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Original article

Micro RNA 34a as a promising biomarker in early diagnosis of adult sepsis patients in ICU: A pilot study

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ABSTRACT

Background: Sepsis is a serious condition, miR-34a is a microRNA (miRNA) that plays a critical role in the onset of inflammatory and autoimmune diseases. Single nucleotide polymorphisms (SNPs) in miRNA-coding genes have the potential to change miRNA function by influencing expression rates and target specificity, implicating them in disease. We conducted a case-control study to determine whether the two miR-34a SNPs (rs2666433 and rs6577555) polymorphism increase the risk of adult sepsis and affect the outcome of patients with adult sepsis in the Egyptian population. **Methods:** 70 critically ill adult septic patients who met the SOFA score criteria for clinical sepsis or septic shock and 70 adult normal controls were enrolled in the study, laboratory tests for sepsis for cases and control were done. Two miR-34a SNPs (rs2666433 & rs6577555) were chosen for analysis and correlated with sepsis parameters. **Results**: The rs6577555 SNP (CC and AC genotypes) and the rs2666433 SNP (GG genotype) were significantly higher in adult sepsis patients than the control. Univariate and Multivariate regression analysis revealed that the rs6577555 genotypes AC+CC, and WBC corelated with susceptibility to adult sepsis, while rs2666433 genotypes GG correlate with adult sepsis only by Univariate regression analysis. The relationship between the demographic data, laboratory investigations for adult sepsis patients and SNP were significant between rs6577555 (CC, AC) genotype and CRP, total serum bilirubin, while insignificant for rs2666433 genotypes. **Conclusion**: miR-34a polymorphism may be a useful indicator of adult sepsis and may provide a new direction for its treatment.

Introduction

Sepsis as defined by the Society of Critical Care Medicine (SCCM) Sepsis-3 criteria is life-threatening organ dysfunction caused by an abnormal host response to infection. Sepsis and septic shock are high causes of mortality worldwide, resulting in the death of one in three to one in every six patients in whom sepsis is identified. Most sepsis screening tools use criteria that include clinical evaluation, vital signs, and laboratory data to screen for sepsis and predict mortality. The Systemic Inflammatory Response Syndrome (SIRS) criteria, quick Sequential Organ Failure Score (qSOFA), Sequential Organ Failure Assessment (SOFA) criteria, National Early Warning Score (NEWS), and Modified Early Warning Score (MEWS) are some of the most used tools for sepsis screening [1].

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The SOFA score, formerly known as the sepsis-related organ failure assessment score, is among the diagnostic criteria for sepsis syndrome that is used to monitor the sepsis patient's condition during their stay in an intensive care unit (ICU) to determine the extent of organ function. The score is composed of six distinct scores, one for the respiratory (PaO2/FiO2), cardiovascular (Mean arterial pressure), hepatic (Bilirubin), coagulation (Platelets), renal (Creatinine), and neurological systems (Glasgow coma scale) each scored from 0 to 4 with an increasing score reflecting worsening organ dysfunction [2].

Sepsis affects over 49 million people and kills approximately 11 million people each year, and the World Health Organization considers it a global health priority. Notably, these figures only include in-hospital deaths; the outcome for the remaining 38 million sepsis survivors discharged from the hospital varies greatly. The mortality rate within one year of hospital discharge ranges from 7% to 43%. The five-year mortality rates following sepsis range between 44% and 82% [3]. The incidence of sepsis is increasing as the population ages, the prevalence of chronic diseases rises, and antibiotic resistance spreads [4].

The pathophysiology of sepsis is complicated and includes elements of immune suppression, endothelial dysfunction, and pro-inflammatory responses. The outcome is a rise in proinflammatory cytokines such as IL6, IL8, TNF-α, and IL-1b as well as a systemic inflammatory response, Immunosuppression makes the host more prone to secondary infections [5].

