Expression of miR-21 in Saliva of Oral Submucous Fibrosis

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Abstract: *miRNAs are short (19-24 nucleotides in length) non coding RNAs that regulate messenger RNA (mRNA)or proteins levels either by promoting mRNA degradation or by attenuating protein translation. miRNA are good biomarkers.[3]Objectives: The present study was aimed to quantitatively investigate miRNA-21expression in saliva and exfoliative cells of osmf patients and to compare miRNA-21expression in saliva and exfoliative cells of osmf.* <u>Methods</u>: *A total no of 40 subjects were selected for the study, out of which 20 were controls and 20 were patients with oral submucous fibrosis. Based on the clinical and histopathological evaluation, these patients were confirmed for oral submucous fibrosis. The samples were subjected for analysis of the presence of miRNA using real-time PCR.* <u>Results</u>: *The results of the study showed that out of 20 study salivary and exfoliative cells samples, 14 salivary samples and 11 exfoliative cells showed the miRNA expression and whereas no control samples showed miRNA. After the analysis we found that the 70% of study sample showed positive results for miRNA in saliva whereas 55% in exfoliative cells.Clinical relevance: miRNAs in body fluids are currently extensively explored for their potential as non–invasive diagnostic tumour markers. Tumour specific miRNAs may improve oral and pre-cancer diagnosis & prognosis. saliva can be used as a potential sample for evaluating miRNA-21.*

1. Introduction

Oral submucous fibrosis (OSF), first described in the early 1950s, is a potentially malignant disease predominantly seen in people of Asian descent. It is a chronic progressive disorder and its clinical presentation depends on the stage of the disease at detection. [4]The etiology of the disease remains elusive till today. A variety of etiological factors have been suggested which include betel nut, capsiaicin, malnutrition and vitamin imbalance, autoimmunity, viral and genetic predisposition. Micro RNAS (miRNAs) are small non coding RNAs that mediate gene expression at the post transcriptional level by degrading or repressing target messenger mRNAs (mRNA). Each miRNA has the ability to regulate many target genes in humans modulating the levels of mRNAs. They are encoded by genes located in non coding regions or in introns of protein coding genes and have been associated with the pathogenesis of oral cancer.[3] Over expression of oncogenic miRNAS result in reduced tumour suppressor proteins and loss of tumour suppressor miRNA expression may cause elevated levels of oncogenic protein which will ultimately result in impaired apoptosis, cell differentiation, increased proliferation and metastasis. (Gomes and Gomez, 2008).[26] Much of the work carried out using miRNA in head and neck cancer has used cancer cell lines and micro array technology (Tran et al, 2007; Hebert et al, 2007; Kozaki et al, 2008).[26,27] miR-21 was first noted as an apoptotic suppressor in various cell lines. In a subsequent large scale study from 540 human samples, it was found that miR-21 is the only miRNA that is over expressed in six solid cancers including that of lung, breast, stomach, prostate, colon and pancreas.44 New evidence suggests that miRNA from cell lines may not show the same profile of the original tumour due to invitro growth conditions affecting miRNA expression. miRNA profiles also appear to be affected by tumour site which has not been controlled for in the majority of studies where cell lines comprising tonsillar tumours, hypopharynx and oral cavity and have been combined.[23]

