ORIGINAL RESEARCH

CORRELATION OF ONCOGENIC MICRORNAS 21 AND 22 EXPRESSION LEVEL TO THE DIFFERENT GRADES OF MUCOEPIDERMOID CARCINOMA OF SALIVARY GLANDS (EX VIVO STUDY)

Mariam Mohamed Zaki*[1], Heba Ahmed Farag[2], Olfat Gamil Shaker[3], Heba Khaled Rizk[4], Sara Abd El Wahed[5]

[1]PhD candidate at Oral Pathology Department, Faculty of Dentistry, Cairo University, Cairo, Egypt & Assistant Lecturer, Oral Pathology Department, Faculty of Dentistry, The British University in Egypt, Cairo, Egypt.

[2]Professor of Oral & Maxillofacial Pathology, Faculty of Dentistry, Cairo University, Cairo, Egypt.

[3]Professor of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University, Cairo, Egypt.

[4]Lecturer of Oral & Maxillofacial Pathology, Faculty of Dentistry, Cairo University, Cairo, Egypt.

[5]Assistant Professor of Oral Pathology, Faculty of Dentistry, The British University in Egypt, Cairo, Egypt.

Corresponding author*: Mariam Mohamed Zaki: PhD candidate at Oral Pathology Department, Faculty of Dentistry, Cairo University, Cairo, Egypt & Assistant Lecturer, Oral Pathology Department, Faculty of Dentistry, The British University in Egypt, Cairo, Egypt. Email: mariam.zaki@bue.edu.eg

ABSTRACT

Background: Mucoepidermoid carcinoma (MEC) counts among the most frequent types of malignant salivary gland neoplasms. Brandwein system classified MEC into low, intermediate and high grade as per histological pattern, but this classification lacks the correlation with the genetic basis such as MicroRNA profile. This study aims to assess the expression level of MicroRNA-21 (miR-21) and MicroRNA-22 (miR-22) in different grades of MEC.

Material And Methods: Formalin-fixed paraffin-embedded blocks of MEC were obtained from Oral and Maxillofacial Pathology Department, Faculty of Dentistry, Cairo University and General Pathology Department, Faculty of Medicine, Cairo University within the period of (2014-2023). Reverse transcription polymerase chain reaction was applied to measure the expression level of miR-21 and miR-22.

Results: The high grade group showed significant upregulation in the level of expression of miR-21 and miR-22 in comparison to all other groups. The levels of upregulation of low group and intermediate group were insignificant. A significant strong positive relationship was recorded between the levels of expression of miR-21 and miR-22 in all the assessed grades of MEC.

Conclusions: As the levels of miR-21 and miR-22 were significantly increased in the high grade-group, these MicroRNAs could be possibly used as prognostic markers in cases of MEC.

Keywords: Mucoepidermoid carcinoma, MicroRNA, miR-21, miR-22.

INTRODUCTION

Mucoepidermoid Carcinoma (MEC) is reported as the most frequent salivary neoplasm that makes up 10-15% of all tumours of salivary glands and one-third of salivary gland malignancies with a broad age spectrum from childhood to elderly age. Parotid gland represents approximately half of the MECs followed the submandibular and sublingual salivary glands. Intraorally, palate is the most frequent site followed by buccal mucosa and other intraoral sites [1,2].

Several histological grading systems of MEC have been proposed. The frequently used Brandwein system classifies MECs into three grades: low, intermediate, and high grade. Low grade MECs are usually confined, contain cystic spaces and have many mucous cells. Intermediate grade MECs have more solid areas, while high grade neoplasms have much more solid areas with fewer mucous cells, and may reveal mitotic figures, nuclear pleomorphism, areas of necrosis, and perineural, lymphovascular or bony invasion [2,3]. However, this histological grading system lacks the correlation with the genetic basis.

MicroRNAs (miRNAs) are short, single-stranded, endogenous Ribonucleic Acid (RNA) molecules that plays a key role in controlling gene activity and RNA silencing. Dysregulated human miRNA expression has been attributed to the growth and evolution of diverse forms of malignancies as Breast cancer, Lung cancer, Colorectal cancer, Ovarian cancer and Oral Squamous cell carcinoma (OSCC) [4-8].

MicroRNA-21 (miR-21) is among the early identified cancer-promoting miRNAs (oncogenic MicroRNA) whose role in carcinogenesis has been widely investigated in many studies. Various studies documented the function of miR-21 in the onset and advancement of numerous types of malignancies

including Colorectal cancer, Breast cancer, Ovarian cancer, Lung cancer as well as Head and Neck cancers [9-14].

Recently, MicroRNA-22 (miR-22) has been documented with its dysregulation contributing to MEC development. It has been involved in control of the cell cycle, apoptosis, and invasion [15]. Some studies revealed that miR-22 was among the maximally upregulated miRNAs in MEC which may indicate its oncogenic role. miR-22 has been implicated to increase invasion and migration of MEC [16,17].

The rationale for carrying out this study is to investigate the expression levels of miR-21 and miR-22 in the various histological grades of MEC.

