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Article

Association of Serum Zinc, Selenium, and Oxidative Stress Markers with Disease Severity in Children with Recurrent Respiratory Infections

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Abstract: Recurrent respiratory infections (RRI) are frequent in childhood and can be affected by nutritional and oxidative factors. Oxidative stress can lead to tissue damage and disease progression, Zinc and Selenium are essential trace elements, involved in Immune regulation and Antioxidant defense. But, the association of these factors with RRI severity in children is still poorly understood. Objectives are to examine the relationship of serum zinc, selenium and some oxidative stress and antioxidant markers in relation to the severity of the disease in children with recurrent respiratory infections. And see whether there is a relationship between the serum levels of zinc and selenium and some oxidative stress and antioxidant markers and the severity of the disease in children with recurrent respiratory infections. The hospital-based Cross Section Analytical Study was carried out at Tikrit Teaching Hospital and Salah Al-Din General Hospital, Iraq, from March 2023 to September 2024. Children in the study were 150 children with RRI and 50 apparently healthy children aged 6 months-12 years. The RRI group was divided into mild, moderate and severe disease groups. Complete blood count parameters were measured, along with serum zinc, selenium, malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx), total antioxidant capacity (TAC) and C-reactive protein (CRP). The statistical analysis was done via SPSS version 26.0. The zinc and selenium levels in the blood were found to be significantly lower in the mild, moderate and severe RRI than in the controls (P<0.001). Conversely, the values of MDA were raised with an increase in disease severity (P<0.001), and those of SOD, GPx, and TAC were lowered (P<0.001). RRI was also significantly correlated with increased CRP levels, increased WBC counts, increased neutrophil count, higher infection rate, hospitalization, respiratory distress and lower oxygen saturation levels in severe RRI patients. The correlation between zinc, selenium and CRP and MDA was negative and the correlation with SOD, GPx and TAC was positive. Multivariate analysis revealed that the zinc, selenium, MDA, SOD, GPx and TAC were independent variables of severe RRI. The results of ROC analysis indicated the good discriminatory performance especially in MDA (AUC=0.91) and zinc (AUC=0.86). In conclusion, a lower level of zinc and selenium in children and increased oxidative stress are significantly associated with higher severity of recurrent respiratory infections in children. The results indicate that micronutrient imbalance and antioxidant defense impairment play a role in clinical burden of RRI. Additional prospective studies are merited to further elucidate the causality and to establish if correction of known micronutrient deficiencies has a beneficial effect on clinical outcomes.

Keywords: Recurrent respiratory infections; Zinc; Selenium; Oxidative stress; Children

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1. Introduction

Recurrent respiratory infections (RRI) are one of the most common clinical issues in childhood and constitute a substantial cause of repeated health care visits, antibiotic use, hospitalisation and unfavorable individual quality of life. While recurrent infections can

be attributed to normal childhood susceptibility (especially the preschool years), more than usual and frequent infections can be a sign of underlying abnormalities with host defense, airway structure, atopy, or nutrition status [1,2]. There are several interacting factors that influence the clinical severity of RRI; identification of biological markers that are linked to severe disease could enhance risk stratification and treatment.

Zinc is a trace element that plays a key role in innate and adaptive immunity. It plays a role in epithelial barrier integrity, in Lymphocyte function, in the regulation of inflammatory responses and in antiviral defence in the respiratory tract [3]. Zinc is also involved in antioxidant processes and in the maintenance of the cellular redox balance. As such, zinc deficiency has been linked to higher rates of respiratory infections in children. There is evidence from randomized trials and systematic reviews to suggest that zinc supplementation can decrease the rate of pneumonia and respiratory infections in selected populations of children, but these benefits seem less consistent when zinc is used to treat established severe respiratory infections [4,5].

Another micronutrient of interest in regards to immune and antioxidant function is selenium. Its biological activities are mainly mediated by selenoproteins, such as glutathione peroxidases, which are involved in cell protection from oxidative damages and inflammation/immune system regulation [6]. The interaction between selenium with glutathione peroxidase and its association with respiratory infection has been demonstrated in previous pediatric studies where children with pneumonia showed lower level of selenium and glutathione peroxidase than healthy children [7].

