after treatment is approximately 50%.[1,2] The delayed detection and recognition of these lesions by the patient or the dental practitioner and the prolongation of the diagnosis process reduce the survival rates of the disease. Studies have revealed that 30% of oral SCC patients had undergone oral cancer screening in the 3 years prior to receiving a diagnosis of oral SCC.[3] Management of advanced-stage tumors includes radical surgery involving neck dissection, radiotherapy, and chemotherapy. The surgical procedures, which result in significant loss of tissue from the head and neck area, lead to devastating psychological and sociological effects on patients. Therefore, early detection and diagnosis of oral SCC are essential to induce overall survival rates and to reduce treatment-related complications and treatment costs.[3] Molecular diagnosis/genetic studies have become more and more prominent in the prevention of oral cancer to identify risk groups before the disease presents.

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Tumor growth, invasion and metastasis is a multistep process that involves numerous genes and pathways. The molecular biology of oral SCC is rather complex. Dysfunction of any of the genetic mechanisms that have an influence on cancer development may cause oral SCC.[4] Therefore, researchers focus on verifying prognostic biomarkers that enable early diagnosis in high-risk patient groups. In genetic studies that using single nucleotide polymorphism as a biomarker, certain factors related to angiogenesis, immune response and thrombosis have been found to be effective in the development of oral cancer.[3–5] Among these factors, cytokines and adipokines that regulate inflammation attract considerable attention as potential biomarkers for their possible relationship with cancer risk.[4] Adipokines contribute to numerous physiological processes such as nutrition, appetite, energy balance, insulin, glucose and lipid metabolism, regulation of blood pressure, vascular remodeling, coagulation, and inflammation. TNF-alpha, IL-6, resistin, leptin, adiponectin, vaspin, visfatin, and omentin are the most well-known adipokines.[6,7] The relationship of adipokines with obesity and obesity-related diseases have been described clearly. Studies have been reported that obesity causes several disorders such as hypertension, type II diabetes (DM), cardiovascular diseases, osteoarthritis, and cancer. Recently, the roles of adipokines as mediators in different physiological mechanisms such as the immune system and inflammatory response, and pathological processes such as malignant tumors, have started to attract more attention.[6] Young et al. have reported that there was an inverse relationship between the levels of leptin, a proinflammatory mediator, and adiponectin, an anti-inflammatory mediator. Additionally, the serum level of leptin was shown to increase in patients with oral premalignant lesions.[8] Guo et al. have observed a significant decrease in circulating adiponectin levels in patients diagnosed with tongue cancer compared to healthy individuals. Furthermore, it has been reported that leptin or leptin receptors stimulate cell proliferation and have an influence on cancer development and metastasis.[9] Yapijakis et al. have analyzed DNA samples from tissues of 150 patients diagnosed with oral SCC and 152 healthy individuals by polymerase chain reaction and reported that leptin gene polymorphism increases the risk of oral SCC.[10] These studies suggest that leptin and adiponectin may be promising candidates as biomarkers that can be useful to determine the risk of oral cancer. Expression levels for leptin and adiponectin in tissue samples diagnosed as healthy, dysplastic and OSCC has not been compared before. The aim of this study is to determine adiponectin and leptin expression levels in samples from healthy, dysplastic, and cancerous oral lesions and to investigate the potential role of these biomarkers in oral cancer development.

