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Assessment of miRNA Serological Levels in Esophageal Cancer Patients from Southern Africa: Insights into Demographic-Specific Variation and Potential Predictor of Disease Severity

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Abstract:

Introduction: Esophageal cancer (EC) is one of the least studied cancers in the world, but also one of the deadliest due to its very aggressive nature and low survival rate.

Aim of the study: To evaluate the demographic influences of serological miRNA levels in esophageal cancer and its use as an indicator of disease progression.

Subjects and Methods The quantification of miRNA was examined in 51 newly diagnosed ESCC patients using quantitative ELISA kits utilized according to the manufacturer's protocol. Demographic parameters were recorded and evaluated against the level of miRNA.

Results: Our study showed that they were no significant relationship between the level of miRNAs and the studied parameters such as age, gender, alcohol, smoking, and esophageal cancer severity.

Conclusion: The study revealed that while age, gender, alcohol consumption, and smoking demonstrated varying associations with miRNA levels, none reached statistical significance, emphasizing the complexity of these relationships. Furthermore, the study found no significant correlation between miRNA levels and the progression of ESCC, challenging the notion of using miRNAs as reliable indicators of cancer severity in this specific population.

Keywords: Esophageal Squamous Cell Carcinoma (ESCC); MicroRNA (miRNA) Dynamics; Cancer biomarkers; Demographic-specific variations; Diagnostic Potential.

1. Introduction

Esophageal cancer (EC) is one of the least studied cancers in the world, but also one of the deadliest due to its very aggressive nature and low survival rate [1].

In Southern Africa, there is a predominance of esophageal squamous cell carcinoma (ESCC) as is seen in most in developing countries. In South Africa, the former Transkei region of the Eastern Cape (EC) is considered the epicenter of this disease [2]. Previous research has proven that microRNAs (miRNAs) are regularly downregulated in the EC, suggesting that miRNAs are vital for tumorigenesis. miRNAs are regulators of RNA translation as they perform a function in biological and pathological techniques such as cell differentiation, apoptosis, proliferation, and metabolism [3].

Since the discovery of miRNAs, they have been proven to play a role in the pathogenesis of cancer through their dual role as an oncogene and a tumor suppressor. A massive range of miRNAs have proven variable expression in esophageal cancer tissues and have hence been investigated for viable diagnostic use. Furthermore, there is a developing interest in their use as prognostic markers and determinants of treatment response [4]. The purpose of this study was, therefore, to investigate the determinants of serum miRNA levels and their use as a potential marker to assess ESCC progression in a rural African population.

2. Subjects and Methods

2.1.Study design

The study was conducted at the Endoscopic Unit of Nelson Mandela Academic Hospital (NMAH) in Mthatha, a rural town situated in the Eastern Cape province of South Africa.

2.2.Subjects

This cross-sectional study recruited participants based on convenience, including all individuals meeting the enrolment criteria. The study cohort comprised patients diagnosed with esophageal squamous cell carcinoma (ESCC) between January 2021 and December 2021. Diagnoses were confirmed through endoscopy and histopathology, while individuals with known underlying chronic conditions such as other cancers, hypertension, diabetes, or digestive problems were excluded.

2.3.Methods

Data Collection

2.3.1. Lifestyle Evaluation

Demographic information and lifestyle data were gathered through a structured questionnaire completed by participating patients.

2.3.2.miRNA Quantification

Professional nurses drew venous blood from enrolled ESCC patients into purple-top tubes containing EDTA as an anticoagulant. After centrifugation at 3200rpm for 10 minutes at 4°C, serum was collected. miRNA quantification was performed using an ELISA technique, detecting both normal and mutated miRNA following the manufacturer's guidelines (Elabscience, Inc, USA).

2.3.3.Cancer Grading

A qualified pathologist at the hospital laboratory evaluated esophageal cancer progression through histopathology assessments. Cancer grading was based on the abnormality of tumor tissue under microscopy:

- Grade 1: Well-differentiated tumors, resembling healthy cells (low grade).
- Grade 2: Moderately differentiated tumors, displaying somewhat abnormal tissue (intermediate grade).
- Grade 3: Poorly differentiated tumors, featuring highly abnormal cells without a distinct architectural structure (high grade).
- Grade 4: Undifferentiated tumors, with the most abnormal-looking cells, typically exhibiting faster growth and spread (highest grade).

2.4.Data Statistical Analysis

Microsoft Excel recorded the data, and Stata 15 was employed for data management and analysis. Descriptive statistics and frequency distributions, including mean and standard deviations, were analyzed. miRNA levels were categorized into two groups based on a threshold of 7 ng/µL. The median and interquartile range assessed miRNA levels, and the Mann-Whitney U test compared groups. The Chi-squared test explored associations between variables, and logistic regression evaluated the relationship between miRNA levels and the esophageal cancer stage. A *p*-value of 0.05 or less was considered statistically significant.