Oxidative stress is more and more acknowledged as a vital element of respiratory inflammatory reaction. During infection too many reactive oxygen species can lead to damage of lipids, proteins and nucleic acids in the cells and can increase the inflammatory response. Malondialdehyde (MDA) is a marker of oxidative damage, while superoxide dismutase (SOD), glutathione peroxidase (GPx) and total antioxidant capacity (TAC) represent significant antioxidant defense mechanisms. In children with acute pneumonia, studies have shown that there is an increase in MDA levels along with changes in enzymatic and non-enzymatic antioxidant systems, indicating that there is an oxidative imbalance that has an effect on respiratory disease in these children [8].

Even though there are data linking individual micronutrients or oxidative stress to respiratory infections, there are relatively few clinical studies that have studied simultaneously the relationship of zinc, selenium, MDA, SOD, GPx and TAC with the clinical severity of recurrent respiratory infections in children. Exploring the connection of these parameters to known markers of severe RRI, such as inflammatory activity or respiratory distress, may help to elucidate the biological processes that underlie severe RRI. Thus, the present study was designed to assess the relationship between serum zinc, selenium and some oxidative stress and antioxidant markers and disease severity in children suffering from recurrent respiratory infections.

2. Materials and Methods

2.1 Study Design and Setting

The cross sectional analytical study was performed in the pediatric departments and outpatient clinics of Tikrit Teaching Hospital and Salah Al-Din General Hospital, Salah Al-Din Governorate in Iraq from March 2023 to September 2024. In this study, the authors examined the correlation between serum zinc, selenium, and oxidative stress markers and the severity of recurrent respiratory infection (RRI) in children.

2.2 Study Population and Sample Size

A total of 200 children between 6 months and 12 years of age (150 children with RRI and 50 presumably healthy control children) were included in the study. The RRI group was divided clinically into mild, moderate and severe disease groups based on pre-defined respiratory and general clinical criteria. The participants were also divided into three age groups: 6 months–2 years; >2–5 years; and >5–12 years.

2.3 Inclusion, and Exclusion Criteria

A pediatrician based on clinical history, medical records and physical examination. Children aged 6 months- 12 years who met the RRI criteria and whose parents gave

informed consent were included. Children who did not have recurrent respiratory infections or chronic systemic diseases were used as controls.

Exclusion criteria included primary or secondary immunodeficiency, CF, significant congenital heart disease, chronic pulmonary, renal or hepatic disease, malignancy, haematological disease, tuberculosis, congenital abnormalities of the respiratory system, severe malnutrition and other chronic diseases affecting inflammation or oxidative stress. Children who used to take zinc or selenium supplements regularly, antioxidant supplements, long-term corticosteroid or immunosuppressive therapy, or who had recently undergone major surgery or trauma were also excluded.

2.4 Clinical Assessment and Disease Severity

Standardized questionnaire was used to collect demographic and clinical data—age, sex, residence, anthropometric measurements, number and type of respiratory infections in the previous year, previous hospitalizations, wheezing, fever, cough, dyspnea, respiratory rate, heart rate, oxygen saturation, retractions of chest wall, and oxygen requirements.

Disease severity was assessed without biochemical data and included respiratory symptoms, respiratory rate, work of breathing, oxygen saturation, general clinical condition and requirement for respiratory support. Respiratory signs were considered mild when there were no other signs of distress or hypoxemia. Moderate disease comprised significant respiratory signs such as tachypnea and/or subtle to moderate respiratory retractions that needed close medical attention or treatment. Severe disease: Respiratory distress (marked chest indrawing, hypoxemia ($SpO_2 < 90\%$) and/or cyanosis, severe tachypnea, inability to accept oral food, altered consciousness, or convulsions, or supplemental oxygen or hospitalization due to respiratory problems [10].

2.5 Blood Collection and Laboratory Investigations

Venous blood was drawn with standard aseptic precautions and the amount was about 5–7 mL. Blood was drawn in serum separator tubes, allowed to coagulate and centrifuged at ~3000 rpm for 10 minutes for biochemical analysis. The serum was separated into trace element-free polypropylene tubes and then kept frozen at –20°C until analyzed. Blood collected into EDTA tubes was used for complete blood count.

To analyze the serum zinc, selenium, malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx) and total antioxidant capacity (TAC), commercially available diagnostic kit were used following the manufacturer's instructions. The concentrations of zinc and selenium were presented as μg/dL and μg/L, respectively, while MDA, SOD, GPx, and TAC were reported in units provided by the tests. Complete blood counts, including hemoglobin, white blood cells, neutrophils, lymphocytes and platelets, were counted with automatic hematology analyzer. Serum C-reactive protein (CRP) was determined by a commercial assay. All the laboratory analyses were conducted with appropriate calibration and internal quality control.

