Prognostic Value of Interleukin-10 and TNF-a in Patients with Ventricular Septal Defect

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Abstract
Background: One of the congenital disabilities most prevalent forms is congenital heart disease (CHD). Previous research found that the incidence of CHD is only 0.1% and that medication may be stopped if it has undesirable results. Ventriculoventricular septal defect is a common CHD (VSD). Abnormalities are frequently seen with VSD and are brought on by insufficient ventricular septal development during the embryonic stage. It poses a serious risk to one's life and requires extensive surgery. All forms of CHD occur 75 times per 1,000 live births, with moderate and severe versions occurring 6 times per 1,000 live births. Methods: Between October 2021 and January 2022, 80 patients (18 men and 22 women) were examined at the Al-Diwaniyah Teaching Hospital for Medicine, the Al-Diwaniyah Center for Cardiovascular Surgery and Cardiac Catheterization, and the AL-Najaf Center for Cardiovascular Surgery and Cardiac Catheterization. Three milliliters of blood were aseptically transferred to a sterile Gel tube using disposable syringes, left to clot at room temperature, and then centrifuged at 2500 rpm for 10 minutes in order to prevent repeated thawing and freezing for the measurement of IL-10 TNF- and using the ELISA method. Once the serum had been separated, it was stored in Eppendorf tubes and kept at -20 C until it was needed again. Results: The mean age of VSD patients was 10.52 ± 6.47, and that of RTI patients was 14.05 ± 9.91 years, and there was no significant difference between patients and RTI patients in mean age (P = 0.065). VSD patients' group included 22 (55.00 %) males and 18 (45.00 %), females. In contrast, RTI patients had 19 (47.50 %) males and 21 (52.50 %) females, and there was no significant difference in the frequency distribution of VSD patients and RTI patients according to gender (P = 0.502). Median levels of serum IL-10 in patients with ventricular septal defect were higher than in comparison the median levels of RTI patients groups, 213.53 (33.13) pg/ml versus 32.75 (71.46) pg/ml; the difference was highly significant (P < 0.001). Median levels of serum TNF-α in patients with ventricular septal defect were higher than in comparison the median levels of RTI patients groups, 5728.14 (1184.34) pg/ml versus 1316.07 (1946.60) pg/ml; the difference was highly significant (P < 0.001). Conclusion: Patients with ventricular septal defect complicated by respiratory tract infection had considerably higher serum levels of IL-10 and TNF- than controls. This discovery may significantly impact the ventricular septal defect disease process's worsening progression.
Keywords: Congenital heart disease, Ventricular septal defect, IL-10, and TNF-α.

1. Introduction

One of the most prevalent forms of congenital disabilities is congenital heart disease (CHD). Earlier research estimated that 0.1% of people had CHD, and poor treatment results may lead to abortion (1). A frequent CHD is a ventricular septal defect (VSD). Abnormalities are frequently seen with VSD and are brought on by insufficient ventricular septal development during the embryonic stage. It poses a severe risk to one's life and requires significant surgery (2).

Congenital abnormalities are also a substantial source of mortality, accounting for 300,000 deaths globally. Due to anomalies in the development of the cardiac tissues and conduction system, several heart diseases manifest in utero. CHDs often affect the blood flow pattern throughout the heart by producing lower pressure channels that interrupt normal flow because of altered oxygenation and the state of the systemic/pulmonary volume, which may later cause an inflammatory reaction (3). 6/1,000 live births result in moderate or severe types of CHD, making up the remaining 75% of CHD cases. Despite the fact that the majority of CHD cases resolve on their own without medical intervention, the frequency of patients who need specialist cardiologic care varies from 2.5 to 13 per 1,000 live births. Furthermore, death rates for neonates in low- and lower-middle-income countries, including those in sub-Saharan Africa, are greater than those for newborns in high-income countries. 90% of infants born with CHD throughout the globe live in places with little to no care and a high mortality rate. (4).

