miR-34a and survivin as biomarkers for oral squamous cell carcinoma surveillance in Fanconi anemia

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Received May 20, 2025; Accepted November 4, 2025

DOI: 10.3892/wasj.2025.422

Abstract. Oral squamous cell carcinoma (OSCC) is a prevalent malignancy with a poor prognosis due to late diagnosis, with a 5-year survival rate of 35-50%. Patients with Fanconi anemia (FA) exhibit a 500-700-fold increased risk of developing OSCC at younger ages. Non-invasive biomarkers are critical for early detection in high-risk groups. The present study investigated the salivary expression levels of miR-34a and the serum levels of survivin, both of which are associated with the PI3K signaling pathway, in patients with OSCC (n=24), patients with FA (n=24) and healthy controls (n=40). The miRNA levels were quantified using reverse transcription-quantitative PCR, and protein levels were measured using ELISA. Compared to the controls, the patients with OSCC exhibited significantly lower miR-34a levels (P=0.012), and patients with FA exhibited a reduced expression of miR-34a (P=0.014). Survivin levels were elevated in patients with OSCC (P=0.04) and FA (P=0.01) compared to the controls. A negative correlation was identified between miR-34a and surviving levels (ρ=-0.52, P=0.005) in patients with OSCC. These findings suggest that miR-34a and survivin are promising non-invasive biomarkers for assessing the risk of developing OSCC in patients with FA and OSCC. However, multicenter studies with larger cohorts are warranted to validate these results for clinical surveillance protocols.

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Key words: oral squamous cell carcinoma, Fanconi anemia, miR-34a, survivin, non-invasive biomarkers, PI3K signaling, early detection

Introduction

Oral squamous cell carcinoma (OSCC), accounting for ~90% of oral cancer cases, and poses a global health challenge with a 5-year survival rate of 35-50%, primarily due to late diagnosis driven by non-specific symptoms and invasive diagnostics (1,2). Fanconi anemia (FA), a rare genetic disorder affecting 1 in 130,000 individuals, markedly increases susceptibility to squamous cell carcinomas (SCC), with OSCC among the most prevalent, exhibiting a 500-700-fold elevated risk, often at younger ages (3,4). This necessitates robust surveillance strategies that are sensitive, comprehensive and sustainable for high-risk patients with FA (5). Non-invasive biomarkers, such as microRNAs (miRNAs/miRs) and proteins, enable patient-friendly monitoring through biofluids, such as saliva and serum, thereby enhancing early detection (6).

miR-34a, a potent tumor suppressor, regulates the Wnt and PI3K pathways by targeting genes [e.g., Wnt family member 1 (WNT1), phosphatase and tensin homolog (PTEN) and baculoviral IAP repeat containing 5 (BIRC5)] and promotes apoptosis (7). However, the downregulation of its expression has been reported in SCCs (head and neck, lung and cervical) and other types of cancer (leukemia and glioma), with this being associated with tumor progression (8). Survivin, an apoptosis inhibitor, suppresses caspase 3/9 activity and is overexpressed in OSCC, head and neck SCC, and cancers such as lung and gastric cancer, is detectable in serum and saliva, and is linked to an aggressive tumor behavior (9). Despite their established roles in SCCs, the combined utility of salivary miR-34a and serum survivin for OSCC surveillance in FA remains underexplored, particularly in a comparative setting with patients with OSCC and healthy controls. Thus, the present pilot study evaluated these biomarkers in patients with FA (n=24), patients with OSCC (n=24) and healthy controls (n=40) cohorts to establish non-invasive tools for early detection of OSCC in FA, aiming to improve clinical outcomes in this high-risk population.

Patients and methods

Study population. The present study included 24 patients with OSCC (mean age, 54.52±10.49 years; 58.3% male; TNM stages I-IV), 24 patients with FA (mean age, 20.03±5.87 years; 58.3% male) and 40 healthy controls (mean age, 42.23±10.22 years, 50% male). OSCC was diagnosed via histopathological biopsy, and FA was confirmed by chromosomal breakage tests (using mitomycin C and diepoxybutane tests). Genetic analysis for specific germline mutations (e.g., FANCA and FANCC) was not performed in the present study due to resource limitations.