2.6 Study Variables

Serum levels of zinc, selenium, MDA, SOD, GPx, and TAC were the primary variables and severity of RRI was the primary clinical outcome. The age, sex, nutritional status, and inflammation status were potential confounding factors, and were considered.

2.7 Statistical Analysis

IBM SPSS Statistics (Version 26.0) was used for the analysis of the data. Continuous variables are presented as means ± SD or median (IQR) depending on the distribution, while categorical variables are presented as frequencies and percentages. Normality was tested using the Shapiro–Wilk test. Two group comparisons were performed with independent t-test or Mann–Whitney U test and comparisons among severity groups with ANOVA or Kruskal–Wallis test. Chi-square or Fisher's exact test was used for categorical variables. Correlations between biomarkers and clinical variables were evaluated using Pearson's or Spearman's correlation. Where appropriate, multivariable logistic regression and ROC curve analysis was used. A two-tailed $P < 0.05$ was considered statistically significant.

2.8 Ethical Considerations

The study was conducted after the approval of the Scientific and Ethical Committee of the College of Medicine, University of Tikrit and the official permission was taken from the two hospitals participated in the study. Parents/legal guardians gave written informed consent. Study identification numbers were used to protect participant confidentiality and standard clinical and laboratory safety procedures were followed.

3. Results

3.1 Demographic and Baseline Characteristics

In this study, 200 children were included, 150 children who had recurrent respiratory infections (RRI) and 50 apparently healthy children. There were 50 children in each of the three categories of mild, moderate and severe disease in the RRI group. The demographic and baseline characteristics of the participants are shown in Table 1.

Table 1. Demographic and baseline characteristics of children with RRI and healthy controls

Variable	RRI group (n=150)	Controls (n=50)	P-value
Age (years), mean ± SD	5.84 ± 3.41	5.71 ± 3.26	0.812
Age groups			
6 months–2 years	32 (21.3%)	10 (20.0%)	0.963
>2–5 years	61 (40.7%)	21 (42.0%)	
>5–12 years	57 (38.0%)	19 (38.0%)	
Sex			
Male	82 (54.7%)	27 (54.0%)	0.934
Female	68 (45.3%)	23 (46.0%)	
Residence			
Urban	91 (60.7%)	32 (64.0%)	0.677
Rural	59 (39.3%)	18 (36.0%)	
Weight (kg), mean ± SD	21.4 ± 10.2	21.9 ± 9.8	0.756
Height (cm), mean ± SD	112.6 ± 24.1	114.1 ± 23.4	0.681
BMI (kg/m²), mean ± SD	16.6 ± 2.3	16.8 ± 2.1	0.594

3.2 Distribution of RRI Patients According to Disease Severity

Among the 150 children with RRI, 50 (33.3%) had mild disease, 50 (33.3%) had moderate disease, and 50 (33.3%) had severe disease. The distribution according to age and sex is shown in Table 2. No significant differences were found in age, age-group distribution, gender or residency across the three RRI severity groups.

Table 2. Distribution of children with RRI according to disease severity

Variable	Mild (n=50)	Moderate (n=50)	Severe (n=50)	P-value
Age (years), mean ± SD	5.61 ± 3.24	5.72 ± 3.37	6.19 ± 3.60	0.679
Age group				
6 months–2 years	12 (24.0%)	10 (20.0%)	10 (20.0%)	0.821
>2–5 years	21 (42.0%)	20 (40.0%)	20 (40.0%)	
>5–12 years	17 (34.0%)	20 (40.0%)	20 (40.0%)	
Male	27 (54.0%)	28 (56.0%)	27 (54.0%)	0.967
Female	23 (46.0%)	22 (44.0%)	23 (46.0%)	
Urban	30 (60.0%)	31 (62.0%)	30 (60.0%)	0.963
Rural	20 (40.0%)	19 (38.0%)	20 (40.0%)	

3.3 Clinical Characteristics of Children with RRI

As for the characteristics of infection, children with more severe RRI had a higher number of infections in the preceding year and a higher number of hospitalizations as well as wheezing, fever, dyspnea, chest retraction and oxygen requirement. The results are summarized in Table 3. The clinical severity increased progressively, from mild to severe, especially for the number of recurrent infections, hospitalizations, wheezing, dyspnea, chest retractions, and oxygen requirement.