2. Literature Survey

Saliva is used as a diagnostics medium for oral squamous cell carcinoma and saliva analytes such as proteins and DNA have been used to detect OSCC According to the **li y et al 2004** thousands of miRNA are present in saliva and a panel of saliva mRNA can be used as oral cancer detection. The salivary mRNA seem to enter the oral cavity through various sources, including the 3 major salivary glands, gingival crevice fluid and desquamated oral epithelial cells. The majority of saliva mRNA seem to be present as partially degraded forms. These degraded forms mRNA maintain their stability in saliva through their association with unidentified macromolecules. **Lu j et al** stated that the miRNA serve important functions in cell growth ,differentiation, apoptosis, stress response, immune response and glucose secretion. **Stadler et al** stated that miRNAs are differentially expressed in various cancer compared with normal cells and it seems that miRNAs more accurately cluster different types of solid tumours than miRNA, suggesting that miRNAs can be used to detect the cancer. Fold change in miRNA between cancer cells and normal cells is relatively small, whereas the expression level of many miRNAs exhibits fold changes of ten to hundreds.[20,30] **Park et al** detected the presence of miRNAs in both the whole and supernatant saliva, and demonstrated that two miRNAs, miR-125a and miR-200a, were lowered in the saliva of oral squamous cell carcinoma patients than in control subjects26.Recent studies have also identified both mRNA and miRNA in 2 types of cell derived lipid vesicles; microvesiceles and exosomes. Microvesiceles are relatively large vesicles released from the cell through blabbing. Exosomes are similar vesicles released when endosomally derived multivesicular bodies fuse with the plasma membrane.[27] miRNA have been identified in both exosomes and microvesicles derived from a sources, including human and mouse mast cells, giloblastoma tumours, plasma, saliva and urine[20]

Li et al first demonstrated that miRNA profile reflects the developmental lineage and differentiation state of the tumors through a global analysis of miRNA expression levels in hundreds of clinical samples. Compared with normal tissues, cancerous tissues generally tend to express smaller amounts of miRNAs, suggesting that miRNA signature is a promising tool to classify human cancers.[27]

Calin et al stated that miRNA expression profiling have been shown the miRNA expression levels are altered in primary human tumours. **Lu et al** stated that significant signatures of miRNA expression profiles can be linked to various types of tumours suggesting that miRNA profiling has diagnostic and perhaps prognostic potential. Certain miRNA can be tumour suppressor because of loss of these miRNA is often associated with cancers .[27]

Only one study to date (Cervigne et al, 2009) has looked at the role of miRNA in malignant transformation of oral dysplasia. A micro RNA signature associated with progression of oral leukoplakia to oral carcinoma was identified using fixed formalin paraffin embedded tissue using samples from 12 patients with progressive dysplastic leukoplakia and 4 with non progressive dysplastic leukoplakia.[2]

3. Materials and Methods

Selection of Patients
The study protocol was approved by Ethics committee for student proposal, Sri Ramachandra university and with the Helsinki Declaration of 1975 that was revised in 2000.Sample size was calculated and total no of 40 subjects were selected for the study, out of which 20 were controls (Group A) and 20 were patients with oral submuous fibrosis (Group B). All 40 patients were those of who reported to the department of oral medicine and radiology. Based on the clinical and histopathological evaluation, these patients were confirmed for oral submucous fibrosis. The patient age group for the oral submucous fibrosis, ranged from 21 to 67 years with mean age of 44. Out of 20 osmf patients one of them was female, 19 of them were males. All patients included in the study group were those who had not undergone any form of therapy for the presenting illness. Samples from 20 controls who has participated in the study were taken from the healthy volunteers of matched age and gender , who are free of oral and medical illness. without any habits of smoking, alcohol, chewing betel nut and tobacco. The age group for control ranged from 21 to 60 years with mean age of 40 years. The patients and controls were explained about the study and written consents were taken.

Sampling of Saliva
2 ml of saliva were collected by spitting method in collecting tube, in a 2 ml sterile eppendroff tube from 20 control group and 20 OSMF patients. The salivary samples collected were transferred to the laboratory and was stored at-80 degree centigrade.

Sampling of Exfoliative Cytology
Buccal cell sample collection was done with the help of cytobrush from 20 control group and 20 OSMF patients. All the participants were instructed to rinse the mouth with tap water for 10 seconds before collection. The affected buccal mucosa was scraped by simple counter pressure by twirling the brush while moving it downward and counter pressure was applied with fingers against the cheek for 30 seconds and the brush was stored in a 2ml eppendroff tube directly without additional processing in -80 degree centrigrade freezer. Same method was followed for the control group.