Material And Methods:

Study design:

An ex vivo experimental design was employed for this research using formalin-fixed paraffin-embedded (FFPE) tissue specimens collected from Oral and Maxillofacial Pathology Department, Faculty of Dentistry, Cairo University and General Pathology Department, Faculty of Medicine, Cairo University. Inclusion criteria included cases of Mucoepidermoid carcinoma during the period from 2014 to 2023, spanning all ages and both genders. The tissue blocks were retrieved, and the histopathological slides were re-evaluated. The study was grouped into control group from normal labial salivary gland tissue, low grade group, intermediate grade group and high grade group of MEC classified according to Brandwein classification. Demographic and diagnostic data was extracted from histopathological reports.

a) RNA extraction:

RNA was extracted from deparaffinized tissue using Qiagen kit (Valencia, CA, USA).

b) Quantitation and assessment of RNA purity:

RNA extracts were done through RNA quantification and purity evaluation using the NanoDrop® (ND)-1000 spectrophotometer (NanoDrop Technologies, Inc. Wilmington, USA).

c) Reverse transcription (RT):

Reverse transcription was conducted on total RNA in a final solution volume measuring 20 uL RT reactions using the miScript II RT kit (Qiagen, Valencia, CA, USA)

d) Calculation of results:

After the Polymerase chain reaction (PCR) rounds, analyses of melting curves were carried out to verify the generation of the expected PCR product. Since there isn't any identified control miRNA in serum, Small nucleolar RNA, C/D box 68 (SNORD 68) was utilized as an endogenous control. The expression level of miR-21 and miR-22 were assessed by the ΔCt method. The cycle threshold (Ct) value is the count of qPCR cycles necessary for the fluorescent signal to surpass a particular threshold. ΔCt was figured out by subtracting the Ct values of SNORD 68 from the ones belonging to target miRNAs. $\Delta\Delta Ct$ was figured by performing subtraction of the ΔCt of the control samples from the ΔCt of the disease samples. The fold change in miR-21 and miR-22 expression were figured out by the equation $2^{-\Delta\Delta Ct}$.

The quantitive association between Ct, ΔCt, $\Delta\Delta Ct$ and FC (Rq) is:

$$\Delta Ct_{(patients)} = Ct_{(target\ genes)} - Ct_{(SNORD\ 68)}$$
$$\Delta Ct_{(control)} = Ct_{(target\ genes)} - Ct_{(SNORD\ 68)}$$
$$\Delta\Delta Ct_{(patients)} = \Delta Ct_{(patients)} - \Delta Ct_{(Control)}$$
$$FC\ (Rq) = 2^{-\Delta\Delta Ct}$$

If the Fold Change is positive, it means that miR-21 or miR-22 are elevated; if the Fold Change is negative, it means they are turned down. Control value was taken to be equal 1, because $\Delta\Delta Ct$ for control cases equals zero and 2^0 equals one.

Statistical methods:

Statistical analysis of the results was carried out using SPSS software. Shapiro-Wilk test of normality was performed to evaluate assumption of normality of all continuous variables. One way-analysis of variance (ANOVA) test was used for evaluation of statistically significant difference among the studied groups, followed by Tukey Kramer Post hoc test for the statistically significant results. Pearson's correlation test was applied to assess the relationship between miR-21 and miR-22 in all cases. P-values ≤ 0.05 were considered statistically significant.

RESULTS

1. Demographic data:

In the current study, 15 cases of Mucoepidermoid carcinoma were included, involving 9 males and 6 females. Patients' ages varied between 9 and 62 years with a mean value of 35.4 years and median of 36 years and a standard deviation of 14 years. Histologically, the cases were equally distributed among 5 cases low grade, 5 cases intermediate grade and 5 cases high grade according to Brandwein classification. Concerning the anatomical distribution of the cases, the most common site where the palate represents 26.7% (4 out of 15 cases) followed by parotid gland 20% (3 out of 15 cases). The lip, tongue, retromolar area and buccal mucosa were equally distributed 13.3% (2 out of 15 cases). 50% of the palate cases were high-grade. All of the lip cases were low-grade. 66.7% of the parotid gland cases were high-grade. The anatomical distribution of the cases is detailed in (Table 1).

Mariam Mohamed Zaki , Heba Ahmed Farag ,Olfat Gamil Shaker, Heba Khaled Rizk, Sara Abd El Wahed

Table 1. Distribution of Mucoepidermoid carcinoma cases by anatomical areas

Anatomical area	Distribution of Mucoepidermoid carcinoma cases
Palate	26.7%
Parotid gland	20%
Lip	13.3%
Buccal mucosa	13.3%
Tongue	13.3%
Retromolar area	13.3%

2. Evaluation of miR-21 expression in different groups:

The greatest value of miR-21 was noted in high grade group with the lowest value being detected in the control group. ANOVA test demonstrated statistical significance difference between all groups ($P<0.05$); with power of study = 0.926, and effect size of one way ANOVA test = 0.613 (Cohen's 1988 large effect > 0.138). Tukey's post hoc revealed that miR-21 expression level in high grade group was statistically significant with all other groups, while there wasn't any significant difference between control, low grade and intermediate group. (Table 2, Fig.1).