In Iraq, the overall prevalence of congenital heart malformation among children attending the outpatient pediatric clinic of IB- IN- Al-Atheer teaching hospital was 6.1 /1000 (5). In Egypt, the prevalence was 21.4/ 1000 patients (6). Every year, some 50 million live infants are born in Africa, and it's thought that 500,000 of them have severe CHD and need specialized cardiological treatment. They have

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little, if any, access to healthcare. One third of the instances happened in the first month of life, and around half of them were deadly within a few years after birth. Furthermore, many older children and CHD patients in sub-Saharan Africa who live longer have disabilities and struggle with ongoing diseases (7). Congenital ventricular and atrial septal abnormalities account for 45.6% and 23.54% of all CHDs, respectively, according to CHD data from the European Commission for the years 2000 to 2018. (8). The most frequent cause of acute lower respiratory tract infection in newborns and young children under the age of five is the human respiratory syncytial virus (HRSV). The fourth most prevalent cause of mortality for this age group. Acute LRTIs are common in children with congenital heart disease, and HRSV infection increases the risk of more serious LRTIs and the length of hospitalization for respiratory problems. Because these children are more often admitted to critical care units, extended oxygen treatment and mechanical ventilation raise their rates of morbidity and death as well as the expense of their medical care. (9).

A pleiotropic cytokine called interleukin-10 (IL-10) is predominantly produced by immune cells such Th2-type T cells, certain T-regulatory cells, B cells, and macrophages. It has inflammatory-reducing qualities. It has a role in the regulation of macrophage inflammatory responses and significantly reduces the development of Th1-type T cells, the presentation of antigens, and the production of cytokines (10). Even though this study only included 71 cases of CHD, the risk of CHD was positively connected with circulating levels of IL-10, with a 1 SD rise in IL-10 being associated with a hazard ratio of 1.34 (95% CI 1.06 to 1.68) for CHD. (11).

Overall, it is thought that IL-10 may be a significant cytokine in controlling inflammation in arterial plaques, where it seems to have an anti-inflammatory effect (11). It may also control endothelial function and leukocyte recruitment in vivo under pro-inflammatory conditions. IL-10 also regulates the production of pro-inflammatory cytokines, particularly the Th-1 cytokines typical of atherogenesis. It may also affect how macrophages and dendritic cells present antigens. Finally, by obstructing foam cell apoptotic pathways, it could stabilize rupture-prone plaques. This has led some scientists to surmise that there is no relationship between the level of circulating IL-10 and the risk of CVD.On the other hand, increased production of an actual "anti-inflammatory" cytokine makes much more sense in the context of ongoing tissue inflammation as a counter-regulatory mechanism to prevent and restrict further inflammation (12). Additionally, elevated IL-10 is linked to a higher risk of CVD. As a result, circulating IL-10, despite being an anti-inflammatory cytokine, may serve as a proxy marker of ongoing systemic inflammation and associated pathophysiological processes and buffering or regulating pro-inflammatory vascular consequences. (13).

A powerful cytokine with pleiotropic effects on immune system performance, cell division, proliferation, and apoptosis is known as tumor necrosis factor (TNF-α). TNF-α may be produced by granulocytes, mast cells, natural killer cells, immune regulatory macrophages, smooth muscle cells, keratinocytes, non-immune endothelial cells, and fibroblasts.Tumor necrosis factor may occur in two different forms: soluble and transmembrane. Higher blood TNF levels in children with CHD may impact the disease's development and course (14).

