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Proteomics in oral cancer: A systematic review

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Abstract--Background: This review aims to develop comprehensive evidence regarding proteomics in oral cancer initiation, regulation, and progress/inhibition. Material and Methods: A systematic review was carried out of all the reported literature on genomics and proteomics as biomarkers from January 2015 to December 2021 across 3 different search engines- Medline/PubMed, Google Scholar and Scopus. There were 11 articles identified after full test review for protein-based studies of oral cancer after following the PRISMA guidelines. Results: Of the 10 articles maximum of them suggested the role of interleukins (IL-6, 8 and 10) along with TNF- α as salivary protein markers in oral squamous cell carcinoma. All of the proteins showed a rise in their levels in cancer cases compared to healthy cohorts and premalignant cases. The levels of these proteins were also raised significantly in the premalignant cases compared to the healthy patients. Conclusion: A conclusion can be drawn that not all markers are raised; some show low expressions and differ according to type and severity of cancer.

Keywords---biomarkers, oral cancer, proteomics.

Introduction

Cancer is an abnormal mass of tissue which undergoes an uncoordinated and excessive growth.[1] The conventional thought process was that neoplasia is monoclonal, but the modern concept suggests otherwise. The understanding of cancer and its types has undergone a drastic change over the past millennia; with more interest in the regulation of the entire cancer progress in the oral cavity and also in metastases.[2] There is a need to understand risk predictors in proteomic factors that regulate the invitation and progression of oral cancer, more specifically, oral squamous cell carcinoma.[3] Understanding their role can help to imitate better tests for early detection and prompt treatment, especially among the high risk and vulnerable groups such as tobacco addicts and those with familial tendencies for cancer.[4–6] Biomarkers such as IL-6, IL-8, IL-10, TNF- α and β are expanded in oral cancer cases. IL-6 and TNF- α are raised even in premalignant lesions such as leukoplakia. Thus high proteomic levels of specific entities are indicators of progress or improvement among cancer cases.[7] This review aims to develop comprehensive evidence regarding proteomics in oral cancer initiation, regulation, and progress/inhibition. Also, this review aims to explore which of these biomarkers have been the most commonly associated with oral cancer.

Methodology

The review was registered with OSF (<https://osf.io/yrqz8>). Three search engines were used for the present search- Medline/PubMed, Scopus and Google Scholar. Only freely available full-text articles from January 2015 to December 2021 were used for the present review. No grey literature was included in the data collection. An initial review was done based on the title and the abstract. Then a full-text review was carried out. Only cross-sectional studies that reported the diagnostic aspects and highlighted the necessity of proteins and genetic markers in oral cancer were finalized for the data extraction. No risk of bias estimation was done for the present review. Since the data were not homogenous, pooling them for meta-analysis was challenging. Hence the present review was only systematic in nature.

Results

Ten original studies were finally selected for full-text evaluation after removing the duplicates (Figure 01)