Table 2. Value of miR-21 in all groups and significance of the difference using (ANOVA) test

POC	Control	Low grade	Intermediate grade	High grade
Mean	1 [b,c,d]	20.54 [b,c,d]	58.11 [b,c,d]	449.37 [a]
SD	0	9.5	18	278.99
Minimum	1	5.5	37.79	249.73
Maximum	1	27.01	84.45	925.58
G power effect size	21			
One-way ANOVA f	8.56			
One-way ANOVA p	0.0021*			

*significant at $p<0.05$

Tukey's post hoc test: means sharing the same superscript letter are not significantly different.
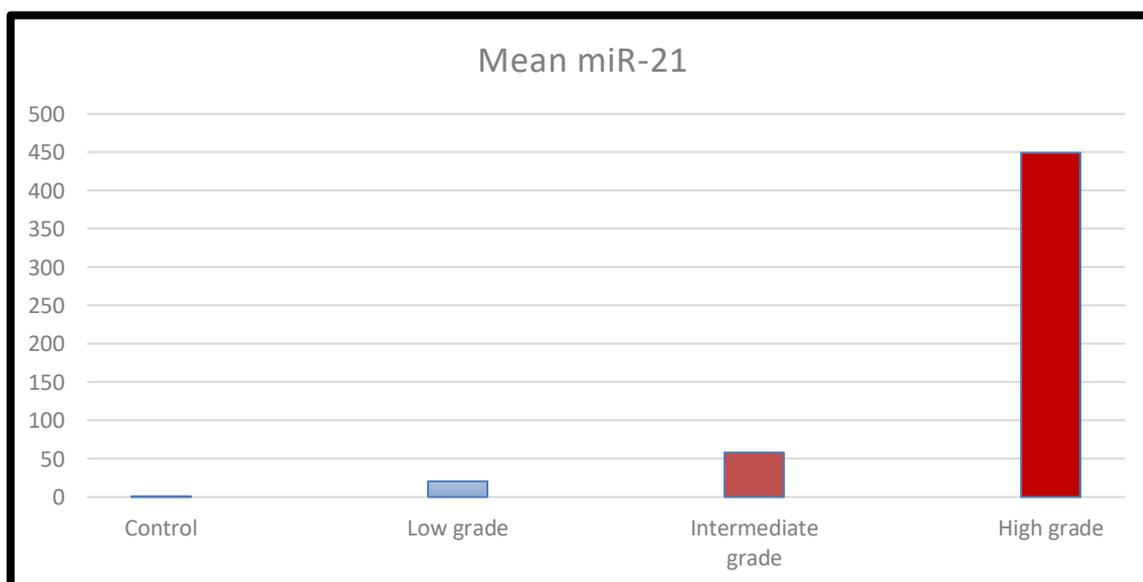

Fig (1) Column chart showing mean value of miR-21 in all groups.

3. Evaluation of miR-22 expression in different groups:

The greatest value of miR-22 was revealed in high grade group, while the lowest value was noted in the control group. ANOVA test revealed that the difference between all groups was statistically significant ($P<0.05$); with power of study = 0.8, and effect size of one way ANOVA test = 0.646 (Cohen's 1988 large effect > 0.138). Tukey's post hoc revealed that miR-22 expression level in high grade group was statistically significant with all other groups, while there wasn't any significant difference between control, low grade and intermediate group (Table 3, Fig.2).

Table 3. Value of miR-22 in all groups and significance of the difference using (ANOVA) test

POC	Control	Low grade	Intermediate grade	High grade
Mean	1 [b,c,d]	1.008 [b,c,d]	1.598 [b,c,d]	3.96 [a]
SD	0	0.1161	0.5216	1.644
Minimum	1	0.82	1.16	2.31
Maximum	1	1.12	2.22	6.5
G power effect size	1.43			
One-way ANOVA f	13.16			
One-way ANOVA p	0.000137*			

*significant at $p < 0.05$

Tukey's post hoc test: means sharing the same superscript letter are not significantly different.
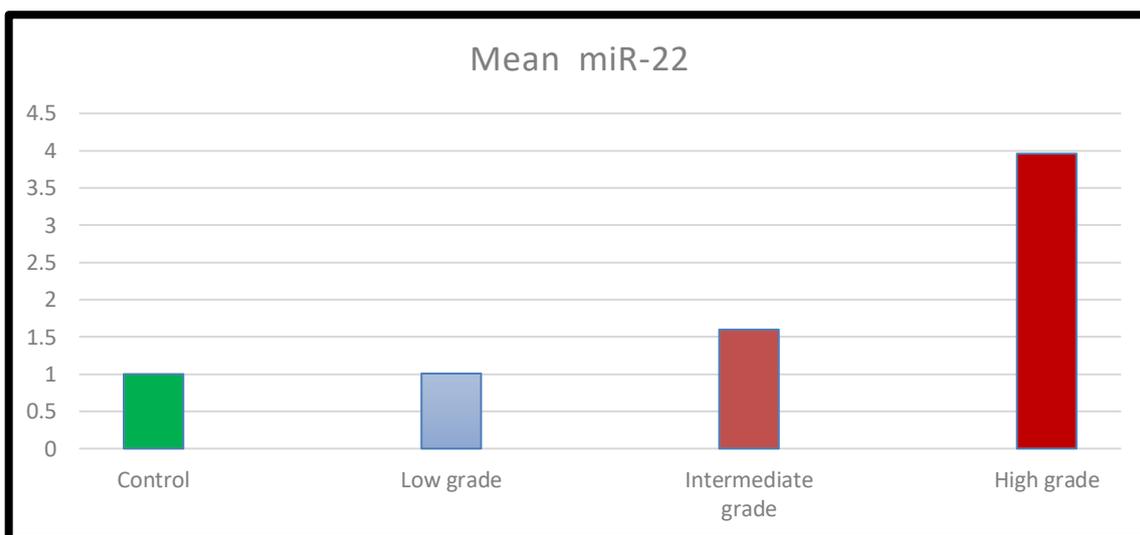