Exclusion criteria included other malignancies, autoimmune disorders, or recent infections. The patients with FA had no history of OSCC at the time of sampling. The demographic characteristics of the study participants (age, sex, oral hygiene and oral lesions) are presented in Table I.

Ethical approval and consent. The present study was approved by the Istanbul University Clinical Research Ethics Committee (Approval no. 1307, November 13, 2019) and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants, with parental consent being obtained for those <18 years of age.

Sample collection. Saliva (4 ml) and serum (6 ml in EDTA tubes) were collected under standardized conditions. Saliva was centrifuged at 3,000 x g for 10 min at 4˚C to remove debris, and serum was separated at 1,500 x g for 10 min at 4˚C. Supernatants were stored at -80˚C until analysis.

miRNA isolation and reverse transcription-quantitative PCR (RT-qPCR). Salivary miRNA was extracted using the miRNeasy Mini kit (Qiagen GmbH). RNA purity and concentration were verified using a NanoDrop spectrophotometer (A260/A280 ratio: 1.8-2.0). cDNA was synthesized using the miRCURY LNA RT kit (Qiagen GmbH). Quantitative PCR (qPCR) was performed using SYBR-Green Master Mix (Qiagen GmbH) on a LightCycler 480 (Roche Diagnostics) under the following cycling conditions: Initial activation at 95˚C for 2 min, followed by 40 cycles of denaturation at 95˚C for 10 sec and annealing/extension at 56˚C for 60 sec, and a melt curve analysis from 65˚C to 95˚C.Primers for hsa-miR-34a-5p (forward, 5'-GCAGTGGCAGTGTCTTAG-3'; reverse, 5´-GGTCCAGTTTTTTTTTTTTTTTACAAC-3') and U6 snRNA (human; forward, 5'-CTCGCTTCGGCA GCACA-3'; reverse, 5' AACGCTTCACGAATTTGCT-3' were purchased from Qiagen GmbH. Relative expression levels were calculated using the $2^{-\Delta\Delta Cq}$ method (10).

Protein analysis. Serum survivin levels were quantified using a commercial ELISA kit (Cat. No: E3904Hu, Human Survivin ELISA kit, BT-Lab). Standard curves were generated according to the manufacturer's instructions, and the optical density was measured at 450 nm using a microplate reader (BioTek; Agilent Technologies, Inc.).

Statistical analysis. Data normality was assessed using the Shapiro-Wilk test for continuous variables (age, salivary miR-34a expression and serum survivin levels). Age satisfied the normality assumption (Shapiro-Wilk, P=0.37) and data were compared between groups using one-way ANOVA with the Bonferroni correction for post hoc pairwise comparisons. Salivary miR-34a and serum survivin distributions deviated from normality (Shapiro-Wilk, P<0.05) and are therefore are presented as the median and interquartile range (IQR). Group comparisons for these biomarkers were performed using the Kruskal-Wallis test, followed by Dunn's post hoc test for multiple comparisons. Categorical variables were compared using the Chi-squared test or Fisher's exact test, as appropriate. All correlation analyses were performed using Spearman's rank correlation. Receiver operating characteristic (ROC) analyses were performed to calculate the area under the curve (AUC), 95% confidence intervals (CIs), cut-off values, sensitivity and specificity. All tests were two-sided, and a value of P<0.05 was considered to indicate a statistically significant difference. Statistical analyses were performed using SPSS v25 (IBM Corp.).

Results

Demographic and clinical characteristics of the study participants. The present study comprised 24 patients with OSCC (mean age, 54.52±10.7 years; 58.3% male), 24 patients with FA (mean age, 20.03±5.87 years; 58.3% male) and 40 healthy controls (mean age, 42.13±10.92 years; 50% male). Age followed a normal distribution (Shapiro-Wilk, P=0.37), while salivary miR-34a and serum survivin did not (Shapiro-Wilk, P<0.05). Therefore, non-parametric tests were applied for these biomarkers. Sex distribution was also comparable (P>0.05). The demographic characteristics of the patient and control groups are summarized in Table I.

miR-34a expression levels. As salivary miR-34a and serum survivin values deviated from normality (Shapiro-Wilk, P<0.05), they are presented as the median (IQR) and were compared using the Kruskal-Wallis test with Dunn's post-hoc (Table II). Salivary miR-34a expression, quantified using RT-qPCR with the $2^{-\Delta\Delta Cq}$ method with U6 normalization, was significantly lower in the patients with OSCC [median (IQR), 1.33 (0.68-3.69); P=0.012] and FA [median (IQR), 0.72 (0.05-3.19); P=0.014] compared to the controls [median (IQR), 3.63 (0.32-14.77)]. No significant difference was observed between the OSCC and FA (P=0.78) groups. These results are presented in Fig. 1 and Table II.