3. Results

3.1.Demographic Characteristics of the Population

A total of 51 patients newly diagnosed with ESCC were recruited, and their demographic data are summarized in Table 1. Among the patients, 61% were female, 53% were over 65 years old, 57% were alcohol drinkers, 53% were smokers, and 65% had education levels not surpassing primary school (**Table 1**).

We recruited a total of 51 patients newly diagnosed with ESCC. Their demographic data is summarized in Table 1 below. We found that 61% of the patients were female, 53% were over 65 years of age, 57% were alcohol drinkers, 53% were smokers and 65% were not educated beyond primary school (**Table 1**).

Table 1: Demography characteristics of oesophageal cancer.

Characteristics		**Frequency (%)**
Gender	Male	20 (39)
	Female	31 (61)
Age	65 and below	24 (47)
	65 and above	27 (53)
Smoking	Yes	24 (47)
	No	27 (53)
Drinking	Yes	29 (57)
	No	22 (43)
Education	None	6 (12)
	Primary	33 (65)
	High School	12 (24)

3.2.miRNA Quantification

Table 2 presents the miRNA levels in ng/µl, expressed as median and interquartile range. The overall miRNA level in the population was 6.8 (3.1;9.7) ng/µl. Male patients exhibited a higher miRNA level at 7.1 (3.5, 11.5) ng/µl

compared to female patients. Older patients also showed a higher miRNA level at 6.7 (2.3;9.7) ng/µl. Smoking and non-drinking patients displayed higher miRNA levels at 7.6 (4.7;9.9) ng/µl and 7.1 (4.2;11.3) ng/µl, respectively. Interestingly, lower-grade cancers (Grade 1 and 2) presented with a higher miRNA level compared to higher-grade cancers (Grade 3 & 4), with respective levels of 7.1 (3.1;11.3) ng/µl and 6.8 (3.4;9.2) ng/µl. Unfortunately, none of these values reached statistical significance (**Table 2**).

Table 2: MiRNA level among oesophageal cancer patients.

		Level of MiRNA (ng/µL)	
		Median (Interquartile range)	***P*-Value#**
Overall		6.8 (3.1;9.7)	
Gender	Male	7.1 (3.5;11.5)	0.354
	Female	6.6 (3.0;9.1)	
Age	65 and below	6.7 (2.3;9.7)	0.503
	65 and above	7.3 (3.8;9.5)	
Smoking	Yes	7.6 (4.7;9.9)	0.124
	No	5.9 (2.1;9.1)	
Drinking	Yes	6.8 (3.0;9.2)	0.628
	No	7.1 (4.2;11.3)	
Grade of cancer	1 - 2	7.1 (3.1;11.3)	0.783
	3 - 4	6.8 (3.4;9.2)	

#Mann-Whitney U test.

3.3.Patient Parameters and Level of miRNA: Relationships

An exploration of the relationship between miRNA levels and selected demographic parameters known to be risk factors for esophageal cancer is presented in **Table 3**. The results revealed no evidence of a relationship between miRNA and demographic parameters. The distribution of patients between higher and lower miRNA levels was almost equal, with no statistical significance found (**Table 3**). Similar non-significant results emerged when examining

the relationship between histological cancer grade and miRNA levels (**Table 3**).

Table 3: Relationship between patient parameters and level of miRNA.

Characteristics		**Low MiRNA level (<7 ng/µL)**	**High MiRNA level (≥7 ng/µL)**	***P*-Value#**
Gender	Male	10	10	0.114
	Female	17	14	
Age	65 and below	14	10	0.529
	65 and above	13	14	
Smoking	Yes	10	14	2.313
	No	17	10	
Drinking	Yes	16	13	0.134
	No	11	11	
Stage of cancer	Stages 1 & 2	11	11	0.134
	Stages 3 & 4	16	13	

#Chi-Square test.

3.4.Patient Parameters and Level of miRNA: Associations

Further analysis using logistic regression, while controlling for well-known esophageal cancer confounders, is summarized in Table 4. The odds of having a high miRNA level were 89% greater in female patients [OR: 1.89; 95% CI: 0.37-9.66] and doubled in older patients [OR: 2.04; 95% CI: 0.57-7.39] (**Table 4**). In contrast, non-smoking participants had 88% lower odds of having a high miRNA level [OR: 0.12; 95% CI: 0.02-0.82] (**Table 4**). Surprisingly, the odds of a high miRNA level were twice as much in patients who did not drink alcohol [OR: 2.48; 95% CI: 0.55-11.20]. Importantly, the odds of a higher miRNA level were evenly distributed between lower and higher stages of esophageal cancer, confirming the independence of miRNA levels from esophageal cancer progression [OR: 0.93; 95% CI: 0.27-3.16] (**Table 4**).