Table 3. Clinical characteristics of children according to RRI severity

Clinical variable	Mild (n=50)	Moderate (n=50)	Severe (n=50)	P-value
Recurrent infections/year, mean ± SD	4.1 ± 0.9	5.3 ± 1.1	6.8 ± 1.5	<0.001
≥6 infections/year	4 (8.0%)	17 (34.0%)	37 (74.0%)	<0.001
Previous hospitalization	5 (10.0%)	15 (30.0%)	32 (64.0%)	<0.001
Wheezing	13 (26.0%)	25 (50.0%)	37 (74.0%)	<0.001
Fever	25 (50.0%)	34 (68.0%)	42 (84.0%)	0.002
Cough	44 (88.0%)	47 (94.0%)	49 (98.0%)	0.156
Dyspnea	5 (10.0%)	21 (42.0%)	41 (82.0%)	<0.001
Chest retractions	2 (4.0%)	17 (34.0%)	42 (84.0%)	<0.001
Supplemental oxygen	0 (0.0%)	5 (10.0%)	36 (72.0%)	<0.001
SpO_2 (%), mean ± SD	96.8 ± 1.4	94.1 ± 1.8	88.7 ± 3.1	<0.001

3.4 Hematological and Inflammatory Parameters

The RRI group with severe RRI had significantly higher WBC, Neutrophil %, platelet count, and CRP level, and the lymphocytes % in the severe RRI group was lower than the other mild and moderate groups. Full results are reported in Table 4. Regarding disease severity, CRP had the highest inflammatory increase, which got higher as the RRI severity went up from mild to severe.

Table 4. Complete blood count and inflammatory markers according to RRI severity

Parameter	Mild (n=50)	Moderate (n=50)	Severe (n=50)	P-value
Hemoglobin (g/dL)	11.8 ± 1.0	11.6 ± 1.1	11.4 ± 1.2	0.184
WBC ($\times 10^3/\mu L$)	8.7 ± 2.1	10.2 ± 2.6	13.4 ± 3.5	<0.001
Neutrophils (%)	49.6 ± 8.7	56.8 ± 9.4	66.7 ± 10.2	<0.001
Lymphocytes (%)	42.8 ± 8.3	36.9 ± 8.5	27.8 ± 8.9	<0.001
Platelets ($\times 10^3/\mu L$)	286 ± 61	309 ± 69	338 ± 76	0.001
CRP (mg/L)	5.8 ± 3.4	12.7 ± 7.1	25.9 ± 14.6	<0.001

3.5 Serum Zinc and Selenium Concentrations

The serum level of zinc and selenium were significantly reduced in RRI children compared to healthy control. In the RRI group, the trends of decreases in both micronutrient levels with disease severity were progressive (Fig. 1). Post hoc analysis showed that significant differences existed between the severe group and mild and moderate groups with regard to serum zinc and selenium. There was also a significant decrease in all RRI severity categories as compared to controls.

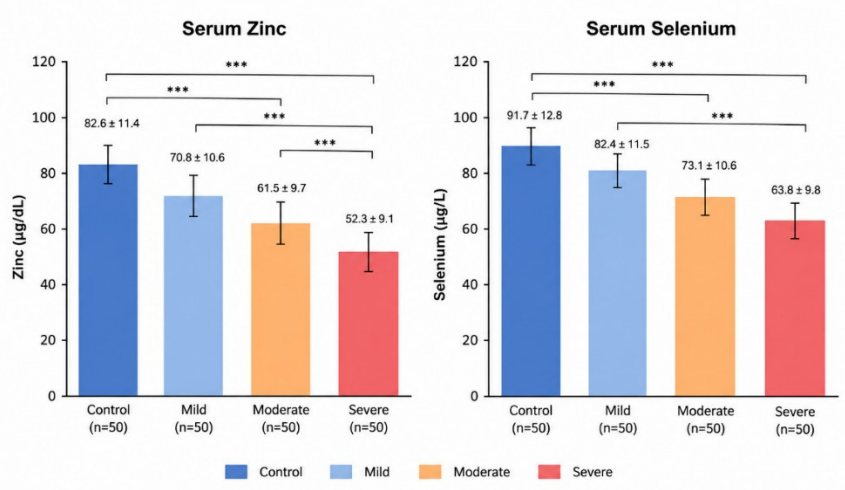


Figure 1. Comparison of serum zinc and selenium concentrations according to RRI severity.