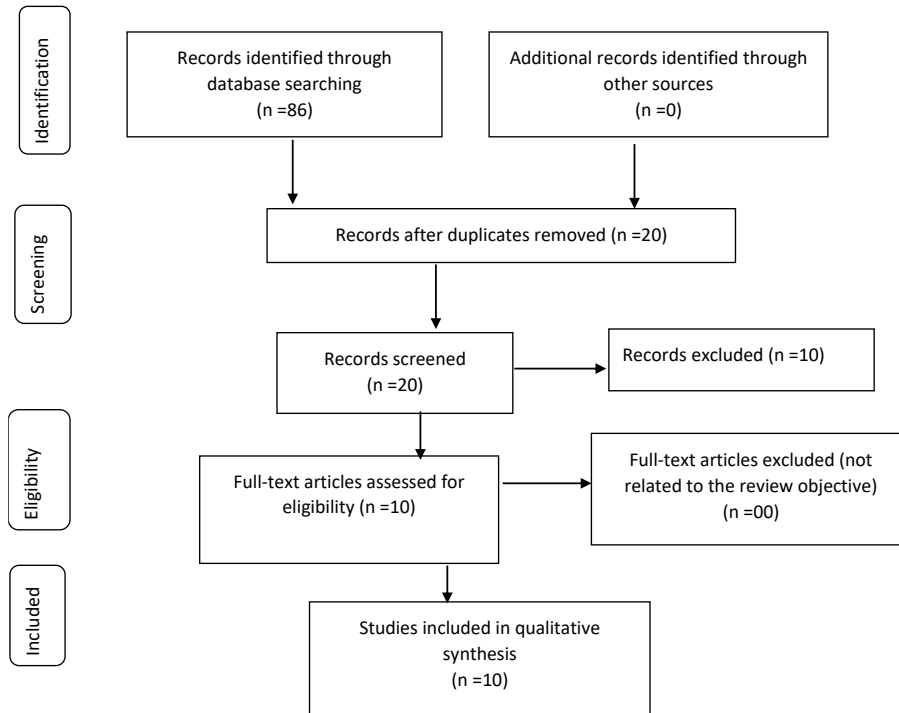


Figure 1. Prisma flow chart

There were 10 articles related to reporting of proteomics and their roles in the regulation of oral cancer (Table 01). Four of these ten studies fulfilled all eight criteria selected for quality check.[8–11] Only 40% of these studies could mention the power of the analysis.[8–11] All of the studies reported the patient characteristics and the major outcomes for the study. None of these ten articles mentioned the standardization and calibration of the examiner. It was not clear if one or all of the authors carried out the procedure. None of the studies has compared any two standard methods for cut off criteria and ease of application while carrying out the tests. Hence combining the studies in terms of methodology is challenging. Also, the kits used for the study samples were different and of different commercial make. Therefore, clarity with regards to the standard universal method is missing. The articles also don't report the standardization cut off of different methods which they employed.

Hence solely depending on the outcome while neglecting the procedure could be farfetched. Though these studies were well spread globally, reports about the European, African and American populations were missing. None of the articles suggested any further comparison of their present findings in association with the genetic predisposition of the cases or commented on the extrapolation of the data. Since the samples were chosen using convenience sampling, a generalization of the results was limited. Also, patient ethnic characteristics were not taken into

consideration. Hence any changes in the parameters due to the underlying genotype is unknown.

Table 1
Study details of reports on Proteomics

Author and year	Study type	Number of oral cancer cases	Biomarker identified	Comparison of oral cancer levels with healthy controls
Polz-Dacewicz et al; Poland; (2016)[12]	Cross sectional	78	IL-10, TNF (alpha), TNF (beta)	Oral cancer cases showed higher levels of all the three factors than the healthy controls
Gleber-Netto et al; Taiwan; (2016)[8]	Cross sectional	60	IL1- β , IL1-8	Oral cancer cases showed higher levels of the two factors than the healthy controls
DineshKumar et al; India; (2016)[13]	Cross sectional	100	IL-6	Oral cancer cases showed higher levels of IL-6 factor than the healthy controls
Chen et al; China; (2018) [14]	Cross sectional	35	thioredoxin-1 (Trx-1), glutaredoxin-1, peroxiredoxin-2	Higher levels in cancer cases as compared to controls as well as potentially malignant cases
Abbas, Rawi et al.; Iraq; (2018)[15]	Longitudinal	25	IL-7	Levels of IL-7 were more in cases at baseline as compared to post-surgery (1.5 months) and also against the controls
Lee et al.; Taiwan; (2018)[16]	Cross-sectional	41	IL-6	IL-6 levels were much higher among the cancer cases than the health control at both stage 1 to stage 4 levels of cancer
Ameena and Rathy; India; (2019)[9]	Cross-sectional	30	TNF α	Higher levels of TNF were found with cases than the controls
Deepthi, Nandan, Kulkarni; India; (2019)[10]	Cross-sectional	30	TNF α	Higher levels of TNF were found with cases than the controls
Val et al; Italy; (2019)[11]	Longitudinal	66	Ila, IL-6, TNF α , HCC-1, MCP-1, PF-4	All six factors were raised among the cancer cases as compared to the controls
Neves et al; Brazil; (2021)[17]	Cross sectional	79	Not stated clearly	Higher levels of- CTDS, CAPN1, CAPN2, MEP1A and CTCS can be

				indicators of recurrence of tumour; CTSS and CAPN2 are indicators of perineural invasion; CTSK, MMP2, MMP11 indicates worsening of condition; MMP25 indicates definitive perineural invasion
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Discussion

The major proteins were identified as risk predictors were- IL-6,7,8,10; TNF- α and β ; thioredoxin-1 (Trx-1); glutaredoxin-1; peroxiredoxin-2; HCC-1; MCP-1; PF-4; CTDS, CAPN1, CAPN2, MEP1A and CTCS; MMP-11 and 25. Across three studies [9,10,13], there is a regular increase in the levels of IL-6, 8, TNF- α . This is seen as the cases move from well-differentiated to the more poorly differentiated categories. Hence these can be considered as associated with disease aggressiveness and indicating the severity of spread. Thus, one can conclude that those cases with early stages of oral cancer; especially squamous cell carcinoma; have higher concentrations of IL-6, IL-8, IL-1 β , TNF- α , IFN- γ , MIP-1 β as compared to the levels among their healthy controls. The longitudinal study [11] also supports the evidence that IL-6, TNF α , HCC-1, MCP-1, and PF-4 are reduced post-intervention among full-blown cancer cases. The levels of premalignant cases were higher for these six factors as compared to the controls. Four studies [9–12] reported higher values of TNF- α and four studies reported higher levels of IL-6.[11,13,16,18] Since only 4 of the ten proteomics studies in the included review mentioned the power of the study, it is difficult to formulate evidence with regards to the use of proteomics alone as an aspect of detection for regulation of oral cancer.

