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Metabolomics approaches in oral submucous fibrosis : Areview

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ABSTRACT

The present reviewexploresthe applications of "OMICS" approaches in the diagnosis and treatment of an oral pre-malignant disease (OPMD) named Oral Submucous Fibrosis (OSF). The study is mainly concentrated on "omics" techniques leading to its identification of novel biomarkers and molecular signatures that are capable of diagnosing OSF and thus are applied in clinical practice for the proper treatment.Various "Omics" techniques were applied to differentiate OSF from control cases in many research works. Several online databases (PubMed, Google Scholar, and Springer) were explored for a detailed study of the papers of these research works that are being published in the last 20 years. The searches were made as "omics + OC diagnosis", "metabolomics + OPMD", "OSF + biomarkers", "metabolic profiling of OSF + control group", "Modern OC therapies", "Molecular approaches + OSF" etc.From the various reported literature on OSF detection and treatment, the obtained result is that metabolic signatures are found inthe saliva, serum, and tumor tissue samples of oral cancer patients that are capable to differentiate among oral cancer, oral-precancerous lesions, and healthy control individuals.Though there are various advancements of Oral Cancer (OC) therapies in the past few decades, the traditional methods of diagnosis that are still in practice can detect oral pre-cancerous conditions like OSF only at their advanced stage,whendisease becomes incurable. Thus, a need for early detection of these diseases before their progression towards OCis essential,at least inpre-malignant conditionswhere it can only be diagnosed by metabolic profiling of the biomarkers. Modern OMICS strategies hold the potential to serve this need by giving a major contribution to metabolomic profiling.

1.1. Introduction

The most common type of cancer worldwide is Oral Cancer (OC) which shows a higher prevalence in males than in females [Chaturvedi, P., et al., 2013]. Almost 1.7 million new cancer cases were diagnosed worldwide in 2019. Among these, OC ranks third in India and the sixth most abundant type of cancer in the world [Lu, M. and X. Zhan, 2018].OSF is an irreversible, chronic premalignant lesion that is characterised by scarring and hardening of the oral mucosa. Though its pathogenesis is unknown to date it is suspected to be induced by the use of tobacco, the habit of chewing betel quid, smoking, consumption of alcohol, and chronic inflammation [Rajalalitha, P. and S. Vali, 2005].

Over the past few decades, the prevalence of

OSF in India increased from 0.03-6.42% [Rajalalitha, P. and S. Vali, 2005]. The disease that was previously constricted only in South East Asia and India has now gained its popularity throughout the United Kingdom, the USA, and other developed countries, thus, is a serious problem to global health [Hazarey, V., et al., 2007.]. The most threatening part of OSF is that it has a high malignant transformation rate that has increased by 7.6% within a span of 17 years [Rai, V., et al., 2018]. The transformation of OSF to OC involves altered thickness of the epithelium and keratin proteins, the presence of micronuclei during dysplasia along with maturation, and interaction of collagen fibers with mast cells and myofibrils [Arakeri, G., et al., 2017]. The sites that are most commonly affected due to OSF are as follows- labial and buccal mucosa, soft palate, retromolar pads, the floor of the mouth, pharynx, esophagus as well as para tubal muscles in eustachian tube of the ear [Rajalalitha, P. and S. Vali, 2005].Alist of histopathological changes observed in oral mucosa during different stages of progression in OSFis presented in Table 1. These are the signs and symptoms observed by clinical practitioners or dentists to diagnose OSF.

Table 1. Depicts histopathological Grading of OSF [7]

Very early (stage I)	Early (stage II)	Moderately advanced (stage III)	Advanced (stage IV)
Dispersion of collagen fibers accompanied by edema	Juxta-epithelial hyalinization	Moderately Hyalinised collagen present	Collagen completely Hyalinised
Blood vessels are often dilated and congested	Blood vessels are dilated and congested	Blood vessels are normal and constricted	Blood vessels are destroyed and narrowed
Strong fibroblastic responses	Presence of young fibroblast cells	Less fibroblastic response; adult fibrocytes seen	Hyalinised areas are devoid of collagen
Normal and non-keratinized epithelium	Shortening of epithelial rete-pegs followed by keratinization	Atrophic epithelium with loss of rete-pegs and muscular degeneration	Epithelial dysplasia with degeneration of muscle cells (malignant transformation)

1.2. Materials and Methods

Extensive studies of related literature are exploredmainly based on the keywords Omics, Metabolomics, OC, OSF on the search engines like Google Scholar, Springer, and PubMed from 2000 till aug 2020. Relevant papers containing specific information about OSF diagnosis are selected, summarized and presented in this review article.