2. Materials and Methods

The current study involved 80 patients (18 men and 22 women) who were seen between October 2021 and January 2022 at the Al-Diwaniyah Teaching Hospital for Medicine, the Al-Diwaniyah Center for Cardiovascular Surgery and Cardiac Catheterization, and the AL-Najaf Center for Cardiovascular Surgery and Cardiac Catheterization. 40 VSD patients with respiratory tract infections and 40 with respiratory tract infections were exclusively separated into two groups. To avoid repeated thawing and freezing for the measurement of IL-10 TNF- and by ELISA technique, three ml of blood samples were transferred to a sterile Gel tube using disposable syringes in an aseptic manner, allowed to clot at room temperature, and then centrifuged at 2500 rpm for 10 minutes. The separated serum was then saved in Eppendorf tubes and frozen at -20 C until further use. Verbal informed consent was obtained from each patient and their family following the medical ethics of the Al-Diwaniyah Teaching Hospital, Al-Diwaniyah center for cardiovascular surgery and cardiac catheterization, and AL-Najaf center for cardiovascular surgery and cardiac catheterization.

3. Statistical Analysis

The data were shown and examined using the Mean SE with a probability threshold of p 0.05 for significant outcomes. The statistical analysis program SPSS version 25 (Statistical Package for the Social Science) was used to conduct the statistical studies.

4. Results

There was no discernible difference in mean age between patients with VSD and patients with RTI (P = 0.065); the mean age of patients with VSD was 10.52 ±6.47, and that of RTI patients was 14.05 ±9.91 years. There were 21 (47.50%) males and 22 (52.50%) females among the group of VSD patients, compared to 22 (55.00%) males and 18 (45.00%) females among the group of RTI patients. However, there was no statistically significant difference between VSD and RTI patients' frequency distribution according to gender (P = 0.502), table (1).

The results of the serum Interleukin-10 levels between the groups of patients with ventricular septal defects and those with RTI are shown in table (2) and figure (1). The difference between the median

serum IL-10 -α was > 4505. concentrations in patients with ventricular septal defects and patients with RTI was highly significant (P 0.001), being 213.53 (33.13) pg/ml versus 32.75 (71.46) pg/ml. Receiver operator characteristic (ROC) curve analysis was done to assess the IL-10 cut-off value and predict the ventricular septal defect as diagnostic or adjuvant diagnostic tests. The results are shown in table (3) and figure (2). The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the curve for the IL-10 cut-off value were 92.5%, 92.5%, 92.5%, and 0.988, respectively (0.972- 1.000). The results of the serum TNF- levels between the groups of patients with ventricular septal defects and those with RTI are shown in table (4) and figure (3). Patients with ventricular septal defects had median serum TNF- levels that were higher than the median levels of RTI patient groups, measuring 5728.14 (1184.34) pg/ml versus 1316.07 (1946.60) pg/ml, respectively. This difference was highly significant (P 0.001). Receiver operator characteristic (ROC) curve analysis was done to assess the TNF-cutoff value and predict the ventricular septal defect as diagnostic or adjuvant diagnostic tests. The results are shown in table (5) and figure (4). The cutoff for TNF47, with sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under curve values of 97.5%, 87.5%, 88.6%, 97.2%, and 0.988, respectively (0.972- 1.000).

5. Discussion

Two of the first organs to mature during pregnancy are the heart and the placenta. The "placental-heart" axis refers to the relationship between their developmental stages. Insufficient human investigations have been done to assess how early placental function affects the later development of CHD because this link is poorly understood. (15). Congenital heart illness with a ventricular septal defect (VSD) is the most prevalent form and the leading cause of morbidity. (16). VSD accounts for 20% of all CHD patients (17). The findings of the current study accord with those of Amel-Shahbaz et al. (18), They discovered that the average patient age at the time of diagnosis was 14.05 years for those with RTI and 8.8 years for those with VSD (at the range of one day to 76 years with a median of 4 years). However, the mean age of the recent discoveries is different from Adel's. (19) reported that the mean age was 15.0 12.78 years. The present findings' mean age is greater than those of Chaw et al. (20), who found that hospitalized patients with underlying CHD had a mean age of 6.3 6.0 months.