Previous studies have also demonstrated that ILs-1, 6, and 8 produced among oral squamous cell carcinoma cases disrupt the normal physiological mechanism of the cells. They also cause stimulation of the growth and invasion processes of irregular cells, bring about inhibition of the immune system, and prevent tumour inhibition. Higher IL-6 and IL-8, and VEGF are found in tumour cells. The TNF- α , along with the IL-6 and eight, can act as endogenous mutagens, causing damage to the DNA directly by the formation of reactive oxygen species. IL- 1 β activates the carcinogenesis process by adding strength to the chemical carcinogens. This causes reproduction of the cells, which are altered and mutated and hence there is also accumulation of genetic damage in the tissues.[19] Previous reports have suggested the presence of other proteins contents such as – *STAT3*, *Repp86*, *SPARC*, *EGFR*, *CD151*, *CD9*, *VEGF*, and *SIRT2*, *p53*, *PCNA*, *Ki-67*, *E2F1*, *Cyclin D1*, and *PTEN*, *GLI3*, *EZH2*, *ER- β* , *SNAI1*, and *ZEB2*, extracellular matrix metalloproteinase inducer (EMMPRIN), *LAMC2*, and *TWIST1*, *E-CAD*, *CD44*, and *delta 1*, *Annexin A1*, *COX-2*, *14-3-3 γ* , *MENA*, *TACE*, and *Vimentin*.[20]

None of the studies in this review was able to categorize the proteins into essential groups such as those involved specifically in the cell proliferation cycle mainly either individually or in a group, proteins that assist in repression of the transcriptional cycle of the RNA, those which induce differentiation, cell adhesion

proteins, those which are associated with signals and with epithelial-mesenchymal transition. Since this cannot be concluded from the available literature, the data may not draw a plan for intervention in the cases that may be actively progressing. An array of proteins were actively involved in the process of cell migration, signaling, as well as in the process of proteolysis. Vimentin has been a protein specifically identified in abundance with the process of epithelial-mesenchymal transition. In those cases which show well-differentiated tumours, Notch signaling pathways and cytoskeleton functioning tumours such as Delta 4 and Delta 1 have been identified.

Among patients who show moderate differentiation of tumour proteins, commonly involved proteins are Wilms' tumour-associated protein, oesophageal cancer-related gene coding leucine-zipper motif, unusual cadherins, and epithelial cells associated proteins called desmosomal proteins. Interesting proteins were detected only in the PD group, including the potential oncoprotein AF1q (17) and numerous proteins involved in cell cycle control, fatty acid metabolism, and membrane trafficking.[21] Inflammation and immunity are major factors concerning the spread of tumours in the oral cavity. The data mentioned above shows that proteins are involved in expressing factors that regulate and upgrade cancer progress. Expression of these factors in the presence of other diseases or conditions is likely to confound the findings. Hence further studies are required to corroborate the findings in cases with only cancer. As far as IL-1 α , IL-1 β , IL-6, TNF- α and IL-8 are concerned, the presence or absence of habits does not affect their levels adversely. This stability can be preferentially used in cases with addiction induced cancers.

The same cannot be said about the genetic indicators also; since they seem to partially act with tobacco and increase the progress of the tumour. The results indicate that protein expression is correlated with metastasis (either regional or distant), clinical stages of the tumour indicating progression, grading correlation with the histopathological findings, tumour localization. Therefore, these protein markers could be used to classify the tumours into premalignant lesions and conditions (since their levels are more than those of healthy controls) and malignant tumours. Using these to identify and differentiate benign and malignant variety and carcinoma in situ is unclear from the reported literature. Though previous reports have suggested that the role of proteins may assist even in this classification easily.[22] These protein markers have successfully provided detailed prognoses, especially regarding salivary gland tumours and in cases of thyroid tumours with an accuracy of up to 91.5%.[23] The process of tumorigenesis has been associated with a lot of proteomics in the head and neck region. 14-3-3 γ , a member of the 14-3-3 proteins, is a family of highly conserved phosphoserine/threonine-binding proteins that regulate diverse cellular processes such as cell cycle progression and apoptosis transcriptional regulation and mainly cell proliferation.

