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Diagnostic Accuracy of Serum Biomarkers in Oral Cancer – A Systematic Review and Meta-Analysis

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Abstract

Oral cancer is one of the most leading causes of death worldwide. Serum biomarkers can be of great help in characterizing oral cancer. Thus the aim of this systematic review and meta-analysis was to determine the diagnostic capability of serum biomarkers in the assessment of oral cancer. The search was performed by PubMed and Studies were gathered from the last 10 years. Studies which focused on serum biomarkers in the diagnosis of oral cancer were considered. Meta-analysis was carried out using R Open source scripting software .The receiver operator characteristic (ROC) curve was plotted and the area under curve (AUC) were calculated based on all included studies. In the analysis, both random effect and fixed effect model were used to calculate the ideal serum biomarker. The study with value lying between 62.07 and 70.75 is considered as the best biomarker. Only single biomarker lies between this values which is serum CIC (circulating immune complexes), therefore it is considered as the best biomarker for assessment of oral cancer.

Keywords: Serum Biomarker, Oral Cancer, Systematic Review, Meta-Analysis.

Introduction

World Health Organization defines a biomarker as “any substance, structure or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease.”[1] Classically, a marker is released into circulation after getting synthesized by the tumor and or expressed in large quantity at the cell surface by malignant cells.[2] In the recent years due to the revolution in molecular biology, the development of valid biomarkers has been a major breakthrough in the field of cancer research and care.[1] They also provide new system for early cancer detection and can be used as an auxiliary approach to assist in clinical decision making.[3] Substantial progress has been made from the discovery to the development and clinical application of biomarkers and matched

targeted therapies.[4] Biomarker targeted therapy; for example: EGFR targeted therapy for lung cancer, wild type KRAS for colon cancer,ERBB2 for breast cancer, BRAF for melanoma is already in practice as a standard treatment protocol.[5] Whereas in oral cancer, despite the numerous identified biomarkers; researches are still going on for further validation of these for stating a strong scientific evidence and clear advantage of use of these biomarker.[(6,7)]

The recent emergence of highly selective technologies has provided global information to observe genetic and proteomic alterations and to facilitate the discovery of new biomarkers with improved sensitivity and specificity.[(1,2)] The use of biomarker-based diagnostics for cancer include non-invasive screening for early-stage disease, detection and localization, risk assessment, disease stratification and prognosis, response to therapy and, for those in remission, screening for disease recurrence.[3] Biomarkers can be derived from one, or a combination of the following; body fluids blood, serum, plasma, body secretions like saliva or from the biopsied tissues.[7]Identification of biomarkers in blood/ serum has advantage over tissue because the specimen can be obtained non-invasively, and also inexpensive and have played an important role in diagnosing and surveying oral cancer compared with invasive tissue biopsies.[5,6]

Even though there is an exponential increase in the discovery of several promising diagnostic biomarkers over the last 2 decades, none of them are effectively implemented into clinical practice.[8] Hence a survey is necessary to find out the highly sensitive and specific biomarkers. Thus, the purpose of this systematic review/ meta-analysis was to answer a focused question namely: “Do serum biomarkers have the capacity to precisely distinguish Oral squamous cell carcinoma patients from non-oral squamous cell carcinoma controls?”

Materials and Methods

Search Strategy and Selection criteria

A systematic, computerized database search was conducted to search MEDLINE (PubMed). The search was conducted using the following MeSH terms: serum biomarker in oral cancer Search url: ("serum"[MeSH Terms]OR "serum"[All Fields]) AND ("biomarkers" [MeSH Terms] OR "biomarkers"[All Fields]) AND ("mouth neoplasms"[MeSH Terms] OR ("mouth"[All Fields] AND "neoplasms"[All Fields]) OR "mouth neoplasms"[All Fields] OR ("oral"[All Fields] AND "cancer"[All Fields]) OR "oral cancer"[All Fields])

Methods

Protocol and reporting

A protocol was developed and registered in the PROSPERO database of systematic reviews. This systematic review was reported in accordance with the PRISMA guidelines