In the patients OSCC, the miR-34a levels tended to be lower in those with advanced tumor stages (T3-T4) and lymph node metastasis; however, these associations were not statistically significant (P=0.34 and P=0.87, respectively; Table III). No significant associations were found with other clinical parameters, including depth of invasion, differentiation, or treatment response (P>0.05; Table III).

Survivin levels. The serum survivin levels, measured using ELISA, were significantly elevated in patients with OSCC [median (IQR), 196.19 (165.83-298.75) ng/ml; P=0.01) and FA [median (IQR), 216.38 (102.89-858.87); P=0.001] compared to the controls [median (IQR), 121.90 (103.85-182.03)]. The survivin levels were higher in patients with FA than in patients with OSCC (P=0.028). These results are presented in Fig. 2 and Table II.

Table I. Demographic characteristics of patients with OSCC ad FA, and the controls.

Feature	Patients with OSCC (n=24)	Patients with FA (n=24)	Control group (n=40)
Age range, years	31-67	14-32	25-53
Age, years (mean ± SD)	54.52±10.49	20.03±5.87	42.13±10.92
Sex, n (%)			
Male	14 (58.3)	14 (58.3)	20 (50)
Female	10 (41.7)	10 (41.6)	20 (50)
Oral hygiene			
Poor	10 (41.6)	14	21 (55)
Good	14 (58.3)	10	19 (45)
Oral lesions			
Present	19 (79.1)	9 (37.5)	14 (20)
Absent	5 (20.9)	15 (67.5%)	26 (80)

Patients with FA were diagnosed in childhood using mitomycin C and diepoxybutane tests and followed-up for 5-15 years by pediatric hematology-oncology specialists. Of the 24 patients with FA, 11 developed SCC (8 patients developed OSCC and 3 patients developed other SCC) during or after the study. OSCC, oral squamous cell carcinoma; FA, Fanconi anemia; SD, standard deviation.

Table II. miR-34a and survivin levels in the OSCC, FA and control groups.

Group	miR-34a ($2^{-\Delta\Delta Cq}$) median (IQR)	P-value vs. control	Survivin (ng/ml) median (IQR)	P-value vs. control
OSCC (n=24)	1.33 (0.68-3.69)	0.012	196.19 (165.83-298.75)	0.01
FA (n=24)	0.72 (0.05-3.19)	0.014	216.38 (102.89-858.87)	0.001
Control (n=40)	3.63 (0.32-14.77)		121.90 (103.85-182.03)	

P-values indicate comparisons with the control group. Pairwise FA vs. OSCC: P=0.78 (miR-34a), P=0.028 (survivin). Shapiro-Wilk test: miR-34a (OSCC, P<0.01; FA, 0.01; control: 0.04; survivin (OSCC: P<0.05; FA, P=0.05; control, P=0.03), indicating non-normal distribution in at least one group. Group comparisons were performed using the Kruskal-Wallis test with Dunn's post hoc. P<0.05 was considered to indicate a statistically significant difference. IQR, interquartile range; OSCC, oral squamous cell carcinoma; FA, Fanconi anemia.

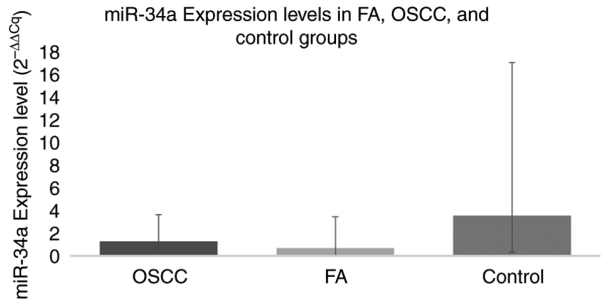

Figure 1. Salivary miR-34a expression levels in the FA, OSCC and control groups. Bar graph illustrating the median (IQR) miR-34a expression levels (quantified by RT-qPCR using the $2^{-\Delta\Delta Cq}$ method with U6 normalization) across the groups [OSCC, 1.33 (0.68-3.69); FA, 0.72 (0.05-3.19); and control, 3.63 (0.32-14.77)]. P-values indicate significant differences: P=0.012 for OSCC vs. control and P=0.014 for FA vs. control. Error bars represent IQR. IQR, interquartile range; OSCC, oral squamous cell carcinoma; FA, Fanconi anemia.