With a ratio of 1.22:1, the current research found that males had a greater prevalence of ventricular septal defects than females. There was no significant difference in the frequency distribution of patients and RTI patients according to gender (P=0.502), table (1). Men had a higher chance of developing a ventricular septal defect (19). In the northern area of Iran, Nikyar et al. (21) found that the risk of CHDs is 1.35 times higher in men than in women (P value > 0.05). Our conclusion that men outnumber women across all age categories is consistent with other research findings. The current findings agree with Adel. (19), who found that out of 50 instances, 26 had a male gender (52%), whereas 24 had female gender (48%). The findings are consistent with earlier research that indicated men were more likely than women to have ventricular septal defects (22). Additionally, the present findings are consistent with research done in Enugu, Nigeria, where men make up the majority. The authors asserted that Nigerian sociocultural practices were to blame for this (23). On the other hand, Cox et al. (24) found that females had a higher prevalence of VSD than males (51.7% vs. 48.3%). The current results also conflict with those of Amel-Shahbaz et al. (18), who found that the proportions of men and females were 54.2 (n = 2014) and 43.8 (n = 1627), respectively. Also, a large clinical register's data reveal no discernible sex-gender variations in the VSD rate, yet the ratio was 1:1. (25).

According to earlier research, CHDs are caused by genetic, epigenetic, and environmental factors (26). Although a connection to the immune system has not been established, clinical investigations have revealed that children with structural CHDs have higher levels of pro-inflammatory cytokines and a decreased cellular immunological response to infection (27). It suggests that the immune system could play a dynamic role in developing CHD-related problems. Additionally, it has been shown that immune cells, such as macrophages, are essential for the development of the heart (28). Given how alterations in the immune response may be found in children with CHDs, a potentially beneficial route to investigate juvenile heart disease can be by examining the immunological systems at play. Regardless of the underlying reason, immunological imbalance in patients with cardiovascular illnesses increases susceptibility to recurrent infections and the risk of chronic disease.

According to the current research, there is a significantly significant correlation between IL-10 concentration and ventricular septal defect (P <0.001), table (2). In the VSD patient group, the median blood IL-10 levels were greater than those in the RTI patient group, 213.53 (33.13) pg/ml vs. 32.75 (71.46) pg/ml, respectively. The state of patients with cardiovascular illnesses is greatly worsened by concurrent disorders linked to recurrent acute respiratory infections, the efficiency of treatment for heart failure is decreased, and congenital heart disease surgical correction is delayed. However, in CHD patients, immunological dysregulation is a factor in the development and exacerbation of the condition. These factors include poor cardiac hemodynamics, arterial hypoxemia, and cardiac surgery (29). The cytokines' mechanism of action in heart failure includes a negative inotropic impact, cardiac remodeling, a reduction in endothelium-dependent dilatation of arterioles, and an increase in

cardiomyocyte mortality as a result of the activation of endogenous nitric oxide synthase. In terms of clinical terms, this is connected to cardiomegaly, decreased contractility, inadequate left ventricular (LV) function, the severity of the clinical symptoms, and the prognosis of the illness. abnormalities in the endothelium-dependent dilatation of arterioles and elevated cardiomyocyte mortality (induced by the activation of endogenous nitric oxide synthetase).This is connected to diminished LV function, diminished contractility, cardiomegaly, the intensity of clinical symptoms, and the prognosis of the disease (30).

The current findings are consistent with those of Istamovna et al. (31), who found that patients with CHD had a 4-fold increase in IL-10 titer compared to the control group, indicating an increase in proliferative processes and hypercoagulation (48.00 ±2.20 vs. 12.00 ±2.30, respectively), and Grosek et al. (32), who found that patients with ventricular septal defects had significantly higher serum IL-10 levels

The present study validated the IL-10 in predicting ventricular septal defect, which is very important for detecting the progression of patients with the ventricular septal defect. The present study shows that 37 of 40 VSD patients (92.5%) had IL-10 higher than the cut-off value (>178.38 pg/ml) compared to 3 of 40 RTI patients (7.5%) had IL-10 higher than the cut-off value (>178.38 pg/ml), and the difference was highly significant (P< 0.001) table (3) and figure (2). ROC curve analysis shows that the IL-10 cutoff value was >178.38 pg/ml with sensitivity, specificity, PPV and NPV levels of 97.5%, 87.5%, 88.6% and 97.2% respectively. The area under the curve of the receiver operating characteristic (R was 0.988 (0.972- 1.000) for this cytokine, indicating that these cytokines could predict the disease severity of the ventricular septal defect.