MicroRNAs (miRNAs) are short, noncoding RNAs that regulate gene expression. MicroRNAs can target many protein-coding genes, influencing inflammation, immunity, apoptosis, and cell differentiation. Furthermore, studies have shown that miRNA expression is modulated early in sepsis and is positively correlated with severity and disease progression, making them powerful endogenous factors regulating the inflammatory signaling cascade during sepsis. Furthermore, blocking their proinflammatory effects can effectively improve sepsis-related organ injury, providing new insights into potential biomarkers and treatment options for sepsis [6]. Recent research has shown that serum miRNAs can serve as early biomarkers in a variety of diseases, including sepsis. Furthermore, miRNAs are considered ideal biomarkers for sepsis because of their selectivity and specificity, stability [7]

miR-34a is a miRNA that plays a critical role in the onset of inflammatory and autoimmune diseases. It has also been demonstrated that miR-34a regulates TLR signaling and the NF-κB-mediated inflammatory response, both of which are critical to the sepsis process [8]. Additionally, it has been demonstrated that overexpression of miR-34a causes the expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), which stimulates monocyte adhesion to endothelial cells and exacerbates endothelial cell inflammation which has a main role in the process of sepsis. [9]. An Egyptian study showed that miR-34a-may be useful as a novel biomarker in neonatal sepsis and could provide a new direction for treatment [8].

Single nucleotide polymorphisms (SNPs) are the most common type of human genetic variation. SNPs in miRNA-coding genes have the potential to change miRNA function by influencing expression rates and target specificity, implicating them in disease [10]. MiRNA-34a is encoded in the second exon of the MIR134AHG gene, located on chromosome 1p36.22 [11]. Two promoter SNPs (*rs6577555* and *rs2666433*) located in the miR-34a gene were selected for the study based on location, minor allele frequency (MAF) and their implications in numerous diseases whose main pathogenesis involve inflammation [12-17]. On the other hand, not much is known about the SNPs within miRNAs linked to adult sepsis. In this paper, we conducted a case-control study to determine whether the two miR-34a SNPs (rs2666433 and rs6577555) polymorphism increase the risk of adult sepsis and affect the outcome of patients with adult sepsis in the Egyptian population.

Patients and methods

This study was a case-control study, the sample size was estimated as follows: as it was gene polymorphism study, the Log fold change was used to calculate the sample size**.** Sample size was calculated as 25 patients per group using the following equation: (Gerald 1998) **,**n = k* s2/ d2, Where**:** n = the sample size per group; k = 2 (Z1-α/2 + Z1-β)2 = 21, given Z1-α/2 =1.96 for 95% confidence interval; Z1-β =1.28 for an 90% power of the study; d = the effect size = log2 (fold change)**,** given the expected fold change equals 2; s = the expected standard deviation of the log2 (fold

change), given that the standard deviation of the fold changes from different samples equals 2. An additional 15% of the calculated sample size was added to compensate for dropout.

By applying this formula, patients who participated were divided into two groups. Group 1 consisted of 70 critically ill adult septic patients who were clinically diagnosed with sepsis or septic shock within the previous 24 hours, and group 2 (a control group) consisted of 70 adults, normal controls from apparently healthy blood donors who came to Suez Canal University Hospital to donate blood. Laboratory tests were carried out at the same hospital's Clinical Pathology Department to diagnose and assess sepsis related organ damage. Patients taking beta-blockers, patients with acute organ dysfunction (SOFA score usually 0), DIC patients, metastatic cancer patients, and mild T.B patients were excluded from the study.

Case definitions

The definition of sepsis, as stated in the "Surviving Sepsis Campaign (SSC): International Guidelines for the Management of Septic Shock, is an acute change in the SOFA score of less than two points, indicating life-threatening organ failure brought on by infection that occurs either during the first 48 hours after ICU admission or at the time of admission. The term "septic shock" refers to sepsis combined with hypotension in which appropriate fluid resuscitation was not enough to maintain a mean arterial pressure >65 mmHg and a serum lactate level >2 mmol/L. This necessitated the use of vasopressors [18].

Study procedure

All subjects were subjected to full history taking (name, age, address, chronic illness, family history). The following physical examination and laboratory tests were used to diagnose and assess sepsis severity according to sofa score [2]: Body temperature >38°C or <36°C, Heart rate >90/min[a], Respiratory rate >20/min or $PaCO_2$ <32 mm Hg, Hypotension (SBP <90 mm Hg, MAP <70 mm Hg, or SBP drop >40 mm Hg), PaO_2/FiO_2 <300 mm Hg (<200 mm Hg in patients with primary diseases of respiratory tract), features of encephalopathy (anxiety, confusion, agitation, delirium, coma), Urine output <0.5 mL/kg/h over >2 h with adequate fluid intake/resuscitation, Leukocyte count $>12\times10^9$/L or $<4\times10^9$/L or >10% immature neutrophil, Platelet count $<100\times10^9$/L or INR >1.5 (using Sysmex 5 differential part ,Siemens AG, Erlangen, Germany), D dimer level (Sterilab Services, smart tester d-dimer, code: RTC-9902-1, Mornington Terrace, Harrogate North Yorkshire, United Kingdom), Elevated C- reactive protein, Serum bilirubin >34.2 micromol/L (2 mg/dL), elevated serum lactate and serum creatinine increase by >0.5 mg/dL (44.2 micromol/L) by using (fully automated auto-analyzer Cobas c 6000 ("Roche Diagnostics, Mannheim, Germany").