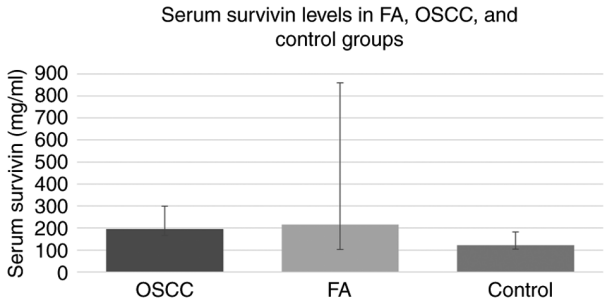

Figure 2. Serum survivin levels in the FA, OSCC and control groups. Bar graph illustrating the median (IQR) serum survivin levels (measured using ELISA) in the different groups [OSCC, 196.19 (165.83-298.75) ng/ml; FA, 216.38 (102.89-858.87) ng/ml; and control, 121.90 (103.85-182.03) ng/ml]. P-values were as follows: P=0.01 for OSCC vs. control, P=0.001 for FA vs. control, and P=0.028 for FA vs. OSCC. Error bars represent IQR. IQR, interquartile range; OSCC, oral squamous cell carcinoma; FA, Fanconi anemia.

In OSCC, the survivin levels did not exhibit a significant association with clinical parameters, including tumor stage, lymph node metastasis, or progression (P>0.05; Table III).

Correlation analysis. ROC analysis was used to evaluate the diagnostic accuracy of miR-34a and survivin in OSCC. Salivary miR-34a exhibited poor discriminatory power (AUC, 0.575; 95% CI, 0.439-0.719; cut-off value, >24.53;

Table III. Association of miR-34a and survivin expression with clinicopathological features of patients with OSCC.

		miR-34a			Survivin		
Clinical characteristics of patients with OSCC	No. of patients (%)	High expression	Low expression	P-value	High expression	Low expression	P-value
Tumor size							0.31
<2 cm	3 (12.5)	1 (33.3)	2 (66.7)		1 (33.3)	2 (66.7)	
2-3 cm	15 (62.5)	4 (26.6)	11 (73.3)		10 (66.7)	5 (33.3)	
>4 cm	6 (25)	2 (33.3)	4 (66.7)	0.08	4 (66.7)	2 (33.3)	
Tumor stage							0.23
T1, T2	11 (45.8)	3 (37.5)	8 (62.5)		10 (58.8)	7 (41.2)	
T3, T4	13 (54.2)	4 (30.8)	9 (69.2)	0.34	8 (61.5)	5 (38.5)	
Lymph node metastasis							0.72
Present	9 (37.5)	2 (22.2)	7 (77.7)	0.87	6 (66.7)	3 (33.3)	
Absent	15 (62.5)	5 (33.3)	10 (66.7)		8 (53.3)	7 (46.7)	
Degree of differentiation							0.84
Poor	2 (8.3)	0 (0)	2 (100)		1 (50)	1 (50)	
Moderate	17 (70.9)	6 (37.5)	10 (62.5)	1.56	10 (58.8)	7 (29.2)	
Well	5 (20.8)	1 (20)	4 (80)		3 (60)	2 (40)	
Depth of invasion							0.95
<10 mm	8 (33.3)	4 (50)	4 (50)		8 (100)	0 (0)	
>10mm	16 (66.7)	5 (31.3)	11 (68.7)	0.058	4 (25)	12 (75)	
Perineural invasion							0.45
Present	14 (58.3)	4 (28.5)	5 (71.5)	0.08	7 (50)	7 (50)	
Absent	10 (41.6)	3 (30)	7 (70)		7 (70)	3 (30)	
Lymphovascular invasion							0.22
Present	9 (37.5)	2 (22.2)	7 (77.8)	0.21	5 (55.6)	4 (44.4)	
Absent	15 (62.5)	5 (33.3)	10 (66.7)		11 (73.3)	4 (26.7)	
Chemotherapy response							0.08
Present	15 (62.5)	5 (33.3)	10 (66.7)	0.07	9 (60)	6 (40)	
Absent	9 (37.5)	2 (22.2)	7 (77.8)		1 (11.1)	8 (88.9)	
Radiotherapy response							0.09
Present	16 (66.6)	4 (25)	12 (75)	0.99	5 (31.3)	11 (68.7)	
Absent	8 (33.4)	4 (50)	4 (50)		2 (25)	6 (75)	
Progression							0.14
Present	7 (29.2)	1 (14.3)	6 (85.7)	0.16	7 (100)	0 (0)	
Absent	17 (70.8)	6 (35.3)	11 (64.7)		7 (41.2)	10 (58.8)	