Helper T cells of types 1 and 2 participate in the body's immuno-regulatory processes. It is generally known that the Th1 and Th2 immune systems produce the cytokines (IL-1, 2, 6, 8, 12, INF, TNF, etc.) that activate cellular immunity and humoral immunity, respectively (IL-4, 5, 10, transforming growth factor-beta, etc.). The interplay between Th-1 and Th-2 helper cells in a healthy organism is roughly balanced. However, when under the influence of any condition, a major change in their behavior may have detrimental consequences on the immune system (29). The development and progression of heart failure are significantly influenced by increased vasoconstrictor and pro-inflammatory cytokines (endothelin, TNF-a, IL-1, IL-6, and IL-8) and neurohormones (31).

TNF-α is a pro-inflammatory cytokine that promotes the host's quick response to inflammatory stimuli and is crucial for managing inflammatory and pro-inflammatory mediators (33). It may be produced by fibroblasts, mast cells, natural killer cells, smooth muscle cells, keratinocytes, immunological regulatory macrophages, T cells, and granulocytes. It may come in soluble or trans-membrane form. (34).

The present study validated the TNF-α in predicting ventricular septal defect, which is very important for detecting the progression of patients with ventricular septal defect. The present study shows that 39 of 40 VSD patients (97.5%) had TNF-α higher than the cut-off value (>4505.47 pg/ml) compared to 5 of 40 RTI patients (12.5%) had TNF-α higher than the cut-off value (> 4505.47 pg/ml), and the difference was highly significant (P< 0.001) table (5) and figure (4). ROC curve analysis shows that the TNF-α cut-off value was >4505.47 pg/ml with sensitivity, specificity, PPV, and NPV levels of 97.5%, 87.5%, 88.6%, and 97.2%, respectively. The area under the curve (AUC) of the receiver operating characteristic (ROC) was 0.988 (0.972- 1.000) for these cytokines, indicating that these cytokines could predict the disease severity of the ventricular septal defect. Also, the median serum concentration of TNF-α was higher in patients with ventricular septal defect 5728.14 (1184.34) pg/ml as compared to RTI patients 1316.07 (1946.60) pg/ml, (p<0.001), table (4) and figure (3). TNF- is generated as a 26 kDa transmembrane monomer that promotes the development of smooth muscle and enhances leukocyte adherence to endothelial cells by increasing the synthesis of cell adhesion molecules (35). Leukocyte activation, the release of various endothelial adhesion molecules, and the excretion of platelet-activating factors are all possible effects of elevated TNF-, which may all play a role in the development and progression of CHD (14). TNF-α during inflammatory events in the heart may lead to misdirected apoptosis, harm the heart's muscle cells, and impair the heart's ability to pump blood. Despite mounting evidence that cardiac injury may significantly impact cytokines like TNF-, the first cytokine stimulation is still unclear (36) . Lopes et al. (37) recognized that heart illness is the cause of an elevation in TNF in patients with congestive heart failure. Unknown molecular processes cause myocardial remodeling and cytokine-induced contractility. The oxidative phosphorylation of troponin-1, intracellular calcium transport, inhibition of pyruvate dehydrogenase, a crucial enzyme for the interaction of fat and protein metabolism, a lack of ATP synthesis and the development of energy deficiency in cardiomyocytes, a reduction in their contractility, and activation of NO synthetase, which raises the level of endogenous intracellular NO are all thought to contribute to the cardio depressive effect of (31). The findings show that children with CHD had higher blood TNF- levels, which may be useful biologic indicators in the early identification of CHD (14).