First Strand CDNA Synthesis
Each of the templates RNA samples were adjusted to concentration of 5ng/µl using nuclease free water. Reagents were prepared and gently thawed at the 5x reaction buffer and nuclease free water and immediately placed on ice mixed by vortexing. Later RNA spike was resuspended in by adding 40 µl nuclease free water to the tube and again mixed by vortexing and left on ice for 15-20 minutes. Then enzyme was mixed by flicking the tubes and placed on ice. The reagents were spinned again. Multiple RNA samples were prepared in RT mastermix of the 5x reaction. Buffer, water, enzyme were mixed and prepared. The required amount of RT master mix was prepared and Placed on ice. The reaction were mixed by very gentle vortexing or pipetting to ensure that all reagents were thoroughly mixed. Later it was incubated for 60 min at 42 c and heat was inactivated in the reverse transcriptase for 5 min at 95 c .Immediately cooled to 4 c and stored at 4 c.

Real Time PCR Amplication
cDNA, nuclease free water and SYBR green master mix are placed on ice and thawed for 15-20 min. SYBR green master mix was mixed by pipetting up and down. The rest of the reagents are mixed by vortexing and Spun down.Immediately after use, cDNA template are diluted for the planned real–time PCR reactions 80x in nuclease free water.395µl nuclease free water to each 5 µl of reaction. Multiple real time PCR reaction were performed with the same microRNA primer set. Later the required amount of PCR master mix was prepared and placed on ice. It was recommended to include all excess reagents in the master mix to compensate for pipetting excess material. Then, placed the relevant volume of PCR master mix in PCR tubes. The reaction was mixed by gentle pipetting to ensure that all reagents were mixed thoroughly and stored at 4c for up 16 hrs. Real time PCR amplication method was done by melting curve analysis. Initial data analysis was done using software to get raw cq value.

Real Time PCR
A real-time PCR reaction mixture of 20 µl prepared (including template) was added in 96 well plate and gently mixed. Non template control (NTC without template only SYBR and primers) added in different well to check the purity of the same.

S.No	Contents	Volume (µl)
1	SYBR Green mix (2x)	10 µl
2	Forward primer	1 µl
3	Reverse primer	1 µl
4	Template	1 µl
5	Sterile water	7 µl
	Total	20 µl

All the samples are loaded and linked to the plate of the equipment (7900 HT Fast Real Time PCR) with the following PCR conditions. The results were analysed with the RQ manager (CT calculated)

4. Results

The expression of miR-21 was studied in both saliva and exfoliative cells in normal & also in biopsy proven oral submucous fibrosis .The study group A (control)comprised of 20 cases with age group 21 to 60 years with mean age of 40. The study group B (OSMF)comprised of 20 cases with age group 21 to 67 years with mean age of 44.These group showed 19 males and 1 female.

Table 1: microRNA expression in saliva of study group A

CS1	Undetermined
CS2	Undetermined
CS3	Undetermined
CS4	Undetermined
CS5	Undetermined
CS6	Undetermined
CS7	Undetermined
CS8	Undetermined
CS9	Undetermined
CS10	Undetermined
CS11	Undetermined
CS12	Undetermined
CS13	Undetermined
CS14	Undetermined
CS15	Undetermined
CS16	Undetermined
CS17	Undetermined
CS18	Undetermined
CS19	Undetermined
CS20	Undetermined

Table 1 shows miRNA expression in saliva of study group A. miRNAs were undetermined in all 20 salivary samples.