Study design

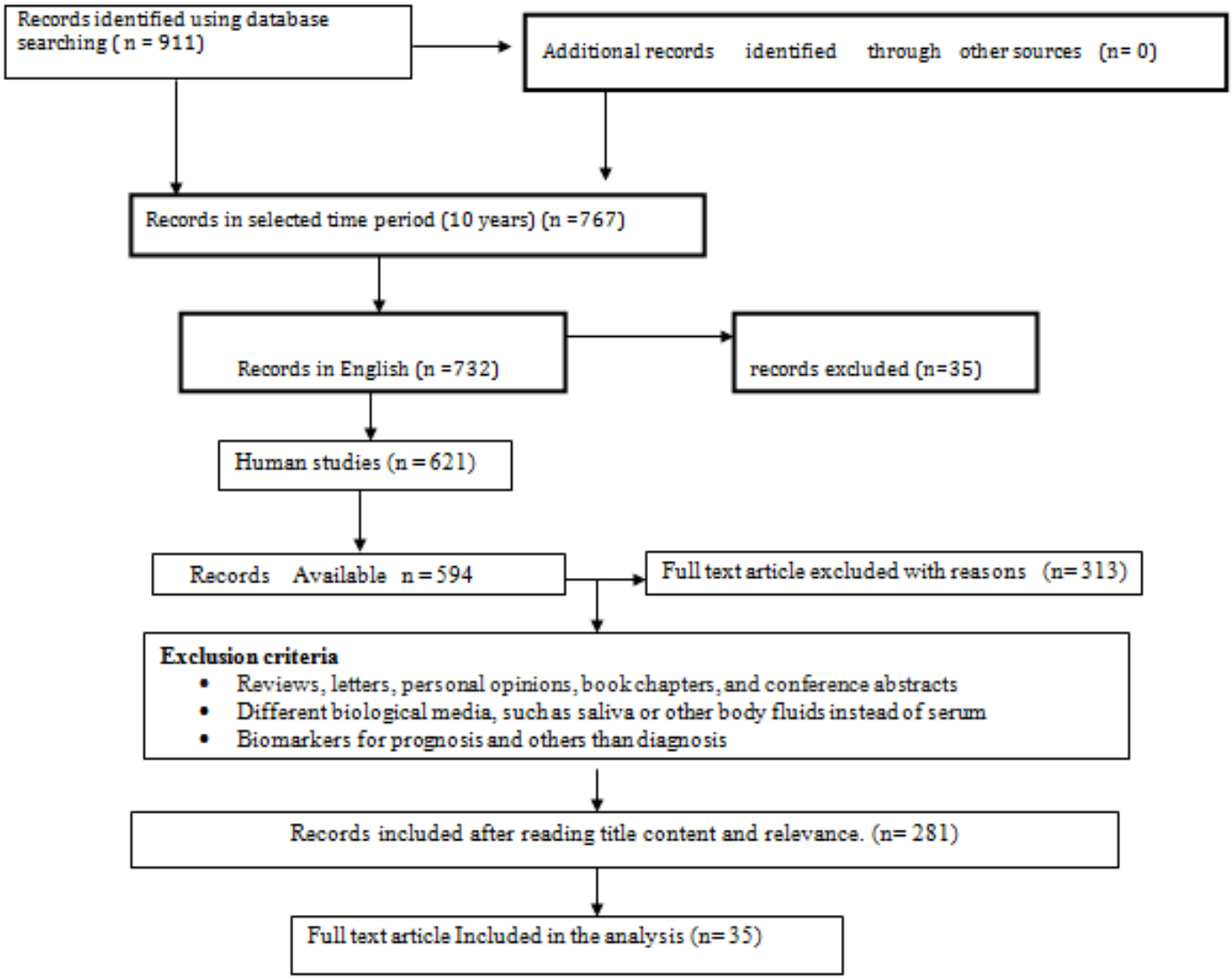
A systematic review of human studies was undertaken to summarize the results of all published studies on serum biological markers and evaluate the diagnostic value of those biomarkers for OSCC.

Inclusion criteria

Articles that focused on serum biomarkers in the diagnosis of oral cancers were selected.

Exclusion criteria

The following exclusion criteria were applied: (1) Different biological media such as saliva (2) Reviews, letters, personal opinions, book chapters, and conference abstracts.(3) Association between saliva and cancer in experimental studies (in vitro or in vivo animal studies). (4) Language restrictions. (5) Full paper copy is not available.