Table 4: Association between patient parameters and level of miRNA.

Characteristics		OR (Adjusted)	95% CI	*P*-Value
Gender	Male	1	0.37-9.66	0.45
	Female	1.89		
Age	65 and below	1	0.57-7.39	0.275
	65 and above	2.04		
Smoking	Yes	1	0.42-2.82	0.160
	No	0.12		
Drinking	Yes	1	0.55-11.20	0.239
	No	2.48		
Stage of cancer	Grades 1 & 2	1	0.27-3.16	0.907
	Grades 3 & 4	0.93		

OR: Odds Ratio, CI: Confidence Interval.

4. Discussion

Over the past few decades, extensive research has established that microRNAs (miRNAs) play a crucial role in the oncogenesis and pathogenesis of various cancers, presenting potential implications for cancer prognosis and treatment [5]. Mitchell et al. (2008) were pioneers in demonstrating the stable detection of miRNA in serum, suggesting its potential as a diagnostic marker, particularly in prostate cancer [6]. The exploration of circulating miRNAs as novel biomarkers has been notable in various cancers, including lung cancer, colorectal cancer, gastric cancer, and breast cancer [7]. In the context of esophageal squamous cell carcinoma (ESCC), several studies have indicated the potential diagnostic value of circulating miRNAs [8, 9, 10, 11]. However, recent studies have highlighted considerable differences in the profile of circulating miRNAs between plasma and serum [12]. This study sought to assess the utility of miRNAs as indicators of ESCC severity in Southern African patients.

Examining age, our study observed that older individuals tend to have higher miRNA levels, although these findings did not reach statistical significance. In contrast, Hooten et al. (2013) reported differential

expression of circulating human serum miRNAs with age, with most miRNAs being downregulated in older individuals, suggesting a potential link between cellular aging and miRNA expression [13]. Despite the discrepancy, this emphasizes the population-specific variability in miRNA expression, considering previous studies focused on healthy participants.

Regarding gender, our results indicated no significant difference in miRNA levels between male and female participants. However, Hasáková et al. (2017) found an up-regulation of certain miRNAs in male participants with colorectal cancer, in contradiction to our findings [14]. The divergence might be attributed to our study's equal distribution of the disease between male and female patients.

Similarly, our study suggested higher miRNA levels in individuals who consume alcohol, but the results did not reach statistical significance. Conversely, Avissar et al. (2009) reported a significant correlation between miRNA and alcohol consumption, showing increased miRNA expression with alcohol consumption, particularly in tumors of pharyngeal and laryngeal origin [15]. Methodological differences, such as the sensitivity of RT-PCR compared to the ELISA procedure used in our study, may explain the disparity.

Similarly, our findings suggested higher miRNA levels in smokers, though not reaching statistical significance. This contradicts previous studies indicating miRNA downregulation in smokers [4, 16]. The equal distribution of smokers and non-smokers in our population may explain this controversial result.

Concerning ESCC severity, our study found no significant correlation between miRNA levels and disease progression, suggesting that miRNA may not be an effective indicator of cancer severity. This contradicts a 2005 study by Lu et al., which demonstrated lower miRNA levels in poorly differentiated tumors [17]. Subsequent studies supported the use of miRNA as cancer biomarkers but deemed them inadequate for assessing cancer progression [18]. The role of miRNA types as biomarkers for disease progression in ESCC remains unclear.

5. Conclusions

The observed higher miRNA levels in older individuals, alcohol drinkers, and smokers, while not statistically significant, underscore the complexity of miRNA

expression patterns influenced by multifaceted factors. Our results deviate from some existing literature, emphasizing the importance of acknowledging population-specific nuances in miRNA studies. Furthermore, the lack of a significant correlation between miRNA levels and ESCC progression challenges the notion of using miRNAs as reliable indicators of cancer severity. This finding contradicts prior studies and underscores the intricate nature of miRNA dynamics in ESCC. Therefore, it is advisable to use other diagnostic tools to evaluate ESCC severity.

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Ethics approval and consent to participate: This study followed the code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. All participants consented to their involvement before the study commenced, and confidentiality was observed at all times. Furthermore, permission to conduct the study was obtained from the Faculty of Health Sciences Human Research Ethics Committee under reference number 140/2021.

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Conflicts of Interest: All authors declare they have no conflicts of interest.

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