3.6 Oxidative Stress and Antioxidant Markers

A greater degree of oxidative stress was seen in children with severe RRI. The serum MDA was found to be progressive with the severity of the disease and the other parameters such as SOD, GPx and TAC were significantly reduced in a progressive manner (Table 5). The MDA level was the highest in the severe RRI group and the SOD, GPx, and TAC levels were the lowest, suggesting that there is increased oxidative stress and reduced antioxidant protection in this group.

Table 5. Oxidative stress and antioxidant biomarkers according to RRI severity

Biomarker	Controls (n=50)	Mild (n=50)	Moderate (n=50)	Severe (n=50)	P-value
MDA	2.14 ± 0.48	2.71 ± 0.61	3.46 ± 0.74	4.38 ± 0.92	<0.001
SOD	142.6 ± 18.7	128.4 ± 17.9	114.7 ± 16.3	98.2 ± 15.6	<0.001
GPx	68.5 ± 9.8	61.9 ± 9.1	54.3 ± 8.7	45.8 ± 8.2	<0.001
TAC	1.42 ± 0.21	1.26 ± 0.19	1.09 ± 0.18	0.91 ± 0.16	<0.001

3.7 Comparison of Main Biochemical Parameters Between RRI and Controls

All children with RRI were directly compared with healthy controls with regard to the major biochemical variables. The zinc, selenium, SOD, GPx and TAC concentrations were significantly lower among the RRI group, and the MDA and CRP concentrations were significantly higher (Fig. 2). The overall biochemical profile thus revealed a pattern of micronutrient depletion, lipid peroxidation and antioxidant deficiency in children with RRI (P value <0.001).

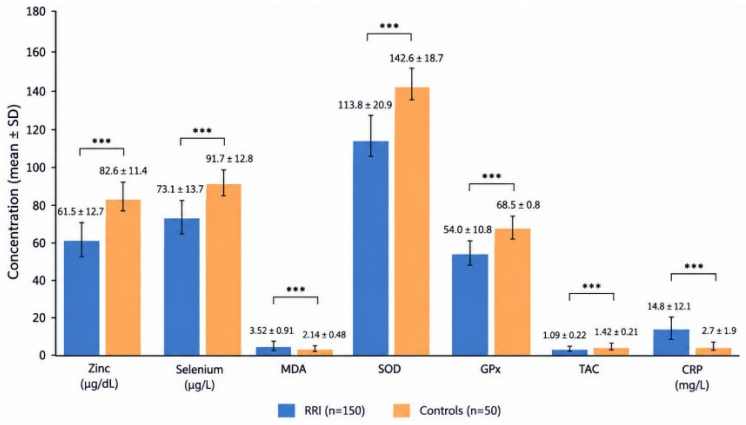


Figure 2. Overall comparison of micronutrient and oxidative stress profiles between RRI children and healthy controls.

3.8 Correlation Between Zinc, Selenium and Disease Severity

Correlation analysis showed that there was significant negative correlation between the markers of disease severity and serum zinc and selenium. More frequent infections, lower oxygen saturation and higher CRP levels were correlated with lower zinc and selenium levels (Table 6). Serum zinc and selenium levels were positively associated with both antioxidant markers and oxygen saturation, and negatively associated with MDA, CRP, infection frequency and hospitalization.

Table 6. Correlation of serum zinc and selenium with clinical and inflammatory parameters

Variable	Zinc r	P-value	Selenium r	P-value
Infection frequency/year	−0.51	<0.001	−0.46	<0.001
CRP	−0.55	<0.001	−0.49	<0.001
WBC count	−0.34	<0.001	−0.30	<0.001
Neutrophils	−0.39	<0.001	−0.35	<0.001
SpO_2	0.48	<0.001	0.43	<0.001
Hospitalization	−0.42	<0.001	−0.38	<0.001
MDA	−0.58	<0.001	−0.53	<0.001
SOD	0.56	<0.001	0.51	<0.001
GPx	0.49	<0.001	0.46	<0.001
TAC	0.52	<0.001	0.48	<0.001

3.9 Correlation of Oxidative Stress Markers with Disease Severity

The results revealed that MDA has a strong positive correlation with the severity and inflammatory activity, while the other antioxidants, namely SOD, GPx and TAC were inversely related with the severity of the disease. The results reveal significant relationships between higher levels of oxidative stress and lower levels of antioxidant defense and between worsening clinical status, respectively (Table 7).