Fig (2) Column chart showing mean value of miR-22 in all groups.

4. Pearson's correlation coefficient (r):

Findings of the Pearson correlation showed that there is a significantly strong positive relationship between values of miR-21 and miR-22 in all cases, ($r(15) = 0.6809$, $p = 0.002622$) (Fig.3).
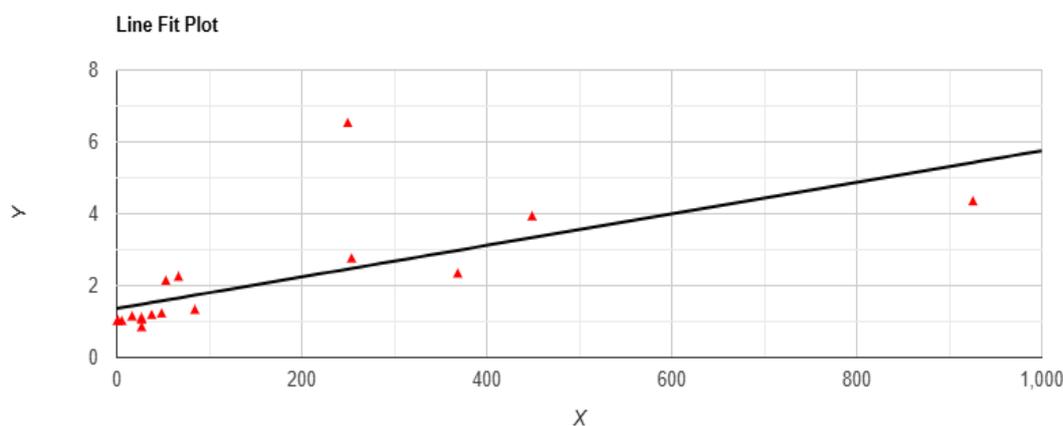

Figure 3. Line plot showing correlation between miR-21 and miR-22 in all groups.

Histopathological Examination:

All the selected tissue specimens were examined and re-evaluated again according to Brandwein classification. low grade cases showed multiple cystic spaces with abundant mucous cells and absence of necrosis or perineural invasion (Fig.4-5). Intermediate grade cases revealed more solid islands and abundant epidermoid and intermediate cells (Fig.6-7). High grade cases showed very few mucous cells with perineural and perivascular invasion (Fig.8-9).
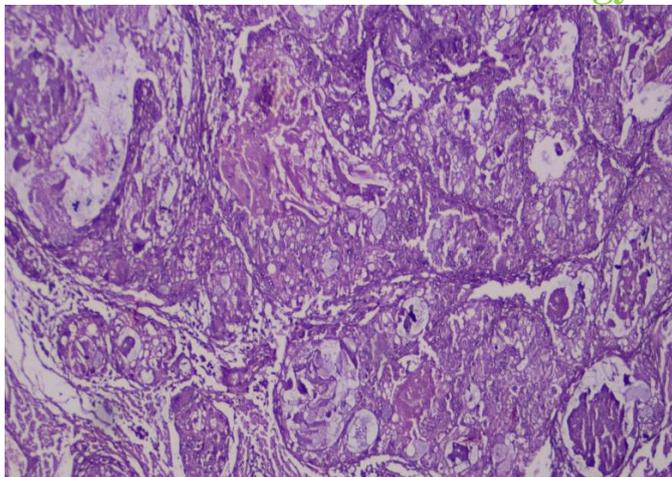
Figure 4. A photomicrograph of (H&E) stained section low-grade mucoepidermoid carcinoma showing multiple cystic spaces filled with mucin and lined with epidermoid and mucous cells (x100)
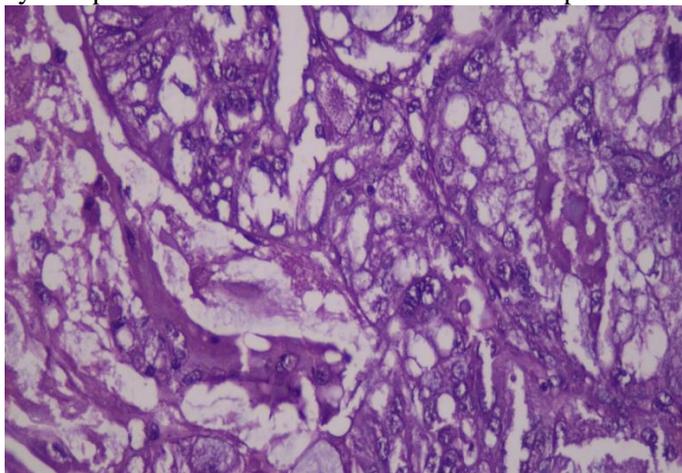