Table 2: microRNA expression in saliva of study group B

PS1	Undetermined
PS2	Undetermined
PS3	32.3682
PS4	36.66063
PS5	38.19478
PS6	35.52382
PS7	Undetermined
PS8	35.86247
PS9	35.20609
PS10	32.61348
PS11	31.96936
PS12	Undetermined
PS13	Undetermined
PS14	Undetermined
PS15	38.22674
PS16	33.45791
PS17	34.97822
PS18	28.70719
PS19	37.43601
PS20	33.16087

Table 2 shows miRNA expression in saliva of study group B

Out of 20 samples in group B, 14 samples showed miRNA expression and no values were obtained from 6 samples. The highest expression of miRNA was obtained 38.22674 and the lowest expression of miRNA was 28.70719..The mean value is 34.597554.
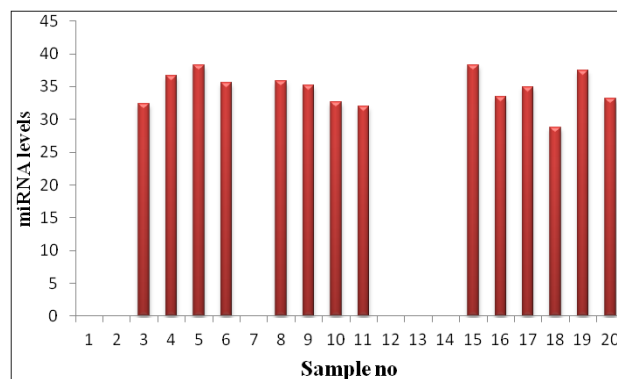

Figure 1: miRNA expression in saliva of study group B

Figure 1 indicates that the highest expression of miRNA levels obtained was 38.22674 and the lowest expression of miRNA was 28.70719.

Table 3: microRNA expression in exfoliative cells of study group A

CE1	Undetermined
CE2	Undetermined
CE3	Undetermined
CE4	Undetermined
CE5	Undetermined
CE6	Undetermined
CE7	Undetermined
CE8	Undetermined
CE9	Undetermined
CE10	39.89007
CE11	Undetermined
CE12	Undetermined
CE13	Undetermined
CE14	Undetermined
CE15	Undetermined
CE16	Undetermined
CE17	Undetermined
CE18	Undetermined
CE19	Undetermined
CE20	Undetermined

Table 3 shows miRNA expression in exfoliative cells of study group A. miRNAs were undetermined in 19 samples and 1 sample value of 39.89007.

Table 4: microRNA Expressions in exfoliative cells of study group B

PE1	34.13953
PE2	Undetermined
PE3	33.98576
PE4	32.72281
PE5	31.18897
PE6	Undetermined
PE7	38.41941
PE8	Undetermined
PE9	Undetermined
PE10	36.63158
PE11	Undetermined
PE12	33.85208
PE13	34.64386
PE14	36.87119
PE15	Undetermined
PE16	30.54784
PE17	Undetermined
PE18	Undetermined
PE19	Undetermined
PE20	32.88863

Table 4 shows miRNA expression in exfoliative cells of study group B

Out of 20 samples 9 samples showed undetermined value and 11 samples showed the miRNA levels. The highest expression of miRNA obtained was 38.41941 and the lowest expression of miRNA was 30.54784.The mean value was 34.171970.
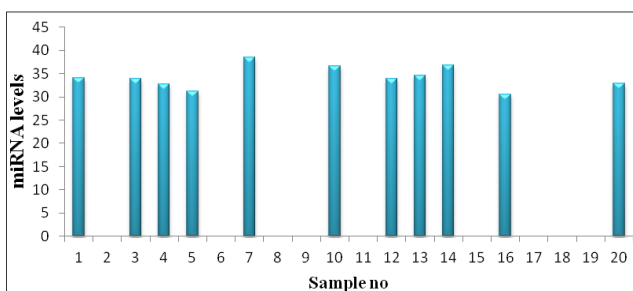