Table 7. Correlations between oxidative stress markers and selected clinical variables

Variable	MDA r	P-value	SOD r	P-value	GPx r	P-value	TAC r	P-value
Disease severity	0.67	<0.001	−0.61	<0.001	−0.57	<0.001	−0.59	<0.001
CRP	0.62	<0.001	−0.48	<0.001	−0.45	<0.001	−0.50	<0.001
WBC	0.43	<0.001	−0.35	<0.001	−0.31	<0.001	−0.33	<0.001
Infection frequency	0.51	<0.001	−0.46	<0.001	−0.41	<0.001	−0.44	<0.001
SpO_2	−0.56	<0.001	0.50	<0.001	0.46	<0.001	0.48	<0.001
Zinc	−0.58	<0.001	0.56	<0.001	0.49	<0.001	0.52	<0.001
Selenium	−0.53	<0.001	0.51	<0.001	0.46	<0.001	0.48	<0.001

3.10 Association Between Micronutrient Status and Oxidative Stress

The lower the serum zinc and selenium levels, the higher the MDA levels and the lower the antioxidant enzyme activity in children. This relationship is summarized in Table 8. The negative association between micronutrients concentrations and MDA along with the positive association with SOD and GPx and TAC, indicates a correlation between zinc and selenium status with antioxidant defense in children with RRI.

Table 8. Association of zinc and selenium with oxidative stress biomarkers

Parameter	Zinc r	P-value	Selenium r	P-value
MDA	−0.58	<0.001	−0.53	<0.001
SOD	0.56	<0.001	0.51	<0.001
GPx	0.49	<0.001	0.46	<0.001
TAC	0.52	<0.001	0.48	<0.001

3.11 Multivariable Logistic Regression Analysis

All the variables were included in a multivariable logistic regression to determine the independent factors associated with severe RRI after adjusting for age, sex, nutritional status, infection frequency, CRP, zinc, selenium, MDA, SOD and GPx. Factors independently associated with severe disease (Table 9) were lower levels of zinc and selenium and higher levels of MDA. Multivariate logistic regression analysis revealed that infection frequency, CRP and MDA were independent risk factors for severe RRI, while zinc, selenium, SOD, GPx and TAC were independent protective factors for severe RRI after adjustment for potential confounders.

Table 9. Multivariable logistic regression analysis of factors associated with severe RRI

Variable	Adjusted OR	95% CI	P-value
Age	1.08	0.94–1.24	0.274
Male sex	1.12	0.63–2.01	0.699
Low nutritional status	1.74	0.91–3.34	0.093
Infection frequency/year	1.42	1.18–1.71	<0.001
CRP	1.06	1.03–1.10	<0.001
Zinc	0.94	0.91–0.97	<0.001
Selenium	0.96	0.93–0.99	0.006
MDA	1.78	1.32–2.41	<0.001
SOD	0.97	0.95–0.99	0.004
GPx	0.96	0.93–0.99	0.009
TAC	0.41	0.20–0.83	0.014

3.12 ROC Curve Analysis

The discriminatory capacity of zinc, selenium and oxidative stress markers were evaluated using ROC curve analysis for severe RRI. MDA, followed by zinc, TAC, selenium, SOD and GPx showed the best individual discriminatory performance (Fig. 3). Good discriminatory ability was demonstrated for severe RRI with the highest area under the curve (AUC=0.91) by MDA. The other two parameters, zinc and TAC, showed good diagnostic performance.