Figure 5. A photomicrograph of (H&E) stained section of low grade mucoepidermoid carcinoma showing multiple mucous cells (x200)
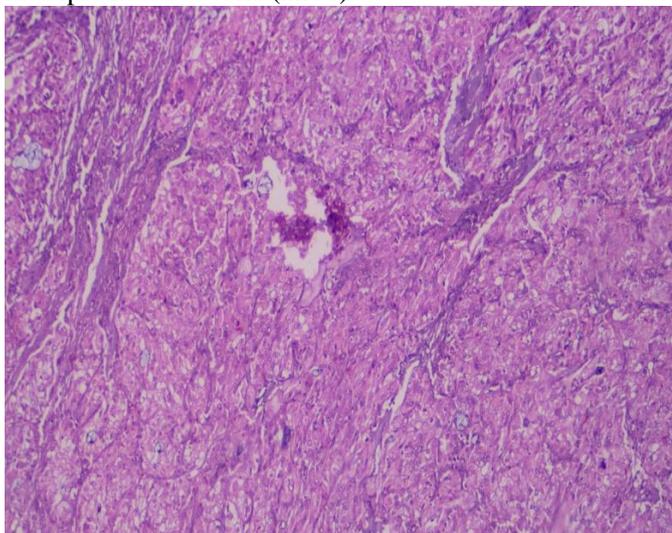

Figure 6. A photomicrograph of (H&E) stained section of intermediate grade mucoepidermoid carcinoma showing more solid areas and few cystic spaces (x100)
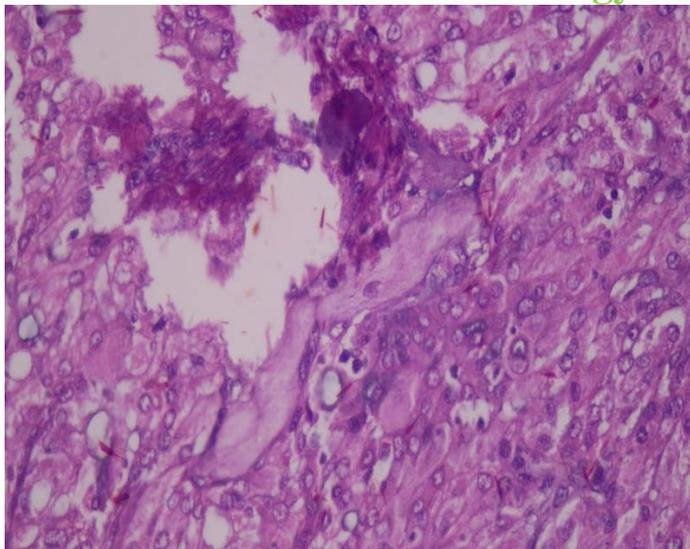
Figure 7. A photomicrograph of (H&E) stained section of intermediate grade mucoepidermoid carcinoma showing solid areas of epidermoid cells and mucous cells (x200)
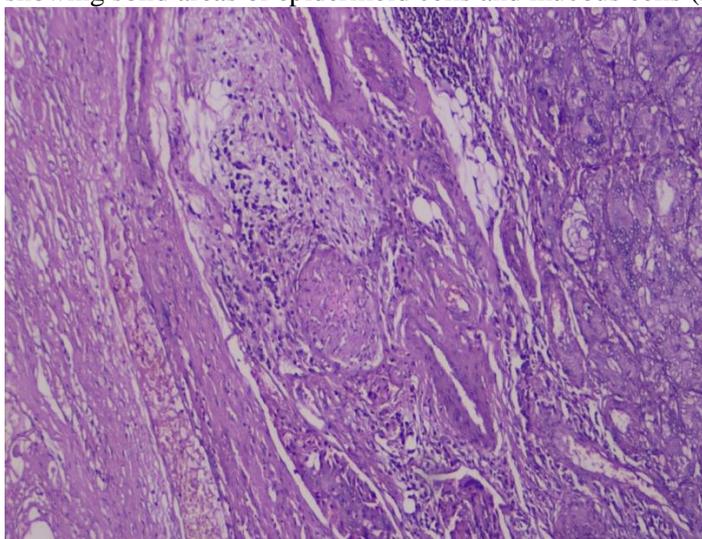