Figure 3: miRNA expression in exfoliative cells of study group B

Figure 3: Indicates that the highest expression of miRNA obtained was 38.41941 and the lowest expression of miRNA was 30.54784.
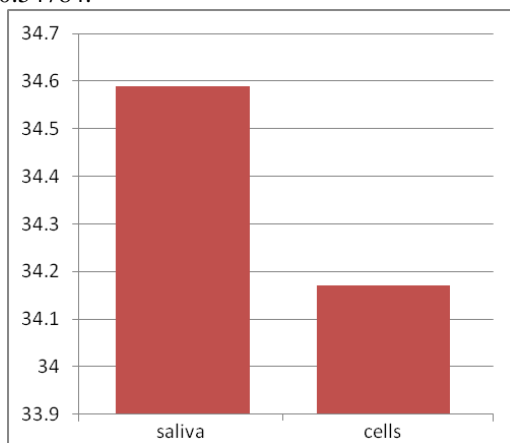

Figure 5: Comparison of mean value between saliva & exfoliative cells of study group B

Figure 5 Indicates a marginal increase in the mean value of saliva when compared with exfoliative cells. The mean value of saliva & exfoliative cells is 34.597554 & 34.171970.

Statistical Analysis
Since Group A (controls) showed no miRNA expression, statistically Odds ratio cannot be calculated to give a significant p value.

5. Discussion

The current study was done to evaluate the expression of miR 21 in saliva and comparing it with exfoliative cells of 20 oral submucous fibrosis patients and with 20 healthy individuals. The present study was aimed for the detection of miRNA in saliva & exfoliative cells of OSMF patients.

miRNAs are found significant number outside of the cells, including various body fluids. These are stable & show distinct expression profiles among different fluid types. miRNA profile reflects the developmental lineage and differentiation state of the tumors through a global analysis of miRNA expression levels in hundreds of clinical samples. Compared with normal tissues, cancerous tissues generally tend to express smaller amounts of miRNAs, suggesting that miRNA signature is a promising tool to classify human cancers.[9]

According to nilva k cervigne, calin and li ye and Danielson et al miRNAs demonstrated significant over expression in leukoplakia & oral squamous cell carcinoma compared with normal. In case of OLP patients, miR -21 showed significantly increased levels when compared to normal oral mucosa . Thousands of miRNA are present in saliva & these miRNAs can be used as oral cancer detection.[2] The salivary miRNA seems to enter the oral cavity through various sources are 3 major salivary glands, gingival crevicular fluid & desquamated oral epithelial cells.[2]

The majority of saliva mRNA seems to be present as partially degraded forms. These degraded forms mRNA maintain their stability in saliva through their association with unidentified macromolecules. St john MA, Li y zou et al 2004 stated that salivary mRNAs can be used as biomarkers for oral cancer that combined measurement of 7 different mRNAs showed a specificity & sensitivity of 0.91 for oral cancer.[21,19]

Li et al. found elevated levels of miR-21 in OSCCs of the tongue, thus relating this miRNA and cell growth, apoptosis, and tumorigenesis. They found that miR-21 over expression was inversely correlated with levels of tropomyosin1 and phosphatase tensin homologue, two apoptotic markers. A multivariate analysis revealed that miRNA-21 is an independent predictor of OSCC .[27]

According Fu et al, showing that miR-21 over expression is associated with a worse average survival rate of carcinomas, in contrast to those that do not show over expression, and a shorter disease-free period, thus confirming the prognostic value of this miRNA.[22]

Stadler et al stated that miRNAs are differentially expressed in various cancer compared with normal cells & Fold change in miRNA between cancer cells & normal cells is relatively small, whereas the expression of many miRNAs exhibits fold changes of ten to hundreds, suggesting that miRNAs can be used to detect the cancer.[23]

The present study was carried out using salivary sample which yielded the similar result to that of Park et al. who detected over 50 stable miRNAs in whole saliva and supernatant in OSCC patients, while miR-125a and miR-200a were significantly subexpressed, compared to healthy control patients. It seems that the degree of degradation of miRNAs in endogenous saliva is slower than that of exogenous miRNAs. It appears that methylation analysis for miRNAs in saliva is also an effective tool for diagnostics and prognostics.[18]

In this study miRNA analysis was done using Real time PCR, it has been proved that it is a superior method for miRNA expression analysis due to sensitivity and specificity of the PCR.