Methods

Paraffine embedded tissue samples from the archives of Ege University, Faculty of Medicine, Department of Pathology which were histologically diagnosed as oral squamous cell carcinoma (OSCC) (n=24), oral dysplasia (n=12) and healthy tissue (n=16) were included in this retrospective study. The study protocol was approved by the Ethics Committee of Ege University (Approval no: 18-5/18). To determine the expression levels of leptin and adiponectin, total RNA extraction was performed using Invitrogen™ PureLink™ FFPE total RNA Isolation kit from a total of 52 tissue samples. Thereafter the concentration and purity of samples were determined with NanodropTM device (Thermo Scientific) by measuring their absorbance at 260/280 nm and 230/260 nm wavelengths. Total RNA's A260/A280 and A230/A260 absorbance ratio >2.0 and the amount of $10 \mu g/\mu l$ were included in the study. Complementary DNA synthesis from isolated RNA was performed using High-Capacity cDNA Reverse Transcription kit. The differences between expression levels of leptin and adiponectin in tissues was evaluated with qPCR. 2X TAQMAN UNIV Master MIX, TaqMan Gene Expression Assays ($Hs00226105_m1$), TaqMan Gene Expression Assays ($Hs00174497_m1$) and TaqMan Gene Expression Assays were used as control qPCR assay. Leptin and adiponectin expression levels related to OSCC was performed according to normalizing gene ACTB. $\delta\delta CT$ method was used for comparison of leptin and adiponectin expression levels. An increase in expression level was accepted in case the $2 - \delta\delta CT$ value was over 2, and a decrease in expression level was noted in case the $2 - \delta\delta CT$ value was under 1. Additionally, dysplastic and OSCC tissue samples were further analyzed together as 'increased risk' group and adiponectin and leptin levels were compared with healthy samples. Student t-test was used to compare the differences in gene expression levels between healthy, dysplastic, and cancerous groups using $2 - \delta\delta CT$ value for each gene ($p<0.05$).

Results

When compared to the healthy group, adiponectin and leptin expression levels were decreased by 1.09 (p=0.49) and by 10.3 (p=0.22) respectively in the OSCC group, however the differences were not statistically significant. The level of adiponectin expression decreased 1.65 times (p=0.62) and leptin expression increased by 3 times (p=0.30) in the dysplasia group when compared to the healthy group. When OSCC and dysplasia groups were compared, it was found that the level of adiponectin expression increased by 1.52 times (p=0.99) and the level of leptin expression decreased by 30.94 times (p=0.16) in OSCC group Figure 1. The samples were re-grouped as increased risk (OSCC+dysplasia) (n=36) and healthy (n=16) and a decrease by 16.5 (p=0.3) in leptin expression and an increase by 1.14 (p=0.6) in adiponectin expression was observed in increased risk group compared to the healthy group.

Discussion

Numerous studies have been conducted to investigate the effects of adipokines on obesity, metabolic diseases, inflammation, cardiovascular and malignant comorbidities. Leptin and adiponectin have antagonistic effects, therefore, in different types of cancer, while an increase in serum leptin concentration is observed, adiponectin level decreases.[6] It has been shown that the increase in the serum leptin level of oral SCC patients causes a decrease in apoptosis rate and accelerates cancer progression.[8] A study has reported a significant decrease in serum adiponectin levels in patients with tongue cancer compared to healthy individuals.[9] Yapijakis et al. have analyzed DNA samples obtained from tissues of 150 patients diagnosed with OSCC and 152 healthy individuals using polymerase chain reaction and reported that leptin gene polymorphism increases the risk of OSCC.[10] In our study, paraffine embedded tissue samples from the archives of Ege University, Faculty of Medicine, Department of Pathology which were histologically diagnosed as oral squamous cell carcinoma, oral dysplasia and healthy tissue were included. Adiponectin and leptin levels in identified tissue samples were determined by gene expression analysis. When compared to the healthy group, adiponectin and leptin expression levels were decreased by 1.09 (p=0.49) and 10.3 (p=0.22) respectively, in OSCC group, however the differences were not statistically significant. The level of adiponectin expression decreased by 1.65 times (p=0.62) and the leptin expression increased by 3 times (p=0.30) in dysplasia group when compared to the healthy group. When OSCC and dysplasia groups were compared, it was found that the level of adiponectin expression increased y 1.52 times (p=0.99) and the level of leptin expression decreased by 30.94 times (p=0.16) in OSCC group. When the samples were re-grouped as increased risk and healthy groups, a decrease by 16.5 (p=0.3) in leptin expression and an increase by 1.14 (p=0.6) in adiponectin expression was observed in increased risk group compared to the healthy group. Since expression levels for leptin and adiponectin in tissue samples diagnosed as healthy, dysplastic and OSCC has not been compared before, it is not pos-