Candidate SNPs selection

Two SNPs were selected: (rs2666433/NC_000001.11:g.9153118A>G and rs6577555/NC_000001.11:g.9152228A>C). The following criteria were used to choose the SNPs for this study: (I) Minor allele frequency>5% in African population (according to data from the 1000 Genome Projects: https://www.internationalgenome.org/); (II) The SNPs should be in the miR-34a gene's regulatory regions; and (III) The SNPs have been previously reported by references. based on earlier reports [19]. These were validated by the Single Nucleotide Polymorphism database (dbSNP) (https://www.ncbi. nlm.nih.gov/snp/) and the Ensembl genome browser 107 (https://www.ensembl.org/index.html).

Total DNA isolation

Each individual provided a two-milliliter venous blood sample. Following the manufacturer's instructions, genomic DNA was extracted from whole-blood samples using the QIAamp DNA Blood mini-Kits (Qiagen, Hilden, Germany). The purity and concentration of DNA were measured using a NanoDropTM 2000 Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, United States). The material was then kept at -80°C until needed again.

PCR analyses and genotyping

The TaqMan SNP Genotyping Assay was used to genotype the samples. The PCR reaction used 5 µl of TaqMan Universal Master Mix, SNP Genotyping assay mix, nuclease-free water, and genomic DNA. The PCR primers used are listed in table 1. The PCR program was set up as follows: 2 minutes at 50°, 10 minutes at 95°, then 40 cycles at 95° for 15 seconds and 60°C for 1 minute. The 384-well ABI 7900HT Real-Time PCR system was used (API, California, USA).

Data management

Data was fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Categorical data were represented as numbers and percentages. The Chi-

square test was applied to compare between two groups. For continuous data, they were tested for normality by the Shapiro-Wilk test. Quantitative data were expressed as range (minimum and maximum), mean, standard deviation, median and Inter quartile range. For normally distributed quantitative variables, the Student T-test was used to compare two groups. On the other hand, for not normally distributed quantitative variables the Mann Whitney test was used to compare two groups. Kruskal Wallis test was used to compare three genotypes. Univariate logistic regression analysis was used to calculate the ratio of the odds and 95% Confidence Interval of an event occurring in patients' group to the odds of it occurring in the control group. The population of the sample studied was explored to find its equilibrium with Hardy-Weinberg equation. The significance of the results obtained was judged at the 5% level.

Ethical considerations

The current study was carried out in accordance with the Declaration of Helsinki's recommendations. The Faculty of Medicine, Suez Canal University, Egypt's Research Ethics Committee granted ethical approval (**5739#**). The patients gave their informed consent, specifying every step of the study as well as their freedom to discontinue participation at any time.

Results

Baseline demographic and clinical characteristics of adult sepsis cases and healthy control

Patients who participated in the study were divided into two groups, 70 critically ill adult septic patients (50.0% men and 50.0% women), and a control group consisting of 70 adults, normal controls (52.9% men and 47.1% women) with mean ages of 56.29 and 54.47 for cases and controls, respectively). Most of the laboratory values showed significant differences between the two groups, including the (creatinine levels, serum lactate, serum total bilirubin, WBC count, and C reactive protein (CRP)) (the case > control). When compared to the control group, the cases' D dimer levels were noticeably lower (p<0.001) (**Table 2**).

Table 3 showed that, in the *rs6577555* A >C SNP, the CC and AC genotypes were significantly higher in adult sepsis patients than the control group. Under the dominant genetic model, the AC+CC (versus AA) genotypes and the C allele were also higher in the cases. In the *rs2666433* **A>G SNP** the GG genotypes were significantly higher in adult sepsis patients than the control group, Under the dominant genetic model, the AG + GG genotypes and the G allele were also higher in the cases than the control.