Clinical parameters were assessed via histopathological and clinical evaluation. Data are presented as number (percentage). Percentages in the first column represent the proportion of patients within the total cohort (n=24), whereas percentages in the expression columns represent the proportion within each category. P-values were calculated using Fisher's exact test, as appropriate, by comparing miR-34a and survivin levels between patients with and without each clinical parameter. A P-value <0.05 was considered to indicate a statistically significant difference. miR-34a levels were quantified using the $2^{-\Delta\Delta Cq}$ method with U6 normalization, and survivin levels were measured using ELISA. OSCC, oral squamous cell carcinoma; T stage, tumor stage.

sensitivity, 24%; specificity, 95%; Fig. 3), while serum survivin exhibited a moderate discriminatory power (AUC, 0.710; 95% CI, 0.657-0.934; cut-off value, >191.1; sensitivity, 70%; specificity, 97%; Fig. 4). In patients with OSCC, salivary miR-34a expression inversely correlated with serum survivin levels (Spearman's Rho=-0.52; P=0.005; Table IV and Fig. 5).

Discussion

The present pilot study was the first to evaluate salivary miR-34a and serum survivin as non-invasive biomarkers for OSCC surveillance in patients with FA and OSCC, with a focus on their roles in cancer pathways. The findings revealed significant alterations in miR-34a (P=0.012 in OSCC and

Table IV. Inverse correlation between miR-34a and survivin expression in OSCC patients.

Group	Correlation coefficient (Rho)	P-value
OSCC (n=24)	-0.52	0.005
FA (n=24)	-	>0.05
Control (n=40)	-	>0.05

Spearman's correlation analysis demonstrated the inverse correlation between miR-34a and survivin expression in patients with OSCC. Correlation coefficients (Rho) were calculated using Spearman's correlation analysis; P<0.05 was considered to indicate a statistically significant difference. '-' indicates no significant correlation. OSCC, oral squamous cell carcinoma; FA, Fanconi anemia.

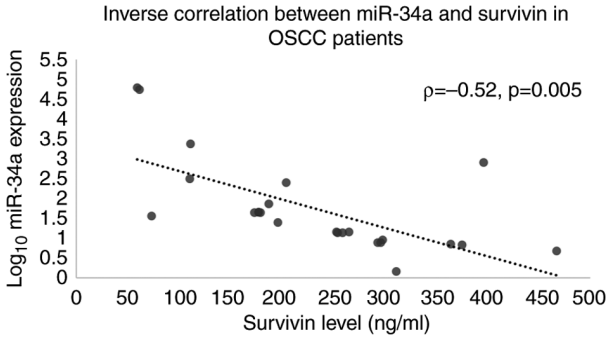

Figure 5. Inverse correlation between miR-34a and survivin in patients with OSCC. Scatter plot demonstrating the correlation between salivary miR-34a relative expression (log_{10}-transformed) and serum survivin levels (ng/ml) in patients with OSCC. miR-34a expression inversely correlated with the survivin concentration (Spearman's Rho, -0.52, P=0.005). OSCC, oral squamous cell carcinoma.