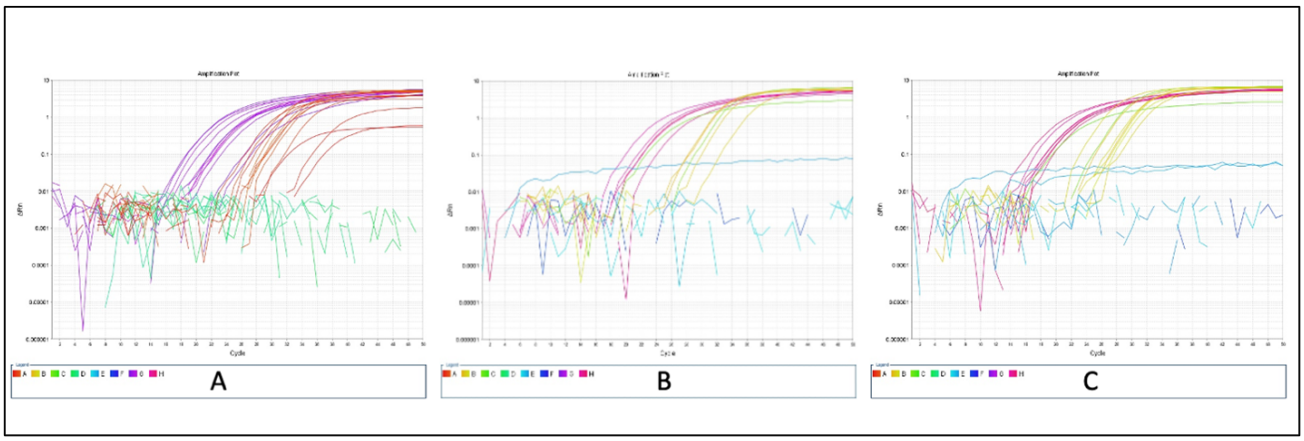

Figure 1. Expression of leptin and adiponectin in (A) OSCC, (B) dysplasia and (C) healthy tissue groups.

sible to compare our results with previous studies. The results of the present study revealed a significant decrease in leptin expression and an increase in adiponectin expression in samples from increased risk group which included dysplasia and OSCC tissue samples. These results conflict with previous studies which reported an increase in leptin expression and a decrease in adiponectin expression in serum levels of OSCC patients. The difference in the number of subjects included may account for this difference. Additionally, the type of tissue that has been investigated regarding the expression of leptin and adiponectin may have an influence on findings from different studies. It may be speculated that the presence of gene polymorphism, tissue levels and serum levels for these adipokines may differ depending on the type of sample collection and the method that have been used for analysis. It is known that the level of adipokines is closely related to several disorders such as obesity, diabetes mellitus etc. There for, it is obligatory to also consider the characteristics of the studied population when interpreting the results. Unfortunately, since this study was conducted in a retrospective manner and tissue samples were obtained from the archive, medical information for these patients were not available. Therefore, further studies with larger study samples in which demographic and medical data of subjects are included are needed to clarify the roles of leptin and adiponectin in oral cancer development. Additionally, it may be interesting to compare the level of these adipokines in both serum and oral tissue samples in the same study population.

Conclusion

The mechanisms between adipokines and oral cancer is still not fully understood. The results of the present study revealed a significant decrease in leptin expression and an increase in adiponectin expression in samples from increased risk group which included dysplasia and OSCC tissue samples. In contrast, an increase in leptin expression and a decrease in adiponectin expression in serum levels of OSCC patients have been reported in previous studies. The limited amount of tissue samples in the present study may account for this difference. Therefore, further studies with larger study samples are needed to clarify the roles of leptin and adiponectin in oral cancer development.

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Author Contributions

All authors made substantial contributions to the present study. BI and PG designed the study, AV and ATV contributed to data collection, PG, BI, ATV and NEÖ contributed to interpretation of data and editing of the manuscript. PG, BI and NEÖ wrote the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

Authors declare that they have no conflict of interest.

Authors' ORCID(s)

N.E.O. 0000-0002-5733-0954
A.T.V. 0000-0001-9890-3256
B.K. 0000-0003-3123-3272
A.V. 0000-0003-0343-9978
P.G. 0000-0001-9423-9191

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