Table 4 showed that, in the *rs6577555* the chance of developing adult sepsis increased by 7.8-fold and 88.4-fold respectively (95% CI=2.742 – 22.186, p=<0.001 and 95% CI=15.869- 492.432, p =<0.001 respectively) with the CC and AC genotypes. Also, the AC+CC genotypes increased the chance of developing adult sepsis by 12.2-fold (95% CI=4.413 – 34.160, p p=<0.001) than the control.

In the *rs2666433* A>G SNP the chance of developing adult sepsis increased by 8-fold (95% CI=2.126 – 30.760, p= 0.002) with the GG genotypes than the control group.

Next, we examined haplotypes of the *rs6577555* and *rs2666433* in relation to adult sepsis. **Table 5,** compared to wild-type (A—A), the haplotypes C—A, and C—G were present in 29.3%, and 35.7% ,respectively of adult sepsis patients and increased the risk of adult sepsis by 8.6, and 4.8-fold, respectively (p<0.001).

We then examined the relationship between demographic data, laboratory investigations of sepsis in cases group with the genotypes of the two studied SNPs. There were significant relationships between CRP and serum total bilirubin and the genotype CC, AC of *rs6577555,* respectively **(Table 6).** There was no significant relationship between the demographic data, laboratory investigations of adult sepsis patients and *rs2666433* genotype **(Table 7).**

Univariate logistic regression analysis revealed that the *rs6577555* genotypes AC+CC (OR=7.8, 95% CI= 2.7 – 22.2, p=<0.001/ OR=88.4, 95% CI= 15.9 – 492.4, p=<0.001) respectively, *rs2666433* genotypes GG (OR=8.1, 95% CI=2.1 – 30.8, p=<0.001) and WBC (TLC) (OR=1.6 95% CI=1.4 – 1.8, p=<0.001) corelate with susceptibility to adult sepsis. Univariate and multivariate regression showed that adult sepsis susceptibility increases with WBC and the presence of *rs6577555* genotype CC **(Table 8)**.

Table 1. Primers for genotyping SNPs in miR-34a.

SNP	Sequences of PCR primers	
rs2666433	Forward primer	5'-TGCCAGGCCAAGATCTAGTGAC-3'
	Reverse primer	5'-CTCTTTCCCCCTTGCACAGAGA-3'
rs6577555	Forward primer	5'-GGAGGATCACTTGAGGCGAGAA-3'
	Reverse primer	5'-ATTGCTTCCCTTTCACGCAGAC-3'

Table 2. Comparison between the two groups studied according to different parameters.

	Cases (n = 70)	**Control (n = 70)**	**Test of sig.**	***P***
Sex				
Male	35 (50.0%)	37 (52.9%)	χ^2= 0.114	0.735
Female	35 (50.0%)	33 (47.1%)		
Age (years)				
Min. – Max.	44.0 – 65.0	43.0 – 65.0	t= 1.798	0.075
Mean ± SD.	56.29 ± 4.68	54.47 ± 7.03		
Median (IQR)	56.0 (54.0 – 59.0)	55.50 (47.0 – 61.0)		
WBCs (×10^3/ul)				
Min. – Max.	2.3 – 27.0	4.0 – 11.0	U= 490.000*	<0.001*
Mean ± SD.	15.9 ± 5.2	6.5 ± 1.6		
Median (IQR)	16.8 (14.70 – 18.50)	6.0 (5.0 – 7.0)		
CRP (mg/l)				
Min. – Max.	69.0 – 212.0	0.0 – 3.10	U= 0.000*	<0.001*
Mean ± SD.	146.6 ± 36.0	0.618 ± 0.690		
Median (IQR)	155.0(123.0 – 174.0)	0.30 (0.13 – 1.0)		
Serum lactate				
Min. – Max.	2.1 – 5.2	0.1 – 2.1	U= 3.000*	<0.001*
Mean ± SD.	3.2 ± 0.8	1.0 ± 0.5		
Median (IQR)	3.1 (2.50 – 3.60)	0.9 (0.50 – 1.30)		
Serum total Bilirubin				
Min. – Max.	0.40 – 7.40	0.20 – 1.30	U= 371.00*	<0.001*
Mean ± SD.	3.69 ± 1.58	0.82 ± 0.26		
Median (IQR)	3.80 (2.80 – 4.70)	0.90 (0.60 – 1.0)		
Creatinine				
Min. – Max.	1.10 – 2.50	0.40 – 1.10	U= 3.000*	<0.001*
Mean ± SD.	1.81 ± 0.42	0.72 ± 0.24		
Median (IQR)	1.75 (1.50 – 2.20)	0.60 (0.50 – 1.0)		
D-dimer				
Min. – Max.	1.0 – 2.0	2.0 – 2.0	U= 980.000	<0.001*
Mean ± SD.	1.40 ± 0.50	2.0 ± 0.0		
Median (IR)	1.0 (1.0 – 2.0)	2.0 (2.0 – 2.0)		