Figure 8. A photomicrograph of (H&E) stained section of high grade mucoepidermoid carcinoma showing solid sheets and nests of epidermoid cells and few mucous cells (x100)
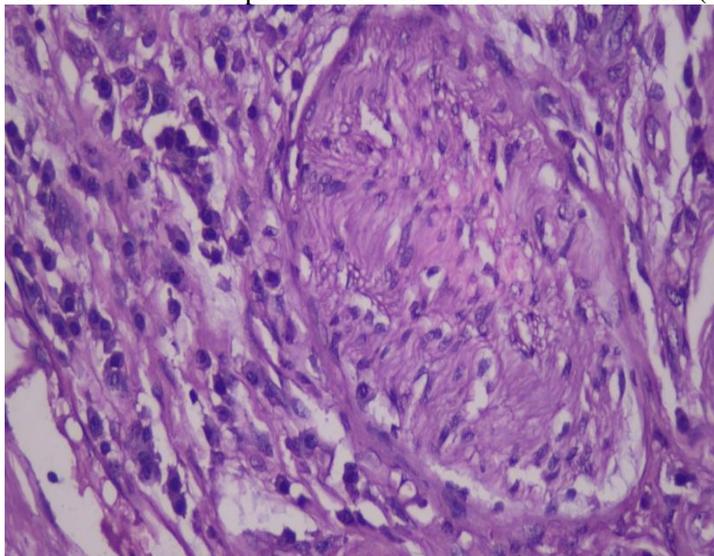

Figure 9. A photomicrograph of (H&E) stained section of high grade mucoepidermoid carcinoma showing perineural and perivascular invasion with tumor cells (x200)

DISCUSSION

MEC is among the most frequent salivary gland malignancies. The survival rate of patients diagnosed with low grade MEC is approximately 90% at 10 years, while survival rate for intermediate grade is 70% and only 25% of high-grade MEC patients are alive after 10 years which reflects the aggressiveness of MEC at higher grades [17]. MECs of salivary glands are inadequately investigated on a molecular scale. Thus, the epigenetic background of MEC particularly miRNA was our field of interest.

miR-21 is identified as a potent oncogenic miRNA which is significantly expressed in tumor cells leading to cancer division and progression. It is widely dysregulated and overexpressed in many types of cancers [18]. Its deregulation affects proliferation, apoptosis and invasion through targeting broad panel of cancer-associated genes (p53, PTEN, TGF) [19]. It was found that inhibition of miR-21 may grant a possible therapeutic strategy for many salivary gland tumours as Adenoid cystic carcinoma [20].

In our study, miR-21 was highly elevated in high-grade group in comparison to the other groups and compared with the control group. A slight increase was noted in the low grade group and intermediate grade group compared to the control group, but this increase was statistically insignificant. The high-grade group was the only group with statistically significant upregulation of miR-21 indicating association between miR-21 and advanced grades of MEC. This revealed the oncogenic function of miR-21 in MEC.

Our Results are in line with earlier studies that have illustrated an increase in miR-21 in MEC. Abdolrahmani et al. [21] demonstrated that miR-21 exhibits a significant difference in MEC than control group using RT-PCR. Also, Binmadi et al. [16] documented increased levels of miR-21 in MEC. Earlier studies reported that miR-21 inhibits tumor suppressor genes as PTEN which is already known to have lower expression in MEC [22, 23]. As far as we know, most studies reported the level of expression of miR-21 in MEC versus normal control group without detailed level of expression of miR-21 across the different grades of MEC. Our Study bridged this gap highlighting the role of miR-21 in the aggressiveness and advancement of MEC.

In the same context, miR-22 has been documented with its complex role in many types of cancers. Mounting studies documented that miR-22 contributes to the processes of senescence, epithelial-mesenchymal transition, progression, metastatic spread and apoptosis [15].

In our research, miR-22 was found to be highly elevated in high grade group compared to the other groups and to the control group. The level of increase in low grade group and intermediate group was insignificant. There was statistically significant increase in the level of expression of miR-22 in high-grade group proving its association with advanced grades of MEC.

Our findings are consistent with other studies that demonstrated the oncogenic role of miR-22 in malignancy. Naakka et al. [17] illustrated that miR-22 knockout reduced migration and invasion of MEC. Also, Binmadi et al. and Costa et al. [16,22] noted overexpression of miR-22 in MEC. Previous studies have reported that miR-22 expression can be induced by MYC and PI3K/AKT which consequently targets PTEN [17,24]. Since AKT is repressed by PTEN, miR-22 could serve as an essential part of a positive feedback of the PI3K/AKT pathway which lead to decreased level of PTEN. This could explain the oncogenic role of miR-22 in advanced grades of MEC. These results provide evidence that miR-22 could be used as a prognostic marker for MEC. On contrary of our results, Feng et al. [25] stated that miR-22 suppressed cell viability, progression and invasion of OSCC which reflects a tumor suppressive function of miR-22 in OSCC. This was explained by the fact that miR-22 reverse the oncogenic role of NLRP3. The presence of contrasting opposing studies could explain the complexity of the role of miR-22 in different types of malignancies.

Based on the present results and to find out whether a correlation exists between miR-21 and miR-22 in MEC, Pearson correlation test was performed. The findings showed a strong positive significant relationship between miR-21 and miR-22 in all cases of MEC suggesting that the levels of expressions of both MicroRNAs are positively associated. This may be explained by the fact that they both target PTEN which in turn leads to tumor aggressiveness. According to the available literature, no studies have conducted this relationship between miR-21 and miR-22 in different grades of MEC. Unlike our results, Paliwal et al. [26] reported that increase of the level of expression of miR-21 is correlated with downregulation of miR-22 in epithelial ovarian cancer. They explained this by the fact that miR-21 acts as oncogenic miRNA that inhibits different tumor suppressors genes as PTEN and BCL2 while miR-22 acts as a tumor suppressor in ovarian cancer that inhibits Notch signalling pathway. Thus miR-22 could have opposite roles in the different types of cancers.