The current findings are consistent with those of Istamovna et al. (31), who found that patients with CHD had a 4-fold increase in the titer of TNF-, indicating an increase in proliferative processes and hypercoagulation (88.00 6.70 vs. 20.00 6.50, respectively), and Moladoust et al., (33), who found that there was no significant difference in the serum levels of TNF- inpatient Patients with CHD had greater levels of TNF- than healthy youngsters, according to a research done in Egypt

(38). Consequently, the findings point to TNF- as having a complicated immune-modulatory function. TNF-α is the only cytokine that significantly increases IL-10 production. This demonstrates the presence of a distinct TNF-self-regulation mechanism based on the idea of IL-10 feedback. The findings are consistent with those of Yadav and YI (39). They found that TNF-serum levels in children with CHD before a percutaneous operation were considerably greater than those in control participants. However, the current findings conflict with those of Takaya et al. (40), who found evidence that TNF- is not raised in congenital heart disease patients who are asymptomatic or have minor symptoms.

6. Conclusions

Although the male gender was slightly more prevalent than the female, no statistical gender difference was found in patients with ventricular septal defect complicated by respiratory tract infection compared to controls with respiratory tract infections without congenital heart disease. Furthermore, no statistical differences were found regarding age in patients compared to controls. Serum levels of Interleukin-10 and Tumor necrosis factor-alpha were significantly higher in patients with ventricular septal defect complicated by respiratory tract infection v than in controls. This finding might have an important role in worsening the disease process of the ventricular septal defect.

Table (1): Demographic characteristics of patients with ventricular septal defect and RTI patients

Characteristic	VSD Patients *n*=40	RTI patients *n*=40	*P*
Age (years)			
Mean ±SD	10.52 ± 6.47	14.05 ± 9.91	0.065 † NS
Range	1 - 24 years	1- 35 years	
< 5, n (%)	4 (10.0 %)	7 (17.50 %)	
5-10, n (%)	20 (50.0 %)	12 (30.0 %)	0.289 ¥ NS
11-19, n (%)	9 (22.50 %)	10 (25.00 %)	
≥ 20, n (%)	7 (17.50 %)	11 (27.50%)	
Gender			
Male, n (%)	22 (55.00 %)	19 (47.50 %)	0.502 ¥ NS
Female, n (%)	18 (45.00 %)	21 (52.50 %)	
Male: female ratio	1.22:1	1:10	
n: number of cases; SD: standard deviation; †: independent samples t-test; ¥: Chi-square test; NS: not significant at P > 0.05			

Table (2): Median levels of Serum IL-10 level of ventricular septal defect patients and RTI patients.

	Cases –control comparison		
IL-10 (pg/ml)	ventricular septal defect patients n=40	RTI patients n=40	P
Range	177.19-285.32	13.00-194.07	< 0.001 † HS
Median (IQR)	213.53 (33.13)	32.75 (71.46)	
n: number of cases; SD: standard deviation; †: Mann Whitney U test; HS: Highly significant at P ≤ 0.001.			

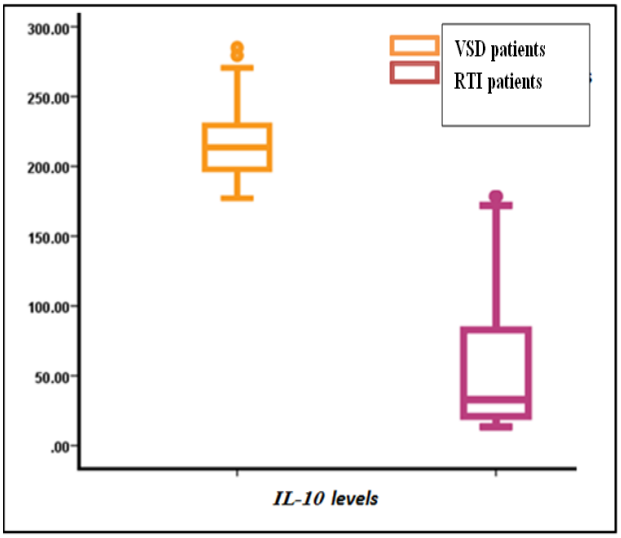

Figure (1): Box plot showing comparison of median serum IL-10 level among patients with ventricular septal defect and RTI patients.