Flowchart I: Prisma Chart

S No	Lead Author	Biomarker	Biomarker Type	Detection Type	Sensitivity	Specificity
1	Tao Jiang (10)	Anti–Mmp-7Antibodies	Proteolytic Enzymes	Real-Time Polymerase Chain Reaction And Western Blot	0.485	0.896
2	Huanxi Xu (12)	Mir-483-5p	Rna	Microarray Analysis And (Rt)-Pcr	0.853	0.746
3	Rewa Malhotra (14)	Cyfra 21-1	Protein	Electrochemiluminescent Immunoassay (Eclia) And Ck19 Messenger Rna (Mrna) Expression In Tissue By Florescent Quantitative Rtpcr	88	78.2 %,
4	Noha A. Ghallab (15)	Chemerin And Mmp-9	Pro-Angiogenic Factors	Enzyme-Linked Immunosorbent Assays	100	100
5	Maximilian Moergel (24)	Albumin	Protein	Electron Paramagnetic Resonance Spectroscopy	72%	80%
6	Sara Ann Maclellan (25)	Micrornas	Rna	Quantitative Pcr	72%	80.0%
7	Cheng-Zhe Yang (26)	Growth Differentiation Factor 15 (Gdf15)	Protein	Enzyme-Linked Immunosorbent Assay	0.75	0.867
8	Shigehiro Tamaki (29)	Major Histocompatibility Complex Class I–Related Chain A (Mica)	Protein	Enzyme-Linked Immunoabsorbent Assay	61.50%	51.00%
9	Stefano Tiziani (31)	Metabolomics	Molecules	Nuclear Magnetic Resonance (Nmr) Spectroscopy	>95%	>95%
10	E. Schiegnitz (32)	Gdf 15	Growth Factor	Enzyme-Linked Immunosorbent Assay	0.75,	0.97
11	Maie A. R. St. John (40)	Il-6 And/Or Il-8	Glycoprotein	Quantitative Realtime Polymerase Chain Reaction Analysis	Il-8 Saliva Protein= 86 Il-6 Serum Protein =57 Il-8 Saliva Protein =99	Il-8 Saliva Protein= 97 Il-6 Serum Protein =100 Il-8 Saliva Protein =90
12	Yang Li, (41)	Rna Biomarkers	Rna	Quantitative Polymerase Chain Reaction (Pcr)	Sensitivity (91%)	Specificity (71%)
13	Chia-Jung Yu (42)	Gbp1		One-Dimensional Gel Electrophoresis In Combination With The Nano Liquid Chromatography Tandem Mass Spectrometry (Gelc Ms/Ms) Approach	Gbp1= 78.9%	Gbp1= 54.1%,
14	Ashish Gupta (43)	Metabolites	Molecules	H Nuclearmagnetic Resonance (1h Nmr) Based Metabolomics	Glutamine, Propionate, Acetone, And Choline Of Cancer Cases 93.5% Hc Vs. Oscc = 8 93.8% 0.980 90.9% 96.0%	Glutamine, Propionate, Acetone, And Choline Of Cancer Cases 93.5%