This family of proteins acts as adaptors and chaperones.[24] The mammalian Ena proteins, also known as Mena proteins, play an essential role in the motility of the cancer cells by bringing about the antagonization of the filament actin. This protein contributes to metastasis promotion.[22] While this review hits at the role of IL-6,7,8,10; TNF- α and β ; thioredoxin-1 (Trx-1); glutaredoxin-1; peroxiredoxin-

2; HCC-1; MCP-1; PF-4; CTDS, CAPN1, CAPN2, MEP1A and CTCS; MMP-11 and 25; there are individual reports which enlist the roles of GLI1, Repp86, CD44, EZH2, H3-K27 and EMMPRIN. The systematic review by Kasradze et al. (2020) included older studies from 2000 and included the roles of these proteins as biomarkers in oral cancer.[25] Glioma associated oncogene or GLI1 is associated with activation of the process of transcription. It also mediates the Hedgehog signal pathway. Repp 86, also known as restrictedly expressed proliferation-associated protein and CD44, is actively involved with cell to cell interaction, adhesion of cell, and migration in full-blown cases of OSSC. EZH2 is another gene not studied by the authors, which acts as a catalyst in the repression complex. Histone methyltransferase that methylates lysine 27 of histone H3 (H3-K27); EZH2 is involved in the process of DNA methylation and gene silencing.

This process is greatly essential for the process of differentiation. These underline the process of carcinoma with epigenetic-genetic-proteomic patterns. Another transmembrane protein EMMPRIIN is involved in the expression of the matrix metalloproteinases. This single protein is involved in 4 major processes of tumor growth and spread, i.e. cell to cell adhesion and modulation, growth of cells, invasion and the process of angiogenesis.[20–25] The studies regarding proteomics were either conducted on the whole of the proteome or were based on immunohistochemistry, ELISA or AQUA. Different studies on diagnostics claim the superiority of each of the diagnostic procedures over the other; but none of them mention the ease of learning and performing them especially in a public health set up. Hence further studies are required to capture this aspect for better applicability of proteomics in diagnostics screening of cancer. But the studies do provide evidence to conclude that- changes in the proteins can be identified by proteomics. These have inadequate levels of expression in diverse cases of cellular processing.

These are altered with progression of cell cycle, in apoptosis, regulation of transcription, proliferation of the tumor cells, invasion (either local or distant) by the tumor cells and communication amongst the cells. This all together helps in the growth of the cancer cells. They can serve as a baseline to distinguish cases of oral cancer from the normal mucosa. Also, these can be correlated with the site of the tumor (salivary gland or mucosal lining or muscle) and also help to some extent in understanding the differentiation of the tumors from primary to fast spreading. These also help to assist to confirm the type of malignancy. Most of the studies included in this review mainly focused on oral squamous cell carcinoma. The role of proteomics in terms of other tumors such as mucoepidermoid carcinoma or in case of secondaries to the jaw from breast or large bones is yet to be explored. One also cannot comment on the economic feasibility of these tests. A battery of such tests would put more emphasis on the profile of tests and rather than the outcome. A salivary biomarker may have the short coming of being overexpressed. There is always a possibility of discontinuity of the oral epithelium and also local inflammation. This may be not always associated with cancer. The data in this review also indicates that the cytokine production is altered in the saliva among oral cancer cases.

This may not co relate to the serum concentration. If the arrays are poorly differential in nature; they can be unreliable for localized lesions. The greatest

advantage is that the tests are based on saliva sample collection. Hence sample obtaining and its handling may not be a challenge for training purposes. More studies comparing the values of such protein biomarkers across the different ethnic groups are also important. Only one of the studies elaborated on the decrease in the levels of the markers post therapy (surgical). More evidence is required to understand the levels of all the identified protein biomarkers post chemo/radio as well as surgical therapy in the different oral cancer cases. The cost effectiveness in terms of large samples has not been discussed across any of the included literature. Hence time and resources into additional training of health care workers; especially in terms of public health will be a challenge. This area needs to be explored further and cost effectiveness of the proteomics component as a biomarker for oral cancer needs to be tested at a larger level.

Conclusion

The proteins that were identified as risk predictors were IL-6,7,8,10; TNF- α and β ; thioredoxin-1 (Trx-1); glutaredoxin-1; peroxiredoxin-2; HCC-1; MCP-1; PF-4; CTDS, CAPN1, CAPN2, MEP1A and CTCS; MMP-11 and 25. All of these proteins were salivary proteins. They showed a rise in their levels in cancer cases compared to healthy cohorts and premalignant cases. On the whole, the cost involved and the logistics with training and screening of a large population at risk for oral cancer using genetic biomarkers are yet to be answered. Further data assessment and more primary studies in this regard is required.

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