Analysis of the expression level of miR-21 and miR-22 in different grades of mucoepidermoid carcinoma in this study could highlight their role as potential prognostic markers of MEC.

CONCLUSION

Present data revealed that miR-21 and miR-22 were significantly upregulated in the advanced stages of MEC. This upregulation suggests that they may serve as potential prognostic markers for MEC. Future research

Mariam Mohamed Zaki , Heba Ahmed Farag ,Olfat Gamil Shaker, Heba Khaled Rizk, Sara Abd El Wahed Correlation of Oncogenic MicroRNAs 21 and 22 Expression Level to the Different Grades of Mucoepidermoid Carcinoma of Salivary Glands (Ex Vivo Study).Bulletin of Stomatology and Maxillofacial Surgery.2025;21(11)223-231 doi:10.58240/1829006X-2025.21.11-223

with higher sample size is needed to understand the mechanism of both microRNAs in the progression and aggressiveness of the advanced stages of MEC.

List of abbreviations:
MEC: Mucoepidermoid carcinoma
miR-21: MicroRNA-21
miR-22: MicroRNA-22
FFPE: Formalin-fixed paraffin-embedded
miRNA: MicroRNA
RNA: Ribonucleic Acid
OSCC: Oral squamous cell carcinoma
RT: Reverse transcription
PCR: Polymerase chain reaction
SNORD 68: Small nucleolar RNA, C/D box 68
Ct: Cycle threshold
ANOVA: One way-analysis of variance
SD: Standard deviation
PTEN: Phosphatase and tensin homolog
TGF: Transforming growth factor
NLRP3: Nod-like receptor protein 3

DECLARATIONS

Competing and conflicting interest: There are no conflicts of interest.
Authors contributions: All authors contributed to the study conception and design. Data collection and manuscript were done by Mariam Mohamed Zaki. All authors reviewed and approved the final manuscript.
Ethical approval:
This study was approved by the Research Ethics Committee of the Faculty of Dentistry, Cairo University (No. 22 10 23) in compliance with Helsinki declaration. In this type of study, informed consent isn't required.
Consent for publication:
Not applicable. This work was conducted on archival tissue blocks.
Data Availability Statement: Data will be made available upon reasonable request. Kindly contact the author via e-mail.

REFERENCES

1. [...] Treatment of Mucoepidermoid Carcinoma. World J Oncol. 2022 Feb;13(1):1-7.
2. WHO Classification of Tumours Editorial Board. Head and neck tumours. 5th ed. Lyon: International Agency for Research on Cancer (IARC); 2022.
3. Brandwein MS, Ivanov K, Wallace DI, Hille JJ, Wang B, Fahmy A, Bodian C, Urken ML, Gnepp DR, Huvos A, Lumerman H, Mills SE. Mucoepidermoid carcinoma: a clinicopathologic study of 80 patients with special reference to histological grading. Am J Surg Pathol. 2001 Jul;25(7):835-45.
4. Aali M, Mesgarzadeh AH, Najjary S, Abdolahi HM, Kojabad AB, Baradaran B. Evaluating the role of microRNAs alterations in oral squamous cell carcinoma. Gene. 2020 Oct 5;757:144936.
5. Buruiană A, Florian ȘI, Florian AI, Timiș TL, Mihu CM, Miclăuș M, Oșan S, Hrapșa I, Cataniciu RC, Farcaș M, Șușman S. The Roles of miRNA in Glioblastoma Tumor Cell Communication: Diplomatic and Aggressive Negotiations. Int J Mol Sci. 2020 Mar 12;21(6):1950.
6. Varrone F, Caputo E. The miRNAs Role in Melanoma and in Its Resistance to Therapy. Int J Mol Sci. 2020 Jan 29;21(3):878.
7. Xin G, Cao X, Zhao W, Lv P, Qiu G, Li Y, Wang B, Fang B, Jia Y. MicroRNA expression profile and TNM staging system predict survival in patients with lung adenocarcinoma. Math Biosci Eng. 2020 Nov 12;17(6):8074-8083.
8. Zhao L, Liang X, Wang L, Zhang X. The Role of miRNA in Ovarian Cancer: an Overview. Reprod Sci. 2022 Oct;29(10):2760-2767.
9. Santos PRB, Coutinho-Camillo CM, Soares FA, Freitas VS, Vilas-Bôas DS, Xavier FCA, Rocha CAG, de Araújo IB, Dos Santos JN. MicroRNAs expression pattern related to mast cell activation and angiogenesis in paraffin-embedded salivary gland tumors. Pathol Res Pract. 2017 Dec;213(12):1470-1476. doi: 10.1016/j.prp.2017.10.012. Epub 2017 Oct 18. PMID: 29108921.
10. Wu Y, Song Y, Xiong Y, Wang X, Xu K, Han B, Bai Y, Li L, Zhang Y, Zhou L. MicroRNA-21 (Mir-

Mariam Mohamed Zaki , Heba Ahmed Farag ,Olfat Gamil Shaker, Heba Khaled Rizk, Sara Abd El Wahed
Correlation of Oncogenic MicroRNAs 21 and 22 Expression Level to the Different Grades of Mucoepidermoid Carcinoma of Salivary Glands (Ex Vivo Study).Bulletin of Stomatology and Maxillofacial Surgery.2025;21(11) 223-231 doi:10.58240/1829006X-2025.21.11-223

21) Promotes Cell Growth and Invasion by Repressing Tumor Suppressor PTEN in Colorectal Cancer. Cell Physiol Biochem. 2017;43(3):945-958.