Table 1: Sensitivity and Specificity

S. No	Biomarker Category	Serum Biomarker	Biomarker Type	Detection Method
I	Genomics	Mir-483-5p	Rna	Microarray Analysis And (Rt)-Pcr
		Micrornas (Mirnas)Mir-16, Let-7b, Mir-338-3p, Mir-223, And Mir-29a ., Mir-223 Yielded An Auc Of 0.81 (95% Ci: 0.69–0.92) With 60.0% Specificity And 96.2% Sensitivity , Mir-16 Yielded An Auc Of 0.84 (95% Ci: 0.73–0.94)With 93.3% Specificity And 61.5% Sensitivity, And Let-7b Yielded An Auc Of 0.82 (95% Ci: 0.71–0.93) With 80.0% Specificity And 80.8% Sensitivity.	Rna	Quantitative Pcr
		Circulating Microrna-21	Rna	Quantitative Real-Time Rt-Pcr (Qrt-Pcr)
		P53	Gene.	
		C-Erbb-2	Oncogene	Enzyme-Linked Immunosorbent Assay (Elisa)
II	Proteomics	Cyfra 21-1	Protein	Electrochemiluminescent Immunoassay (Eclia) And Ck19 Messenger Rna (Mrna) Expression In Tissue By Florescent Quantitative Rtpcr
		Ceruloplasmin	Glycoprotein	Erba Chem 5 Plus
		Epidermal Growth Factor Receptor (Egfr)	Glycoprotein	Enzyme-Linked Immunosorbent Assay
		Sialic Acid	Glycoproteins	Diphenylamine Method
		Gelsolin, Fibronectin,Angiotensinogen And Haptoglobin	Protein	Tandem Mass Spectrometry And Isobaric Tagging
		Label-Free Serum Proteomics	Protein	Quantitative Proteomic Analysis (Hdms
		Galectin (Gal)-1 And Galectin (Gal)-3	Proteins	Quantitative Real-Time Pcr
		Survivin, Carcinoembryonic Antigen (Cea) And Erbb2	Proteins	Usingenzyme-Linked Immunosorbent Assay
		Leucine-Rich A2-Glycoprotein (Lrg), Alpha-1-B-Glycoprotein (Abg), Clusterin (Clu), Pro2044, Haptoglobin (Hap), Complement C3c (C3), Proapolipoprotein A1 (Proapo-A1), And Retinol-Binding Protein 4 Precursor (Rbp4)	Proteins	Two-Dimensional Gel Electrophoresis (2-De) And Silver Staining
		Growth Differentiation Factor 15 (Gdf15)	Protein	Enzyme-Linked Immunosorbent Assay
		Major Histocompatibility Complex Class I–Related Chain A (Mica)	Protein	Enzyme-Linked Immunoabsorbent Assay
		Serum Albumin	Protein	Bromocresol Green Method
		Serum Adenosine Deaminase (Ada)	Protein	Calorimetric Method Of Galanti And Guisti
		Albumin	Protein	Electron Paramagnetic Resonance Spectroscopy
		Serum N-Glycomes And Anti-Carbohydrate Antibodies	Antibodies	Maldi-Tof-Mass Spectrometry
		N-Glycomes And Anti-Carbohydrate Antibodies	Protein And Antibody	Maldi-Tof-Mass Spectrometry
		Serum Ferritin, Copper And Zinc	Micronutrients	Immunoenzymatic Kits,Colorimetric Test
		Glutathion	Intracellular Peptide	Pectrophotometry
		Il-6 And/Or Il-8	Glycoprotein	Quantitative Realtime Polymerase Chain Reaction Analysis
III	Metabolomics	Fucose	Sugar	Uv-Visible Spectrophotometry Based On The Method As Adopted By Winzler Using Cysteine Reagent
		Anti–Mmp-7 Antibodies	Proteolytic Enzymes	Real-Time Polymerase Chain Reaction And Western Blot
		Lactate Dehydrogenase	Enzyme	Autoanalyzer For Spectrometry
		Serum Il-17f Combined With Vegf	Inflammatory Cytokine	Qrt–Pcr
		Chemerin And Mmp-9	Pro-Angiogenic Factors	Enzyme-Linked Immunosorbent Assays
		Cxcl12, Cxcr4	Chemokines	Elisa
		Total Sialic Acid	Monosaccharides	
		Circulating Immune Complexes, (Copper, Iron And Selenium)	Trace Elements	Oxalyl Dihydrazide Method, Colorimetric Dipyridyl Method And The Differential Pulse Cathodic Stripping Voltametry
		Lipid Bound Sialic Acid	Monosaccharides	Spectrophotometry
		Metabolomics	Molecules	Nuclear Magnetic Resonance (Nmr) pectroscopy
		Gdf 15	Growth Factor	Enzyme-Linked Immunosorbent Assay
		Serum Fucose	Monosaccharides	Cysteine Reagent
		Serum Levels Of Copper, Iron And Circulating Immune Complexes (Cics)	Molecules	Colorimetric And Spectrophotometric Methods
	Genomic &Proteomic	Long Non-Coding Rnas (Lncrnas- Ac007271.3, Ac007182.6, Loc283481, And Rp11-893f2.9), Tumor-Specific Growth Factor (Tsgf),And Squamous Cell Carcinoma Antigen (Scca)	Lncrna Is Rna Scca & Tsgf Is Protein	Lncrna By Microarray Analysis & Real-Time Quantitative Pcr. Scca & Tsgf By Elisa , ,

Table 2: Biomarker Type And Detection Method

Quality rating

A methodological quality rating was performed according to the PRISMA statement criteria in order to verify the strength of scientific evidence in clinical decision-making.