*IQR: **Inter quartile range***   *SD: **Standard deviation***   ***t: Student t-test***   ***U: Mann Whitney test*** χ^2: ***Chi square test***

*p: **p value** for comparing between the two studied groups*   **: Statistically significant at $p \le 0.05$*

Table 3 . Comparison between the two studied groups according to *rs6577555* and *rs2666433* single nucleotide polymorphisms.

	Cases (n = 70)	Control (n = 70)	χ^2	*p*
rs6577555				
AA	5 (7.1%)	34 (48.6%)	42.478*	<0.001*
AC	39 (55.7%)	34 (48.6%)		
CC	26 (37.1%)	2 (2.9%)		
$^{HW}p_0$	**0.060**	**0.056**		
Allele				
A	49 (35.0%)	102 (72.9%)	40.378*	<0.001*
C	91 (65.0%)	38 (27.1%)		
Dominant [AC + CC]	65 (92.9%)	36 (51.4%)	29.891*	<0.001*
rs2666433				
AA	23 (32.9%)	31 (44.3%)	12.653*	0.002*
AG	29 (41.4%)	36 (51.4%)		
GG	18 (25.7%)	3 (4.3%)		
$^{HW}p_0$	**0.162**	**0.060**		
Allele				
A	75 (53.6%)	98 (70.0%)	8.002*	0.005*
G	65 (46.4%)	42 (30.0%)		
Dominant [AG + GG]	47 (67.1%)	39 (55.7%)	1.929	0.165

*χ^2: **Chi square test** $^{HW}p_0$: p value for Chi square for goodness of fit for Hardy-Weinberg equilibrium (If p<0.05-not consistent with HWE.) p: p value for comparing between the two studied groups *: Statistically significant at p ≤ 0.05*

Table 4. Comparison between the two studied groups according to genotype.

Genotype	Frequency	Cases (n = 70)	Control® (n = 70)	Crude Odds ratio	
				p	OR (LL – UL 95%C.I)
rs6577555	AA®	5 (7.1%)	34 (48.6%)		1.000
	AC	39 (55.7%)	34 (48.6%)	<0.001*	7.800 (2.742 – 22.186)
	CC	26 (37.1%)	2 (2.9%)	<0.001*	88.400 (15.869 – 492.432)
	AC + CC	65 (92.9%)	36 (51.4%)	<0.001*	12.278 (4.413 – 34.160)
rs2666433	AA®	23 (32.9%)	31 (44.3%)		1.000
	AG	29 (41.4%)	36 (51.4%)	0.825	1.086 (0.524 – 2.249)
	GG	18 (25.7%)	3 (4.3%)	0.002*	8.087 (2.126 – 30.760)
	AG + GG	47 (67.1%)	39 (55.7%)	0.166	1.624 (0.818 – 3.227)

*OR: Odds ratio **®: reference group** CI: Confidence interval LL: Lower limit UL: Upper Limit*

Table 5. Haplotype frequency among the studied population.

rs6577555	*rs2666433*	Cases (n = 140)	Control® (n = 140)	*p*	OR (LL – UL 95%C.I)
A	A	34 (24.3%)	86 (61.4%)		1.000
A	G	15 (10.7%)	16 (11.4%)	0.036*	2.371 (1.056 – 5.323)
C	A	41 (29.3%)	12 (8.6%)	<0.001*	8.642 (4.058 – 18.405)
C	G	50 (35.7%)	26 (18.6%)	<0.001*	4.864 (2.621 – 9.026)