1.3.Molecular signatures of OSF based on metabolomics

Prerequisite of biomarkers for early dectection of OSF is need of the hour. The progression of

OSF towards malignancy is a very subtle process that does not exhibit any early signs or symptoms. India has seen a rise of OSF cases from 25000 to 2 million from 1980 to 1993 [Rai, V., et al., 2018]. Image analysis is used by physicians for diagnosing OSF. Since, ischemia or hypoxia does not occur in this disease, so it becomes very difficult to assess it accurately unless significant stromal changes appear [Rai, V., et al., 2018, Bari, S., et al., 2017]. Presently, doctors rely on incisional biopsy to confirm OSF[Guta, M. and S. Mhaske, 2008]. But the process being invasive and painful is mostly not preferred by patients. Moreover, biopsy can only be done once the symptoms or lesions arise. By that time, the disease becomes incurable [Rajendran, R., et al., 2005]. Under such circumstances, development of non-invasive diagnostic procedure over surgical procedure is significantly important [Lee, C.-K., et al., 2009].The statisticsof the published papers on various samples are embodied from 2000-2020 in (Fig.1).

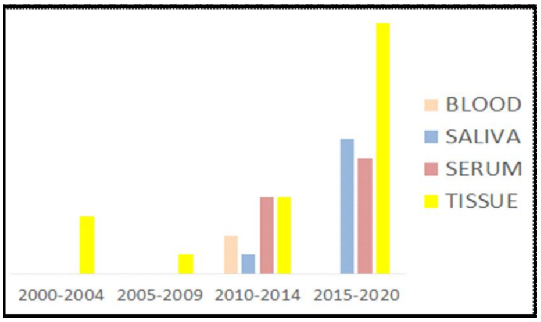

Figure 1. Graphical representation of number of studies conducted on OSF on the biological specimens from 2000-2020

1.4.Molecular Approaches for OSF

Juxta-epithelial inflammatory reactions in OSF are often succeeded by the fibro-elastic changes that occur in lamina propria which is also accompanied by epithelial atrophy [Lee, C.-K., et al., 2009].miRNAs regulate both the transcriptional and posttranscriptional regulatory mechanisms of epithelial-mesenchymal transition (EMT) and thus may serve as biomarkers and therapeutic targets for EMT-based pathological conditions, including OSF[Pandya, S., et al., 2009]. There is a notable rise in p63 level and CD105 expressions in OSF that is accompanied by loss of E-cadherins in the oral mucosal membrane [Zhou, S., et al., 2017]. These above mentioned molecular markers of OSF can easily be assessed by computer-aided quantitative assessment framework based on the altered status of cellular lesions, neo-angiogenesis and hypoxia in malignant potential diseases like OSF [Anura, A., et al., 2016].

1.5. “Omics” Science in OSF Detection

Among various developments in modern biological era, one of the most notable works are applications of “omics” approaches that include genomics, transcriptomics, proteomics and metabolomics in detecting abnormalities at the level of DNA, RNA, protein, metabolite and development in the field of medical imaging [Anura, A., et al., 2016]. OSF during its progression towards malignancy cause copy number changes in chromosome along with loss of heterozygosity (LOH) [Lu, M. and X. Zhan, 2018]. Genetic sequencing have shown p53 expression in precancerous cells have positive correlation with its increase in malignant

transformation [Bathi, R.J., 2003]. Various changes are observed at protein expression level along with their structural modification and deferred activity during conversion from healthy cell into neoplastic cells. Altered localization of these proteins affects cellular functions and can easily be diagnosed by cancer proteomics [Srinivas, P.R., et al., 2001]. RT-PCR, Western Blotting and other immunohisochemical analysis are applied to observe CYPA (Cyclophilin A) expressions, a potential biomarker of OSF. Results revealed that cell proliferation and apoptotic processes are stimulated by RNA interferences due to inhibition of CYPA expressions [Hou, X., et al., 2017].Quantitative proteomics using isobaric tags for relative and absolute quantification (iTRAQ) for tissues showed that ANXA4 and FLNA are proteins found to be up regulated in OSF [Liu, W., et al., 2016]. Other studies using proteomic analysis with two dimensional electrophoresis (2DE) and MALDI TOF mass spectrometry showed that Hsp 70 1B, Calreticulin, and Lumican variant levels in OSF tissues were significantly increased whereas Enolase 1 was decreased[Das, T., et al., 2018]. Various technologies examined for different biomarkers in the last 20 years are listed in Table 2.

1.6.Metabolomics in OSF

Metabolism is defined as a collection of processes that generate energy and various cellular level building blocks by the cell utilizing the molecules, substances, and nutrients accumulated from the surroundings. These products formed along with the various cellular intermediates formed during these processes constitute the metabolites [Adeola, H.A., et al., 2017]. Metabolomics is the detailed

Table 2. Summarises modern techniques along with different biomarkers identified and applied for detection of OSF.