Information sources and search strategy

Detailed individual search strategies for each of the following bibliographic databases were developed: PubMed

Study selection

Results from the database search were transferred into a document making up one long list of publications. Publications written in English were assessed for duplicates. After this, authors independently screened the titles and abstracts for potentially relevant publications. Disagreements were discussed and in cases of doubt, the discussion was settled by 3rd author

Eligibility criteria

Data extraction was extracted independently by the first author. The study selection was completed in two phases. In phase one; the author reviewed the titles and abstracts of all the references independently. The author selected articles that appeared to meet the inclusion criteria based on their titles and abstracts. Any doubts in selecting the article were resolved by the second author. In phase two, both authors independently evaluated all full articles to determine whether the data as presented enabled these diagnostic assessments to be extrapolated.

Data collection process: Two authors collected the required information from the selected articles. A Third author (Lalitha) crosschecked the collected information and confirmed its accuracy. For all of the included studies, the following information was recorded: study characteristics (author, year of publication, country), population characteristics (sample size, cases of HNSCC and controls), age (mean and range), study characteristics (type of serum biomarkers) and main conclusion

Risk of bias in individual studies

Two authors independently assessed the quality of each included study. Disagreements were resolved by a third reviewer.

Results

Search results and study characteristics

Summary of our systematic search is interpreted in table I and flowchart I. Records identified using PUBMED database searching were included in this systematic review. A total of 911 records were identified of which only 767 records were published in the last 10 years. 35 studies were excluded as they were not in English . Out of the rest 732 records, only 621 were human studies. When titles and abstracts were reviewed, 334 full text articles were excluded with reasons. (Reviews, letters, personal opinions, book chapters, conference abstracts, different biological media, biomarkers for prognosis). Hence total of 200 records were obtained out of which sensitivity and specificity were mentioned in only 35 articles. Therefore overall 35 articles were included in this metanalysis. Individual characteristics of the included 35 studies are summarised in Table 1. Studies that have sensitivity and sensitivity values are summarized in table II. All the biomarkers and detection methods from the included studies are mentioned in table III.

Meta-analysis was carried out using R Open source scripting software (version 3.4.3, R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/ 34). The inbuilt packages used for analysis were Meta and Meta R packages. In the analysis, both random effect and fixed effect model were used to calculate the ideal serum biomarker for the treatment of

oral squamous cell carcinoma and I^2 statistic (to measure inconsistency). The τ^2 statistic was also calculated to measure the heterogeneity among the studies. Number of studies combined: k = 22. The mean of fixed effect model is 0.1996 [95 % confidence interval (0.1904; 0.2087)].As there is heterogeneity, the results of the Random effects model is considered. The random effects model is 66.4108. [Confidence interval of 95%, (62.0734, 70.7481)]. Hence, the study with value lying between 62.07 and 70.75 is considered as the best biomarker. Only single biomarker lies between this values which is serum CIC(circulating immune complexes), therefore it is considered as the best biomarker for assessment of oral cancer.

Cochrane Q test was used to assess heterogeneity of effect-size estimates from the individual studies. Cochrane Q value was found to be 72209.39 and the degree of freedom (df) was 21. (P value: 0). Inverse variance method, DerSimonian-Laird estimator for tau^2 and Untransformed (raw) means were the meta-analytical method used.

A Receiver Operating Characteristic curve (or ROC curve) was plotted with the sensitivity and specificity values. It is a plot of the true positive rate/Sensitivity against the specificity for the different possible cut-points of a diagnostic test. When AUC is 0.667, it means there is 66.7% chance that model will be able to distinguish between specificity and sensitivity values. An ROC curve demonstrates several things: It shows the tradeoff between sensitivity and specificity (any increase in sensitivity will be accompanied by a decrease in specificity).The closer the curve follows the left-hand border and then the top border of the ROC space, the more accurate the test. The closer the curve comes to the 45-degree diagonal of the ROC space, the less accurate the test. The slope of the tangent line at a cut-point gives the likelihood ratio (LR) for that value of the test. The area under the curve is a measure of text accuracy.

Discussion

Summary of evidence

This systematic review investigated whether serum biomarkers can be useful in the diagnosis of oral squamous cell carcinoma. Despite the increasing number of researches done on biomarkers for OSCC, there is no clear evidence regarding which assays constitute the most accurate type of biomarker (i.e., proteins, nucleic acids, or metabolites), which possess the best diagnostic value, or which is best detection method to use.