In present study, comparison of OSMF patients against controls have shown that salivary sample is showing high positive results that is 70% of miRNA expressions whereas analysis of exfoliative cells revealed significant positive value that is 55%. So, Out of 20 osmf salivary samples, 14 samples showed miRNA expressions & 6 samples showed undetermined value and 20 osmf exfoliative cells sample, 11 samples showed miRNA levels and 9 samples showed undetermined value and out of 20 control salivary samples, all the samples showed undetermined values and in 20 exfoliative cells sample, 19 samples showed undetermined value and 1 sample showed expression for which the reasons may be due to technical /sampling error.

6. Summary and Conclusion

The Present study was conducted to evaluate the expression of miRNA -21 in saliva and comparing it with exfoliative cells of oral submucous fibrosis. The analysis of miRNA was done using Real time PCR method. The study group comprised of 20 patients with mean age of 44 yrs and all the patients who were clinically diagnosed as oral submucous fibrosis were confirmed histopathologically and 20 controls, age and sex matched with mean age of 40 yrs were included in the study for collection of salivary and exfoliative cells samples.

The results of the study showed that out of 20 study salivary and exfoliative cells samples, 14 salivary samples and 11 exfoliative cells showed the miRNA expression and whereas no control samples showed miRNA. After the analysis we found that the 70% of study sample showed positive results for miRNA in saliva whereas 55% in exfoliative cells

Though high cost, regulated availability of high end equipment and limited sample size may be a limitation to draw a conclusion, however the present study confirmed the abundant expression of miRNA in saliva and hence saliva can be used as a potential sample for evaluating miRNA-21.

miRNAs in body fluids are currently extensively explored for their potential as non–invasive diagnostic tumour markers. Tumour specific miRNAs may improve oral and pre-cancer diagnosis & prognosis, since several promising miRNAs have already been described as non invasive biomarkers for different tumour entities.

References

[1] **K. Danielsson, Y.B Wahlin,X .Gu,L. Boldrup.** Altered expression of miR-21,miR-125b, miR-203 indicates a role for these microRNAs in oral lichen planus. *J Oral Pathol Med 2012 41:91-95.*

[2] **Nilva K.Cervigne, Patricia P. Reis, Jerry Machado.** Identification of a microRNA signature associated with progression of leukoplakia to oral carcinoma. *Human Molecular Genetics 2009 Vol 18 No 24.*

[3] **Ramesh S. Pillai.** MicroRNA functions: *Multiple mechanisms for a tiny RNA.RNA,Vol.11,No.12.*

[4] **W.M. Tilakaratne, M.F. Klinikowski, Takashi Saku, T.J. Peters, Saman Warnakulasuriya.** Oral submucous fibrosis: Review on aetiology and pathogenesis. *Oral Oncology. 2006;42:561–8.*

[5] **F. Sarto, S. Finotto, L. Giacomelli.** The micronucleus assay in exfoliated cells of the human buccal mucosa. *Mutagenesis Vol.2 No.1 pp11-17 1987.*

[6] **Brian E . D. Cooke.** Exfoliative cytology in evaluating oral lesions. *J Dent Res 1963 42:343.*

[7] **T. Toyoshima, F. Kochi, P.Kaemmerer, E.Vairaktaris.** Expression of cytokeratin 17mRNA in oral squamous cell carcinoma cells obtained by brush biopsy: preliminary results. *J Oral Pathol Med 2009 38:530-534*

[8] **Christian A. Heid, Junko Stevens, Kenneth J. Livak, and P.** Mickey Williams. Real time Quantitative PCR. *Genome Res. 1996;6:986-94.*

[9] **Kang-Dae lee, Hyoung-shin lee, Chang-ho-jeon.** Body Fluid Biomarkers for Early Detection of Head and Neck Squamous Cell Carcinomas. *Anticancer Research. 2011;31:1161-68.*