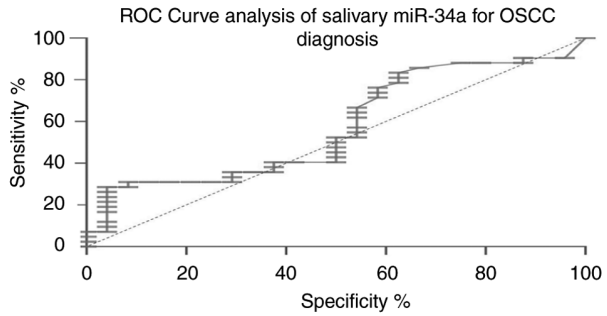

Figure 3. ROC curve analysis of salivary miR-34a for OSCC diagnosis. ROC curve plotting sensitivity vs. specificity for miR-34a as a diagnostic marker for OSCC. AUC, 0.575 (95% CI, 0.439-0.719); cut-off value, >24.53; sensitivity, 24%; specificity, 95%. ROC, receiver operating characteristic; OSCC, oral squamous cell carcinoma; AUC, area under the curve; CI, confidence interval.

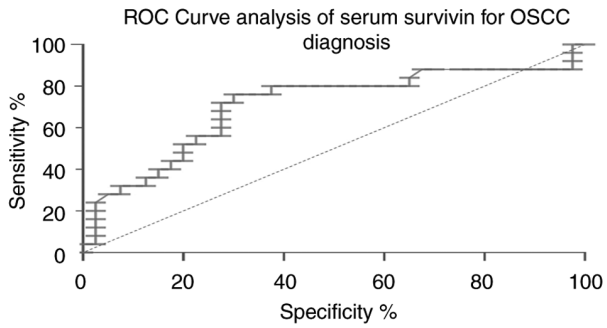

Figure 4. ROC curve analysis of serum survivin for OSCC diagnosis. ROC curve demonstrating the diagnostic performance of survivin for OSCC. AUC, 0.710 (95% CI, 0.657-0.934); cut-off value, >191.1; sensitivity, 70%; specificity, 97%. ROC, receiver operating characteristic; OSCC, oral squamous cell carcinoma; AUC, area under the curve; CI, confidence interval.

P=0.014 in FA) and survivin (P=0.04 in OSCC and P=0.01 in FA) compared to the controls, providing molecular insight into the risk of developing OSCC in patients with FA. Another key finding and limitation is the marked age disparity between patients with FA and OSCC, which reflects the biology of FA-related carcinogenesis, but introduces potential age-related confounding factors.

The marked reduction of miR-34a levels in both OSCC and FA aligns with its tumor-suppressive role, as it regulates the Wnt and PI3K pathways by targeting genes, such as WNT1, PTEN and BIRC5 (11). However, miR-34a did not exhibit a significant association with clinical parameters, such as T stage or lymph node metastasis (P=0.34 and P=0.87, respectively). It exhibited poor diagnostic performance (AUC, 0.575), indicating limitations as a standalone early predictive marker for OSCC. Future studies are thus required to investigate the use of combined biomarkers to improve the diagnostic accuracy. In OSCC, a decreased miR-34a expression was associated with enhanced tumor proliferation and metastasis, consistent with studies reporting its downregulation in head and neck SCCs and an association with advanced tumor stages (12). In the present study, the findings of a reduced miR-34a level in OSCC and FA are consistent with those of the study by Kalfert *et al* (8), who reported the downregulation of miR-34a in head and neck cancers, emphasizing its potential as a salivary biomarker for early detection.

In FA, the reduction of miR-34a expression, despite elevated p53 activity, suggests alternative pathways that suppress tumor-suppressive mechanisms, potentially indicating early malignant transformation. This finding is in contrast to that of reports of miR-34a upregulation in acute graft-vs.-host disease in FA, highlighting the context-specific regulation of this miRNA (13).

Elevated survivin levels in OSCC and FA underscore its anti-apoptotic function via the PI3K/AKT signaling pathway, promoting tumor survival (9). Overexpressed in SCCs (e.g., head and neck, lung) and detectable in biofluids, survivin is associated with an aggressive tumor behavior and a poor prognosis (9,14). In the present study, in patients with FA, significantly higher survivin levels (P=0.001 vs. controls and P=0.028 vs. OSCC) may reflect the evasion of apoptosis under cellular stress. However, its lack of specificity limits its utility as an OSCC-specific marker in FA. These findings align with those in the study by Xie *et al* (15), who identified survivin as a prognostic biomarker in oral cancers through a meta-analysis, highlighting its role in tumor progression. The inverse correlation observed between salivary miR-34a and

serum survivin (Spearman's Rho=-0.52, P=0.005) in patients with OSCC supports a potential regulatory axis, which may be disrupted in FA. Although the present study did not directly investigate mechanistic regulation, previous studies suggest that miR-34a can downregulate survivin directly via the PI3K/AKT pathway (16). This possible regulatory axis warrants functional validation in future *in vitro* and *in vivo* studies.