11. Dioguardi M, Spirito F, Sovereto D, Alovisi M, Troiano G, Aiuto R, Garcovich D, Crincoli V, Laino L, Cazzolla AP, Caloro GA, Di Cosola M, Lo Muzio L. MicroRNA-21 Expression as a Prognostic Biomarker in Oral Cancer: Systematic Review and Meta-Analysis. Int J Environ Res Public Health. 2022 Mar 14;19(6):3396.

12. Najjary S, Mohammadzadeh R, Mokhtarzadeh A, Mohammadi A, Kojabad AB, Baradaran B. Role of miR-21 as an authentic oncogene in mediating drug resistance in breast cancer. Gene. 2020 May 15;738:144453.

13. Talaat A, Helmy MA, Saadawy, SF. Evaluation of mirna-21 and CA-125 as a promising diagnostic biomarker in patients with ovarian cancer. Egypt J Med Hum Genet. 2022;23:123.

14. Wang W, Li X, Liu C, Zhang X, Wu Y, Diao M, Tan S, Huang S, Cheng Y, You T. MicroRNA-21 as a diagnostic and prognostic biomarker of lung cancer: a systematic review and meta-analysis. Biosci Rep. 2022 May 27;42(5):BSR20211653.

15. Wang J, Li Y, Ding M, Zhang H, Xu X, Tang J. Molecular mechanisms and clinical applications of miR-22 in regulating malignant progression in human cancer (Review). Int J Oncol. 2017 Feb;50(2):345-355.

16. Binmadi NO, Basile JR, Perez P, Gallo A, Tandon M, Elias W, Jang SI, Alevizos I. miRNA expression profile of mucoepidermoid carcinoma. Oral Dis. 2018 May;24(4):537-543.

17. Naakka E, Barros-Filho MC, Adnan-Awad S, Al-Samadi A, Marchi FA, Kuasne H, et al. miR-22 and miR-205 Drive Tumor Aggressiveness of Mucoepidermoid Carcinomas of Salivary Glands. Front Oncol. 2022 Feb 9;11:786150.

18. Jaksic Karisik M, Lazarevic M, Mitic D, Milosevic Markovic M, Riberti N, Jelovac D, Milasin J. MicroRNA-21 as a Regulator of Cancer Stem Cell Properties in Oral Cancer. Cells. 2025 Jan 10;14(2):91.

19. Cinpolat O, Unal ZN, Ismi O, Gorur A, Unal M. Comparison of microRNA profiles between benign and malignant salivary gland tumors in tissue, blood and saliva samples: a prospective, case-control study. Braz J Otorhinolaryngol. 2017 May-Jun;83(3):276-284.

20. Jiang LH, Ge MH, Hou XX, Cao J, Hu SS, Lu XX, Han J, Wu YC, Liu X, Zhu X, Hong LL, Li P, Ling ZQ. miR-21 regulates tumor progression through the miR-21-PDCD4-Stat3 pathway in human salivary adenoid cystic carcinoma. Lab Invest. 2015 Dec;95(12):1398-408.

21. Abdolrahmani A, Kardouni Khoozestani N, Azmoudeh-Ardalan F, Shamshiri AR. Prognostic impact of MUC1 and potential regulatory miR-145 and miR-21 expression in salivary mucoepidermoid carcinoma. Head Neck Pathol. 2022 Dec;16(4):1134-1145.

22. Costa RF, de Oliveira CA, Gomes ÁNM, Lourenço SV, Coutinho-Camillo CM. Molecular Aspects of Mucoepidermoid Carcinoma and Adenoid Cystic Carcinoma of the Salivary Gland. Head Neck Pathol. 2024 Apr 24;18(1):34.

23. Rhim J, Baek W, Seo Y, Kim JH. From Molecular Mechanisms to Therapeutics: Understanding MicroRNA-21 in Cancer. Cells. 2022 Sep 7;11(18):2791.

24. Centomo ML, Vitiello M, Poliseno L, Pandolfi PP. An Immunocompetent Environment Unravels the Proto-Oncogenic Role of miR-22. Cancers (Basel). 2022 Dec 19;14(24):6255.

25. Feng X, Luo Q, Wang H, Zhang H, Chen F. MicroRNA-22 suppresses cell proliferation, migration and invasion in oral squamous cell carcinoma by targeting NLRP3. J Cell Physiol. 2018 Sep;233(9):6705-6713.

26. Paliwal N, Vashist M, Chauhan M. Evaluation of miR-22 and miR-21 as diagnostic biomarkers in patients with epithelial ovarian cancer. 3 Biotech. 2020 Mar;10(3):142.