[10] **Marcio Diniz Freitas, Abel Garcia Garcia, Antonio Crespo Abelleira, Jose Luis Martins Carneiro, Jose Manuel Gandara Rey.** Application of cytology in the diagnosis of oral cancer. *Med Oral. 2004;9:355-61.*

[11] **Ravi Mehrotra, Anurag Gupta, Mamta Singh, Rahela Ibrahim.** Application of cytology and molecular biology in diagnosing premalignant or malignant oral lesions. *Molecular Cancer. 2006;5:11:1-9.*

[12] **J. Campo trapero, J. cano sanahez, B. Palacis Sanchez, JJ.Sanchez gutierrrez, M.A. Gonzalez.** Update on Molecular Pathology in Oral Cancer and Precancer. *Anticancer Research. 2008; 28:1197–206.*

[13] **R. Shaw.** The Epigenetics of oral cancer. *Int. J.Oral Maxillofac. Surg. 2006;35: 101-08.*

[14] **Punnya V.Angadi, Sanjay S. Rao.** Arecanut in pathogenesis of oral submucous fibrosis. *Oral Maxillofac Surg. 2011;15:1-9.*

[15] **Ogden GR, Cowpe JG, Wight AJ.** Oral exfoliative cytology: review of the methods of assessment. *J Oral Pathol Med 1997:26:201-5.*

[16] **Ning Li,Xinchin Jian, Yanjia.** Discovery of novel biomarkers in oral submucous fibrosis by microarray analysis. *Cancer epidermiol Biomarkers Prev 2008, 17(9)September 2008.*

[17] **Flavia Maziero, Andreghetto, MariaFathiama.** Evaluation of microRNA expression in head and neck squamous cell carcinoma cell lines and in primary culture of oral keratinocytes. *Einstein,2011;9 442-8.*

[18] **Noh Jin Park, Hui Zhou, David Elastoff.** Salivary microRNA: Discovery, characterization, and clinical utility for oral cancer detection. *Clin Cancer res 2009; 15 (17)2009.*

[19] **Alessia gallo, Mayank Tandon, Llis Alavizos.** The majority of microRNAs detectable in serum and saliva is concentrated in exosomes. *March 2012 Vol7 issue 3.*

[20] **Jessica a weber, David H baxter, Shile Zhang.** The microRNA spectrum in 12 body fluids. *Clinical chemistry 56:11(2010).*

[21] **Alton Etheridge, Inyoul Lee, Leroy Hood.** Extracellular microRNA :A new source of biomarkers. *Mutation Research / Fundamental and molecular mechanisms of mutagenesis .2011.*

[22] **Bartel DP.** MicroRNAs: Genomics, Biogenesis, Mechanism, and function. *cell 116 :281-297.*

[23] **Calin GA, Croce.** MicroRNA signature in human cancers. *Nat Rev Cancer 6:857-866.*

[24] **Chan JA, Krichevsky AM.** MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. *Cancer Res 65:6029-6033.*

[25] **John B, Enright AJ, Aravin et al.** Human microRNA targets. *Plos Biol 2:e363.*

[26] **Krutzfeldt J, Stoffel.** MicroRNA: A new class of regulatory genes affecting metabolism. *Cell Metab 4:9-12.*

[27] **Li J,Getz G,Miska EA.** MicroRNA expression profiles classify human cancers. *Nature435:834-838.*

[28] **Shivadasani RA.** MicroRNA: Regulators of gene expression and cell differentiation. *Blood 108: 3646-3653.*

[29] **P.Rajalalitha, S.Valli.** Molecular pathogenesis of oral submucous fibrosis a collagen metabolic disorder. *J Oral Pathol Med (2005)34:321-8.*

[30] **Rathy Ravindran, Deepa MG**. Salivary tumour markers in oral cancer: brief review. *Oral and maxillofacial pathology vol -2 no-2 jul-dec 2012.*