Of the 35 records included in this review, a total of 47 biomarkers are identified from various studies. These biomarkers can be categorized into four types: genomics, proteomics, metabolomics and genomic & proteomic. The summary of the biomarker types analysed in this review is given in table II. Few biomarkers were investigated in more than one study which include “albumin (R METGUD, MAXIMILIAN MOERGEL), [33, 24] sialic acid (MANJIRI JOSHI, SONIKA ACHALLI),[27,20] copper (SUNALI S KHANNA, RITU TIWARI),[28,36] GDF15 (CHENG ZHE YANG, SCHIEGNITZ) [26,32] 2 times each. In the reviewed 35 studies 24 articles have studied on single biomarkers (TAO JIANG,TREVILLE PEREIRA, HUANXI XU, REWA MALHOTRA, PALAK H, LAURA ZANOTTI,ATESSA PAKFETRAT, SONIKA ACHALLI, MAYANK SARASWAT, MAXIMILIAN MOEGEL, CHENG ZHE YANG,MANJIRI JOSHI, SHIGEHIRO TAMAKI, KINNARI B, STEFANO TIZIANI, SCHIEGNITZ, R METGUD, RAJKUMAR N, DEEPAK, POOJA SINGH, YANG LI, CHIA JUNG

YU,ASHISH GUPTA,GOKUL SRIDHARAN) [10 ,11, 12,14,16,17,18,20,21,24,26,27,29,30,31,32,33,34,35, 37,39,40,41,42] whereas the rest 11 (MARYAM BAHARVAND, LIANG DING, NOHA A GALLAB, FATEMAH LAVAEE, SHU XIA LI, YENG CHEN, SARA ANN MACLELLAN, SUNALI S KHANNA, RITU TIWARI, MAIEA R, CHIA JUNG YU) [9,13,15,19,22,23,25,28,36, 38,43]have studied on more than one biomarker. Out of the 36 researches, biomarker chemerin and MMP-9 (NOHA A. GHALLAB) [15] shows excellent sensitivity and specificity which is 100% each. Anti MMP antibodies are the ones with least sensitivity (48.5%) (TAO JIANG)[10]. MICA (major histocompatibility complex class I related chain A) has the least specificity (51%) (SHIGEHIRO TAMAKI)[29].This study revealed that single biomarkers resulted in diagnostic values with higher sensitivity and specificity than combined biomarkers.Chemerin,MMP-9(matrix metalloproteinase) ,RNA, metabolites and metabolomics had sensitivity of ≤ 90% whereas the others reported with a sensitivity of less than 90%. Specificity of only GDF 15, H nuclear magnetic resonance based metabolomics; IL -8, glutamine propionate, acetone and choline was ≤ 90% whereas other showed a specificity value of less than 90%.

Chemerin also called as tazarotene -induced gene 2, is a novel member of adipokines. They are found as natural ligand of the previously orphan receptor ChemR23. In plasma they circulate as prochemerin (inactive precursor) which is activated by extracellular proteases. Chemerin stimulate intracellular signal path such as p38 causing the regulation and induction of proinflammatory cytokines such as interleukin-1β and tumor necrosis factor alpha. [15]

MMP-9 is family of zinc-dependent endopeptidases. Studies have reported that MMP-9 polymorphism is associated with increased risk for developing oral cancer. [15]

As per our analysis serum CICs were found to be the best biomarker in diagnosing oral cancer. Chester K et al for the first time suggested CIC as a useful tumor marker system. They stimulate local secretion of cytokines by binding to inflammatory cells. They also cause secretion of vasoactive mediator's thereby increasing vascular permeability, adhesion of leukocytes to the endothelium leading to amplification of disease and tissue injury. Elevated levels of CICs were observed in advanced stages of oral cancer. [36]

There are certain limitations in this meta-analysis that should be taken into account while interpreting our results. Although we tried our level best to include all the relevant studies, it is possible that we may have missed some significant studies. Many of the studies had to be excluded due to lack of reporting sensitivity and specificity.

Conclusion

This review demonstrates that serum biomarkers emerge as potentially promising in the diagnosis of oral cancer. The biomarker that has been proven to be more effective in diagnosing oral cancer is serum CIC. Therefore, by this meta-analysis we have concluded that serum CIC might be used as screening tests for OSCC. Nevertheless, further studies and greater improvements of serum CIC in diagnosis are also required to validate this marker.

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