The consistency of miR-34a and survivin alterations in saliva and serum supports non-invasive sampling, which is particularly beneficial for patients with FA who are intolerant to invasive procedures. Salivary biomarkers, such as miR-34a, could enhance annual OSCC screening protocols for high-risk populations such as patients with FA by enabling non-invasive monitoring during routine dental check-ups. Although limited by sample size (FA, n=24; OSCC, n=24; controls, n=40), which prevents the identification of significant associations with clinical parameters, the trends of lower miR-34a expression in advanced OSCC stages align with previous research (8,12). The ROC analysis for survivin (AUC, 0.710; sensitivity, 70%; specificity, 97%) indicates moderate diagnostic potential, which is lower than that reported in other cancer types (e.g., AUC, 0.729; sensitivity, 57%; specificity, 82.6% in colon cancer) (17). By contrast, miR-34a exhibited poor diagnostic performance (AUC, 0.575), further indicating that it cannot be considered a reliable standalone marker and supporting the need for multi-marker approaches.

The findings of the present study align with those in the study by Chen *et al* (18), who linked miR-34a suppression to cutaneous SCC progression, and studies identifying survivin as a prognostic marker in head and neck cancers (9,14,15). By demonstrating alterations in miR-34a and survivin in FA and OSCC, the present study lays the groundwork for non-invasive biomarker strategies to enhance early detection of OSCC in FA. However, larger studies are warranted to validate their clinical utility.

The present study has certain limitations which should be mentioned. The key limitations of the present study include the small sample size, the genotypic heterogeneity of FA (e.g., FANCA mutations) and demographic variability between groups. In particular, the significant age disparity between the patients with FA (mean age, 20.03±5.87 years) and patients with OSCC (mean age, 54.52±10.49 years) reflects the biology of FA-related carcinogenesis, but also introduces potential age-related confounding factors. The recruitment of age-matched patients with FA and sporadic OSCC was not feasible in the current single-center sample, and the limited cohort size precluded adequately powered age-adjusted multivariable analyses. Additionally, the lack of tumor stage stratification in OSCC further limits the generalizability of the findings. Consequently, the biomarker differences observed herein should be interpreted cautiously and validated in larger, multicenter cohorts that are age-balanced or stratified and prospectively followed for OSCC development and treatment outcomes.

In conclusion, the present study demonstrates that miR-34a and survivin are promising non-invasive biomarkers for the surveillance of OSCC in patients with FA and OSCC. Their integration into clinical screening protocols could enhance early detection and improve survival outcomes in patients with FA and OSCC.

Acknowledgements

The authors would like to thank the organizing committee of the European Hematology Oncology Congress (EHOC) 2021, held as a hybrid event on November 10-13, 2021, at the Hilton Istanbul Bomonti Hotel and Conference Center, for providing a platform to present a preliminary version of this study (Abstract, published with DOI: 10.1016/j.htct.2021.10.1085), which helped refine the research through valuable feedback.

Funding

The present study was supported by the Scientific Research Projects Coordination Unit of Istanbul University (Project no. 34946) as part of a doctoral thesis project.

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

ZAK conceptualized and designed the study, performed the experiments, analyzed the data, drafted the manuscript and supervised the project. NY contributed to the study design, performed the clinical examinations of patients with Fanconi anemia, and contributed to the writing of the manuscript and evaluation of the results. TTC conducted the clinical examinations of patients with Fanconi anemia and evaluated the study outcomes. BB, HMY and OK performed clinical the examinations of patients with oral squamous cell carcinoma and contributed to the evaluation of the study outcomes. MGG conducted the statistical analyses and contributed to data interpretation. ZAK and NY confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Istanbul University Clinical Research Ethics Committee (Approval no. 1307; November 13, 2019) and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants, with parental consent being obtained for those <18 years of age.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Use of artificial intelligence tools

During the preparation of this work, AI tools were used to improve the readability and language of the manuscript or to

generate images, and subsequently, the authors revised and edited the content produced by the AI tools as necessary, taking full responsibility for the ultimate content of the present manuscript.