Technique	Observed parameter	Samples examined	References
Two-dimensional gel electrophoresis and Mass Spectroscopy	Protein levels	Tissue and Serum	Srinivasetal.,2001[17]
Staining	P53 expression	Tissue	Bathi etal.,2003[16]
Next-generation sequencing (NGS)	DNA, RNA and Protein metabolites	Tissue	Luetal.,2016 [2]
Optical Coherence Tomography (OCT)	Epithelium Thickness	Tissue	Leeet al.,2009[12]
Computer based quantification of chromogenic immune-histochemical (IHC) images	Cytoplasmic, nuclear and stromal expression	Tissue	Anura et al,2016 [15]
NanoLC MALDI MS/MS	Lipid metabotypes	Tissue	Bagetal.,2016[21]
Itraq	Protein expression	Tissue	Liuetal.,2016[19]
2-DE MALDI TOF MS	Protein expression	Tissue	Dasetal.,2018[20]
FTIR	Metabolic Profiling	Serum	Rai et al., 2019[22]
^{1}H Nuclear Magnetic Resonance Spectroscopy	Glucose metabolytes	Serum	Raietal.,2019[23]

analysis and technical study of these substances with low molecular weights called metabolites present in cellular level, tissue level or of whole organisms and are dependent upon can be manipulated by various factors. It enables the analysis of a wide range of exogenous and endogenous metabolites which include substances like lipids, peptides, amino acids, nucleic acids, vitamins, organic acids, carbohydrates and thiols[Kapila, Y.L., 2015].

Upregulation of miRNA in serum [Singh, P., et al., 2018], over-expressed alpha-enolase [Bag, S., et al., 2018] are some of the indicators of OSF observed in metabolic profiling.

1.7."Omics" Application in OSF diagnosis

5-aminolevulinic acid (ALA) induced cellular accumulation of protoporphyrin IX (PpIX, the photosensitizer) issuccessfully used todistinguish between normal and neoplastic cells andtodiagnose tumour tissues. Though Auto fluorescence spectroscopy can also diagnose malignant cells but cannot differentiate pre-malignant conditions like OSF from normal cells.Due to atrophic epithelium in pre-malignancy, the conversions of ALA into PplX in epithelial cells are very specific and thus results in accurate diagnosis [Wang, C.-Y., et al., 2009]. Optical coherence tomography (OCT) is a non-invasive, real-time, three-dimensional imaging technique that can replace incisional biopsy for diagnosis of OSF. The scanning images from Swept-source OCT (SS-OCT) shows that the boundary between epithelium (EP) and lamina propria (LP) becomes smoother due to thinning of EP layer in collagen-rich OSF tissues while EP-LP boundary is reported to be irregular in control cases [Bag, S., et al., 2016].

Combination of *invivo* fluorescence spectroscopy with principal component analysis (PCA) and partial least square discriminate analysis (PLS-DA) provide a rapid, acute, cheap, non-invasive and technically advanced procedure for screening of OSF [Musharraf, S.G., et al., 2016]. Matrix Assisted Laser Desorption Ionization Mass Spectrometry (MALDI MS) can indicate the alterations in lipid biogenesis during OSF and cancer by significant changes in expression of crucial lipid metabolites. Gas chromatography-Mass spectroscopy (GC-MS) along with chromo-metric analysis reveals the up-regulated fatty acid (FA) synthesis and reduced amount of histidine, threonine, arginine, tyrosine, isoleucine, leucine and glucose in OSF tissues along with increased levels of alanine and methionine [Goel, R., et al., 2014, Misra, B.B., et al., 2019].^{1}-H nuclear magnetic resonance (NMR) spectroscopy revealed up-regulated glucose metabolism, increase in lipid metabolites and considerably altered FA metabolic pathway as a potential biomarker for OSF detection [Rai, V., et al., 2019].

1.8. Discussion

The present review extensively discussed the "Omics" studies that is analysis of high-throughput data like protein-protein interaction, protein-DNA interaction or allosteric regulation of biological sample with decreasing cost and time to reveal critical biological network and help us to promoteour knowledge in abnormality detection as well as treatment.

Elaborate biochemical and genetic changes were observed in oral mucosa when it progresses towards hyperplasia and then advances up to metastasis. Variety of transcription factors that gets altered during malignancy are now considered as potential biomarkers and are studied through Western Blotting, RT-PCR and other image analysing techniques. There is also considerablechanges in the metabolic products in OSF patients that are studied and comes under the field of metabolomics. Spectroscopy, optical coherence tomography (OCT), chromatography and nuclear magnetic resonance (NMR) are some of the techniques thatprovide us with information regarding the metabolic profile of an individual. The altered levels of glucose, lipid and amino acid regulation detected via these techniques benefitus to distinguish betweennormal individualsandthat of OSF or OSCC patients.

1.9. Conclusion

From the above discussion it may be resolved that in modern times, metabolic profiling of biomarkers present in blood, saliva and serum have proved their significant need in detection and treatment of OSF. A number of studies performed in the last decadeidentified the deregulations of those biomarkers taking place in OSF that are capable to differentiate them from normal individuals. Detection of OPMD cases at such an early stage makes OSF curable and the non-invasive techniques used in their treatment and detection makes it more acceptable to the patients since there is no risk of infection, excessive blood loss and pain. The use of advanced techniques like HPLC, GS-MS, OCT, NMR and FTIR has also made the diagnosis cost-effective thus, can be used foreconomically backward people towardstreatment of OSF. Higher specificity and accuracyof these advanced technologies on the way to detecting novel metabolomic markers and molecular signatures hold enough potential to be applied in clinical medicine.

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