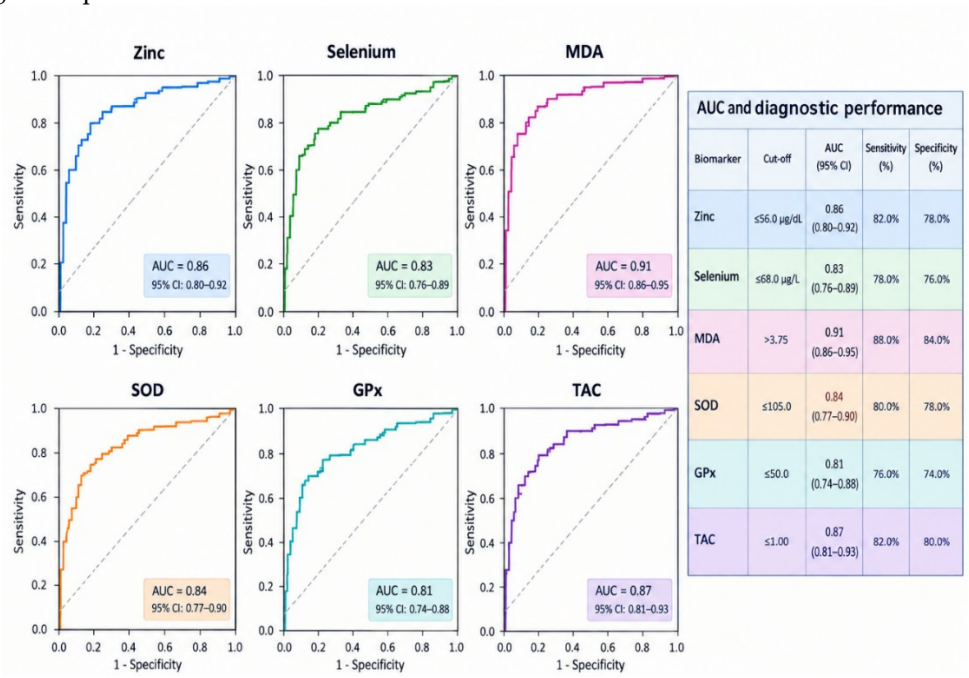


Figure 3. Receiver operating characteristic curves of zinc, selenium, MDA, SOD, GPx, and TAC for predicting severe RRI.

4. Discussion

In the present study, it was found that there is a definite correlation between micronutrient status, oxidative stress and the severity of recurrent respiratory infections (RRI) in children. The most essential conclusion was that as the disease severity increased from healthy to mild to moderate, serum zinc and selenium levels decreased. Meanwhile, MDA was gradually increased while SOD, GPx and TAC were gradually decreased with the increase of the severity of the disease. This pattern implies that recurrence of respiratory infections in children may be related to alteration of the oxidant-anti-oxidant balance along with decreased levels of essential trace elements related to immune and anti-oxidant capacities.

Children with RRI had significantly lower serum zinc levels, especially those with severe disease, and this is biologically plausible since zinc plays a vital role in maintaining the integrity of epithelial barriers, innate immunity, the function of lymphocytes, and the regulation of inflammatory signaling pathways. It has been shown in previous experiments and clinical studies that Zn deficiency affects immune competence and susceptibility to infectious diseases [11,12]. Specifically, zinc is involved in antiviral defense and immune regulation in the RT, a mechanism which could explain the association found in the present study [13]. Our results also corroborate with pediatric clinical studies that have shown that zinc status is associated with respiratory morbidity. Zinc supplementation has been shown to decrease the number of acute lower respiratory infections among young children in a randomized controlled trial of 45% [14]. Likewise, a meta-analysis of randomized trials showed that zinc supplementation decreased the prevalence of lower respiratory tract infections (LRTIs) in children, using more specific clinical definitions of LRTIs. There is, however, not complete agreement in the literature: a randomized study in children with severe acute lower respiratory tract infection found no significant difference in recovery period after short-term zinc supplementation [16]. These differences suggest that the zinc-respiratory infection association may be dependent on the nutritional status prior to the infection, and on the type and severity of the infection, the duration of supplementation or the cause of respiratory disease.

This decrease in selenium found in the present study is another significant finding. Selenium is part of selenoproteins, including some enzymes that help protect against oxidative stress, as well as proteins that are important for the function and regulation of immune cells and inflammatory responses [17]. This progressive depletion of selenium across disease severity categories could be due to either insufficient micronutrient status or greater utilization of selenium during inflammatory stress or both. Selenium and glutathione peroxidase levels have been shown to be lower in children with pneumonia than in healthy children in previous studies [18]. However, the data on selenium supplementation and immunity are not as clear as the data on zinc. A recent systematic review and meta-analysis of the effects of selenium supplementation on human immune parameters also did not support the routine use of selenium supplementation for immunity enhancement because of the conflicting results [19]. Our findings have confirmed the need for evaluating selenium status during severe respiratory illnesses, but must not be taken as proof of therapeutic usefulness of selenium supplementation.