[31] **Aron J Schatter, Nielis H, H.Hegaard**. Inflammation and cancer :Interweaving microRNA, Free Radical, Cytokine and p53. *Carcinogenesis vol 32 no-1 37-49.*

[32] **Anastasios K, Markopoulos.** Salivary markers for oral cancer detection. *The open dentistry journal 2010,4, 172-178.*

[33] **Bernhard G Zimmermann.** Salivary mRNA targets for cancer diagnostics. *Oral oncol2008may ;44(5);425-429.*

[34] **James V, Tricoli and James W.Jacobson.** microRNA: Potential for cancer detection, diagnosis and prognosis. *Cancer res 2007;67;4553-4555.*

[35] **Jan C Brase, Daniel wuttig.** Serum microRNA as non-invasive biomarkers for cancer. *Molecular cancer 2010*

[36] **Noh Jin Park, Yank li.** Characterization of RNA in saliva. *Clinical chemistry 52:6*

[37] **Erson AE, Petty EM.** MicroRNA in development and disease. *Clinical genet 2008 ;74;296-306*

[38] **Manashi shiiba, Katsuhiro uzawa.** MicroRNA in head and neck squamous cell carcinoma and oral squamous cell carcinoma. *Cancer 2010.2.653-669.*

[39] **Miska, E.A.** How microRNA control cell division, differentiation and death. *Curr .opin. genet 2005, 15, 563-568.*

[40] **Garzon R, Calin, G.A.** MicroRNA in cancer. *Annu rev med 2009,60,167-179.*

[41] **M.K Gupta,** Shubahangi Mhaske.Oral submucous fibrosis-current concept s in etiopathogenesis. *Peoples Journal of Scientic Research vol 1-july -08.*

[42] **R. Rajendran.** Oral submucous fibrosis: etiology, pathogenesis and future research. *WHO Bulliten OMS Vol 72 1994.*

[43] **Chun-ju yang, Wilma Grace Shen.** miR-221 and miR-222 expression increased the growth and tumorigensis of oral carcinoma cells. *J Oral Pathol 2011 40,: 560-566.*

[44] **Leigh-AnnMac farlane.** MicroRNA: Biogensis, function and role in cancer. *Current genomics, 2010, 11,537-561.*

[45] **Ayse E. Erson, Elizabeth M Petty.** miRNA and cancer. *Cancer biology and therapy 8:24,2317-2322.*

[46] **Sunit k Singh, Manika Pal Bandra.** MicroRNA –macro in size but macro in function-FEBS *Journal 275 (2008) 4929-4944.*

[47] **Joel L. Schwartz, Craig beam.** RNA from brush oral cytology to measure squamous cell carcinoma gene expression. *J Oral Pathol Med (2008) 37;70-77.*

[48] **Cong Liu, Bailong Li, Ying Cheng, Jing Lin.** MiR-21 plays an Important Role in Radiation Induced Carcinogenesis in BALB/c Mice by directly targeting the Tumor Suppressor Gene Big-h3. *Int. J. Biol. Sci. 2011, 7.*

[49] **Sanjeev Mittal, Vikram Bansal, Sushant Garg**. The diagnostic role of Saliva -A Review. *J Clin Exp Dent. 2011;3(4):e314-20.*

[50] **Yanzhao li, Wen li, Quchang Ouyang**. Detection of lung cancer with blood microRNA-21 expression levels in Chinese population. *Oncology Letters 2011;2: 991-994*

[51] **Regalla Kumarswamy, Ingo Volkmann.** Regulation and function of miRNA-21 in health and disease. RNA *Biology 8:5, 706-713; september/October 2011.*

[52] **Graham Richard Ogden, Jonathan George Cowpe, Michael Green.** Cytobrush and Wooden Spatula for Oral Exfoliative Cytology. A Comparison. The International Academy of Cytology. *Acta Cytologica. 1992; 36(05):706-10.*

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