*OR: Odds ratio **®: reference group** CI: Confidence interval LL: Lower limit UL: Upper Limit*
*p: p value for **Univariate regression analysis** *: Statistically significant at p ≤ 0.05*

Table 6. Relation between *rs6577555* with demographic data and laboratory investigations in cases group (n = 70)

	rs6577555			Test of Sig.	*p*
	AA (n = 5)	**AC** (n = 39)	**CC** (n = 26)		
Sex					
Male	3 (60.0%)	18 (46.2%)	14 (53.8%)	FFT= 0.664	0.754
Female	2 (40.0%)	21 (53.8%)	12 (46.2%)		
Age (years)					
Mean ± SD.	52.6 ± 7.4	56.9 ± 4.4	56.1 ± 4.3	F= 1.961	0.149
Median (Min. – Max.)	51.0 (45.0 – 63.0)	56.0 (44.0 – 65.0)	55.5 (47.0 – 64.0)		
WBCs ($\times 10^3$ / ul)					
Mean ± SD.	13 ± 6	16.7 ± 4.4	15.4 ± 6.1	H= 2.541	0.281
Median (Min. – Max.)	13.5 (3.4 – 20)	16.7 (3.2 – 25.2)	16.9 (2.3 – 27)		
CRP (mg/l)					
Mean ± SD.	118.8 ± 30.4	138.7 ± 35.5	163.8 ± 30.8	H= 11.678*	0.003*
Median (Min. – Max.)	132 (78 – 154)	143 (69 – 209)	169.5 (76 – 212)		
Serum lactate					
Mean ± SD.	3 ± 0.4	3.3 ± 0.9	3.1 ± 0.8	H= 0.425	0.809
Median (Min. – Max.)	3.1 (2.4 – 3.5)	3.1 (2.1 – 5.2)	3.1 (2.1 – 5.1)		
Serum total bilirubin					
Mean ± SD.	1.9 ± 0.8	3.9 ± 1.5	3.7 ± 1.5	H= 9.158*	0.010*
Median (Min. – Max.)	2.1 (0.5 – 2.6)	3.9 (0.4 – 7.4)	3.7 (0.4 – 7.1)		
Creatinine					
Mean ± SD.	1.82 ± 0.29	1.80 ± 0.41	1.82 ± 0.46	H= 0.034	0.983
Median (Min. – Max.)	1.9 (1.4 – 2.2)	1.80 (1.1 – 2.5)	1.7 (1.1 – 2.5)		
D-dimer					
Mean ± SD.	1.0 ± 0.0	1.44 ± 0.50	1.42 ± 0.50	H= 3.549	0.170
Median (Min. – Max.)	1.0 (1.0 – 1.0)	1.0 (1.0 – 2.0)	1.0 (1.0 – 2.0)		

SD: Standard deviation F: F for One way ANOVA test H: H for Kruskal Wallis test χ^2: Chi square test
*p: p value *: Statistically significant at p ≤ 0.05*

Table 7. Relation between *rs2666433* with demographic data and laboratory investigations in cases group (n = 70)

	rs2666433			Test of Sig.	*p*
	GG (n = 23)	**AG (n = 29)**	**AA (n = 18)**		
Sex					
Male	8 (34.8%)	17 (58.6%)	10 (55.6%)	χ^2= 3.215	0.200
Female	15 (65.2%)	12 (41.4%)	8 (44.4%)		
Age (years)					
Mean ± SD.	54.8 ± 4.3	57.7 ± 4.7	55.9 ± 4.7	F= 2.602	0.082
Median (Min. – Max.)	55 (44 – 61)	57 (47 – 64)	55.5 (45 – 65)		
WBCs (×10^3 / ul)					
Mean ± SD.	17.1 ± 2.3	15.2 ± 6.5	15.7 ± 5.5	H= 0.832	0.660
Median (Min. – Max.)	16.9(13.5 –22.0)	16.8 (2.3 – 27.0)	15.9 (3.4 – 25.2)		
CRP (mg/l)					
Mean ± SD.	147.3 ± 38.0	151.8 ± 24.9	137.5 ± 47.3	H= 1.121	0.571
Median (Min. – Max.)	154(69.0 –206)	156(87.0 – 183)	115.5(76.0 212)		
Serum lactate					
Mean ± SD.	3.2 ± 0.7	3.3 ± 1.0	3.0 ± 0.6	H= 0.965	0.617
Median (Min. – Max.)	3.1 (2.2 – 4.5)	3.1 (2.1 – 5.2)	3.0 (2.1 – 4.3)		
Serum total Bilirubin					
Mean ± SD.	3.6 ± 1.6	3.7 ± 1.8	3.9 ± 1.2	H= 0.214	0.898
Median (Min. – Max.)	3.6 (0.5 – 7.1)	3.7 (0.4 – 7.4)	3.9 (1.9 – 5.9)		
Creatinine					
Mean ± SD.	1.7 ± 0.4	1.8 ± 0.5	1.9 ± 0.3	H= 1.464	0.481
Median (Min. – Max.)	1.6 (1.1 – 2.4)	1.7 (1.1 – 2.5)	1.9 (1.1 – 2.2)		
D-dimer					
Mean ± SD.	1.4 ± 0.5	1.5 ± 0.5	1.2 ± 0.4	H= 3.981	0.137
Median (Min. – Max.)	1.0 (1.0 – 2.0)	2.0 (1.0 – 2.0)	1.0 (1.0 – 2.0)		