A significant advantage of the current results is the measurement of multiple oxidative stress and antioxidant markers at the same time. The level of MDA, a marker of lipid peroxidation was significantly higher in children with RRI and increased in parallel to the clinical severity. On the contrary, SOD, GPx and TAC significantly decreased. This pattern suggests a progression towards oxidative stress with disease progression. These findings were consistent with earlier findings in the field of pediatrics. Cemek et al. concluded that oxidative parameters like MDA were found elevated and SOD and GPx were found low in children with acute pneumonia, hence oxidative imbalance was seen with respiratory infection [20]. Likewise, in children with acute bronchiolitis, higher oxidative status was associated with lower TAC and more severe the disease, was associated with lower oxygen saturation, which was inversely correlated with oxidative stress [21]. A similar relationship between elevated oxidative stress markers and the

severity of pulmonary disease has also been shown in more recent studies of children with Mycoplasma pneumonia pneumonia [22].

In the present study, the association of lower levels of zinc and selenium concentration to higher levels of MDA and CRP was also significant, and both micronutrients were significantly correlated with higher levels of SOD, GPx, and TAC. These relationships offer a comprehensive perspective on the interplay between micronutrient status, inflammation and oxidative stress. Zinc is also tightly linked to redox regulation and intracellular signaling processes in immune cells whereas selenium is essential for the biological activity of several selenoproteins with antioxidant functions [12,17]. Therefore, the associations observed may be related to a biological pathway where there is a lack of micronutrients that leads to lower antioxidant defenses in repeated infections. However, this cross-sectional study does not allow for conclusions to be drawn about whether low zinc and selenium are a risk factor for severe RRI or whether chronic/invulnerable infections lead to low micronutrient levels.

The biochemical findings were corroborated by the inflammatory findings. Higher proportions of both WBC and neutrophils were seen in severe RRI, which was also significantly associated with higher levels of CRP, with lower proportions of lymphocytes. In addition, the inverse relationship between CRP and zinc and selenium and positive association between CRP and MDA was also demonstrated. This implies that there might be a parallelism between the inflammatory response and the oxidative imbalance as the severity of the disease progresses. The relationship is biologically sound because activated inflammatory cells can produce reactive oxygen species which may further enhance inflammatory signaling and excessive oxidative stress can contribute to inflammatory signaling. The decrease in SpO_2 and the increase in the need for supplemental oxygen in severe cases also suggest that the biochemical changes were associated with clinically significant respiratory compromise.

Results of the multivariable analysis confirmed the independent relationship between the studied biomarkers and the severity of disease. A higher frequency of infection, CRP, and MDA were associated with higher odds of severe RRI, whereas higher zinc, selenium, SOD, GPx, and TAC were associated with lower odds. These results indicate that the biochemical differences found were not solely due to age or sex. The interpretation must, however, be kept within a reasonable margin of caution as there is a possibility of residual confounding (dietary intake, socioeconomic status, specific infectious pathogens, previous antimicrobial exposure).

Finally, the results of ROC analyses indicated excellent discrimination for a number of biomarkers, with MDA having the highest AUC followed by TAC and zinc. Based on the results of these tests, oxidative stress and micronutrient markers may be useful as supplementary markers of disease severity. Importantly, it is not at the present time recommended as a substitute for clinical assessment. Instead, their value may be as an adjunct to current clinical tests like respiratory rate, oxygen saturation, work of breathing, and inflammatory markers. Additional prospective, multicenter trials are required to confirm predictive value of these biomarkers for future deterioration and the impact of correction of known micronutrient deficiencies on clinical outcomes.

5. Conclusion

This study showed that there was a significant relationship between micronutrient status, oxidative stress and disease severity among children who had recurrent respiratory infections. As the disease became more severe, the concentration of zinc and selenium in the serum of children decreased, while the levels of the antioxidant enzymes SOD, GPx, and TAC decreased, and the level of MDA increased. A higher frequency of infection, hospitalization, respiratory distress and lower oxygen saturation were also correlated with these biochemical changes. There were significant positive correlations between zinc and antioxidant capacity as well as significant negative correlations between zinc and inflammatory and oxidative stress parameters.Zinc had significant positive correlation with antioxidant capacity and inverse correlation with inflammatory and oxidative stress parameters. Further analysis with multiple variables showed zinc,

selenium, MDA, SOD, GPx and TAC to be independently associated with severe disease, with good discriminatory ability shown by ROC analysis, especially for MDA and zinc. In conclusion, there seemed to be an important relationship between the three micronutrient imbalances, oxidative stress, and clinical severity in children with RRI. There is a need for prospective studies to further elucidate the causality of adverse respiratory outcomes and to assess if micronutrient deficiencies can be corrected to improve respiratory outcomes.

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