References

1. Rivera C and Venegas B: Histological and molecular aspects of oral squamous cell carcinoma (Review). Oncol Lett 8: 7-11, 2014.
2. Warnakulasuriya S: Global epidemiology of oral and oropharyngeal cancer. Oral Oncol 45: 309-316, 2009.
3. Mehrotra R and Yadav S: Oral squamous cell carcinoma: Etiology, pathogenesis, and prognostic value of genomic alterations. Indian J Cancer 43: 60-66, 2006.
4. Kutler DI, Auerbach AD, Satagopan J, Giampietro PF, Batish SD, Huvos AG, Goberdhan A, Shah JP and Singh B: High incidence of head and neck squamous cell carcinoma in patients with Fanconi anemia. Arch Otolaryngol Head Neck Surg 129: 106-112, 2003.
5. Singh T and Andi K: Fanconi anemia and oral squamous cell carcinoma: Management considerations. N Z Med J 130: 92-95, 2017.
6. Smetsers SE, Velleuer E, Dietrich R, Wu T, Brink A, Buijze M, Deeg DJ, Soulier J, Leemans CR, Braakhuis BJ and Brakenhoff RH: Noninvasive molecular screening for oral precancer in Fanconi anemia patients. Cancer Prev Res (Phila) 8: 1102-1111, 2015.
7. Mazumder S, Datta S, Ray JG, Chaudhuri K and Chatterjee R: Liquid Biopsy: miRNA as a potential biomarker in oral cancer. Cancer Epidemiol 58: 137-145, 2019.
8. Kalfert D, Ludvikova M, Pesta M, Ludvik J, Dostalova L and Kholová I: Multifunctional Roles of miR-34a in cancer: A review with the emphasis on head and neck squamous cell carcinoma and thyroid cancer with clinical implications. Diagnostics (Basel) 10: 563, 2020.
9. Pickhard A, Gröber S, Haug AK, Piontek G, Wirth M, Straßen U, Rudelius M and Reiter R: Survivin and pAkt as potential prognostic markers in squamous cell carcinoma of the head and neck. Oral Surg Oral Med Oral Pathol Oral Radiol 117: 733-742, 2014.
10. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
11. Fu J, Imani S, Wu MY and Wu RC: MicroRNA-34 family in cancers: Role, mechanism, and therapeutic potential. Cancers (Basel) 15: 4723, 2023.
12. Kumar B, Yadav A, Lang J, Teknos TN and Kumar P: Dysregulation of microRNA-34a expression in head and neck squamous cell carcinoma promotes tumor growth and tumor angiogenesis. PLoS One 7: e37601, 2012.
13. Wang L, Romero M, Ratajczak P, Lebœuf C, Belhadj S, Peffault de Latour R, Zhao WL, Socié G and Janin A: Increased apoptosis is linked to severe acute GVHD in patients with Fanconi anemia. Bone Marrow Transplant 48: 849-853, 2013.
14. Khan Z, Khan AA, Yadav H, Prasad GBKS and Bisen PS: Survivin, a molecular target for therapeutic interventions in squamous cell carcinoma. Cell Mol Biol Lett 22: 8, 2017.
15. Xie S, Xu H, Shan X, Liu B, Wang K and Cai Z: Clinicopathological and prognostic significance of survivin expression in patients with oral squamous cell carcinoma: Evidence from a meta-analysis. PLoS One 10: e0116517, 2015.
16. Shen Z, Zhan G, Ye D, Ren Y, Cheng L, Wu Z and Guo J: MicroRNA-34a affects the occurrence of laryngeal squamous cell carcinoma by targeting the antiapoptotic gene survivin. Med Oncol 29: 2473-2480, 2012.
17. Jakubowska K, Pryczynicz A, Dymicka-Piekarska V, Famulski W and Guzińska-Ustymowicz K: Immunohistochemical expression and serum level of survivin protein in colorectal cancer patients. Oncol Lett 12: 3591-3597, 2016.
18. Chen S, Yuan M, Chen H, Wu T, Wu T, Zhang D, Miao X and Shi J: MiR-34a-5p suppresses cutaneous squamous cell carcinoma progression by targeting SIRT6. Arch Dermatol Res 316: 299, 2024.