SD: Standard deviation *F: F for One way ANOVA test* *H: H for Kruskal Wallis test* *χ^2: Chi square test*
p: p value **: Statistically significant at p ≤ 0.05*

Table 8. Univariate and multivariate logistic regression analysis for WBCs and Genotype to discriminate sepsis cases from control (n = 70 vs. 70) .

	Univariate		**#Multivariate**	
	p	**OR (LL – UL 95%C.I)**	***p***	**OR (LL – UL 95%C.I)**
rs6577555				
AA		1.0		1.0
AC	<0.001*	7.8 (2.7 – 22.2)	0.461	1.9 (0.3 – 11.1)
CC	<0.001*	88.4 (15.9 – 492.4)	<0.001*	145.4 (10.6 – 1996.2)
rs2666433				
AA		1.0		1.0
AG	0.825	1.1 (0.5 – 2.2)		
GG	0.002*	8.1 (2.1 – 30.8)	0.173	6.9 (0.4 – 109.6)
WBCs	<0.001*	1.6 (1.4 – 1.8)	<0.001*	1.7 (1.4 – 2.1)

Hosmer and Lemeshow Test (χ^2=18.796*; p=0.016*)
OR: Odd`s ratio C.I: Confidence interval LL: Lower limit UL: Upper Limit
#: All variables with *p*<0.05 were included in the multivariate *: Statistically significant at *p* ≤ 0.05

Discussion

Microbial pathogens interacting with the host immune system to produce severe systemic inflammation, and ultimately multisystem failure is known as sepsis, a potentially fatal illness [1]. MiRNAs, or short non-coding RNAs, regulate a variety of biological processes. There is mounting evidence that miRNAs play a critical role in immune regulation in autoimmune and infectious diseases. Numerous biomarkers have been studied to aid in

the diagnosis and prognosis of sepsis, despite limitations in severe and early cases [20]. Serum miRNAs, which are stable in circulation and released outside of cells into serum, have recently gained a lot of attention as a potential biomarker for the diagnosis and prognosis of sepsis due to their ease of detection [21]. In the human genome, single nucleotide polymorphisms, or SNPs, are the most prevalent variations at particular base positions. SNPs found in the coding genes of miRNAs can alter the expression of miRNAs, which in turn alters a range of biological functions [22].

In the current study, the adult sepsis group had significantly higher WBC count, CRP, serum lactate, serum total bilirubin, and creatinine levels than the control group. **Mahmud et al.** found that more than two-thirds (68.3%) of sepsis patients had elevated C-reactive protein levels, while 65% had elevated serum lactate levels [23]. **Mustafić et al.** found a strong positive correlation between CRP and lactate concentration and fatal outcome of sepsis [24]. WBC count, CRP, serum lactate, serum total bilirubin, and D dimer were higher significantly between the sepsis group than the control group, according to **Meng et al.** [25]. This demonstrates how crucial inflammatory markers are for identifying and assessing the degree of sepsis.

Unlike fibrin/fibrinogen degradation products (FDP), D-dimer (DD) is a specific degradation product of stabilized fibrin. As a result, the presence of an increased value for this biomarker indicates both thrombin generation and fibrinolytic degradation. Over the last few decades, several studies have investigated the potential prognostic value of DD in sepsis, yielding inconsistent or even controversial results. Although increased DD values have been associated with worse clinical outcomes in some studies, others failed to validate such findings, revealing that the prognostic value of DD may be modest or poor in sepsis patients [26].

In our study, the level of D dimer was significantly lower in the adult sepsis cases than in the control group (p<0.001). **Tang et al.** study revealed that the emergency patients with sepsis have D-dimer levels higher than 10 times of the upper normal limit [27]. Notably, a recent study also showed that the risk of death was almost four times higher for sepsis patients whose DD values were within the normal reference range [28]. In contrast, **Han et al.** found that patients with very low DD values paradoxically had the highest overall risk of death, up to 60% [29]. The low level of D-dimer in our study may be attributed to small sample size.

It has been widely reported that single nucleotide polymorphisms found in miRNA are the reason behind the aberrant functions of the miRNAs. SNPs in the miR-34a gene have previously been linked in some studies to an increased risk of developing a number of human diseases, including osteosarcoma [30], colon cancer [17], and type 2 diabetes [14]. Our results suggest a positive association between rs6577555 (The CC and AC genotypes) and adult sepsis with a chance of developing adult sepsis of 7.8 fold and 88.4 fold, respectively, also this is proved by Univariate logistic regression as independent risk factor for adult sepsis development. The rs2666433 polymorphism significantly increased the chance of developing adult sepsis by 8 fold in patients with GG genotypes compared to the control group as approved by univariate regression analysis; however, the chance of developing adult sepsis was only insignificantly increased by 1.6 fold in those with AG + GG (versus AA) genotypes.

According to **Wei et al.** the AA genotype of rs2666433 was linked to a notably higher risk of Ischemic Stroke (IS) than the GG genotype, and this relationship remained significant when comparing the AA genotype to the GG+GA genotype. Furthermore, compared to the G allele, the A allele of rs2666433 was also linked to a noticeably higher risk of IS. Meanwhile, rs6577555 was not linked to the development of IS [17]. **Choi et al.** also failed to find any wide differences in rs6577555 polymorphism between the control group and the cases of Ischemic stroke in a Korean population [16]. According to **Fawzy et al.** rs2666433 AA and AG were 2.8 and 5.7 times more likely to develop cancer than GG, respectively, and an allele that was highly prevalent in cancer tissues [31] . It could be explained by different distribution of SNPs among different populations.

In the current study, we also investigated the link between demographic information and laboratory tests of sepsis in the cases group and the miR-34a polymorphisms. While there was no significant correlation found between the rs2666433 genotype and the demographic information or laboratory tests of adult sepsis patients, there were significant relationships found between CRP and serum total bilirubin and the genotypes CC and AC of rs6577555, respectively that might control miR-34a level which in turn leads to increase of

inflammatory markers of sepsis.
A different study found no evidence of a significant correlation between the severity of IS and the miR-34a polymorphisms [17]. MiR-34a was shown by **Abdelaleem et al.** to have significant correlations with TLC, RDW, RBS, and CRP, suggesting that these variables are related to the severity of neonatal sepsis [8]. **Chen and colleagues** also demonstrated that miR-199a negatively regulated IKK-beta, facilitating the activation of NF-κB signaling [32], this, in turn, contributed to the induction of endogenous C-reactive protein [33]. Additionally, Xie et al. discovered that in patients with systemic lupus erythematosus and rheumatoid arthritis, miR-34a has a positive correlation with a number of inflammatory markers, including CRP [34].

Limitation: The main limitations of this study are the relatively small sample size and the ethnic restriction to the Egyptian population, which may limit the generalizability of the findings. Furthermore, the current study focused exclusively on miR-34a, leaving out other miRNAs that may be associated with adult sepsis. It also focuses on the miR-34a SNP but lacks follow-up on miRNA expression levels. Also, in addition to the current gene variant, other genetic/epigenetic and environmental factors play important roles in sepsis etiopathology.

Conclusions

Based on these findings, we deduced that: The miR-34a polymorphism may be a useful indicator of adult sepsis. Also, miR-34a polymorphism was significantly correlated with the severity of adult sepsis. Future research must be conducted in-depth to validate these SNPs in larger and more diverse populations to explore the genotype-phenotype association details of these variants, and to explore functional studies of miR-34a in sepsis.

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