Table (3): Sensitivity and specificity of IL-10 level (> 178.38 -fold) in ventricular septal defect.

IL-10 level	ventricular septal defect patients n = 40	RTI patients n = 40
> 178.38	37 (%)	3 (%)
< 178.38	3 (%)	37 (%)
Sensitivity %	92.5 %	
Specificity %	92.5 %	
PPV %	92.5%	
NPV %	92.5 %	
AUC (95% CI)	0.988 (0.972- 1.000)	
CI: Confidence interval, AUC: Area under curve.		

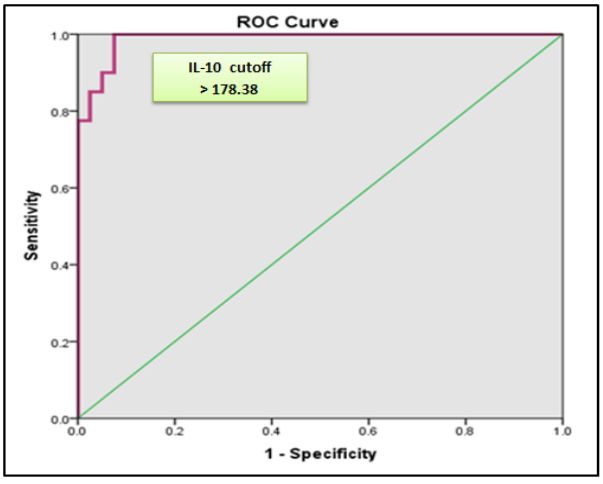

Figure (2): Receiver operator characteristic curve analysis of IL-10 for the calculation of possible diagnostic cut-off value.

Table (4): Median levels of Serum TNF-α level of ventricular septal defect patients and RTI patients

TNF-α (pg/ml)	Cases –control comparison		
	ventricular septal defect patients n=40	RTI patients n=40	P
Range	4465.76 - 7854.35	321.92 - 4910.08	< 0.001 † HS
Median (IQR)	5728.14 (1184.34)	1316.07 (1946.60)	
n: number of cases; SD: standard deviation; †: Mann Whitney U test; HS: Highly significant at P ≤ 0.001.			

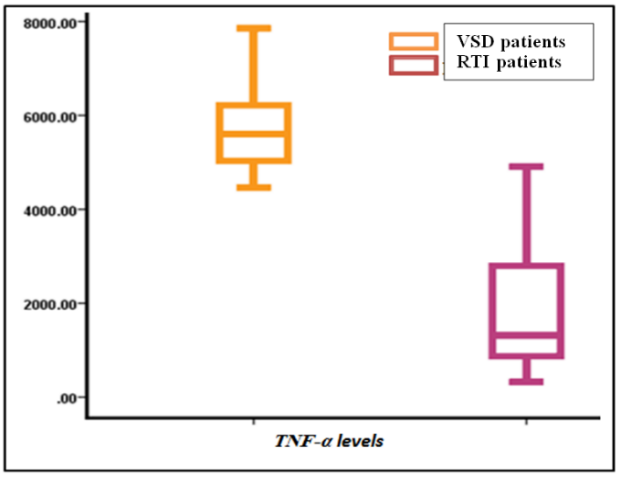

Figure (3): Box plot showing comparison of median serum TNF-α level among patients with ventricular septal defect and RTI patients.

Table (5): Sensitivity and specificity of TNF-α level (> 4505.47-fold) in ventricular septal defect.

TNF-α level	ventricular septal defect patients n = 40	RTI patients n = 40
> 4505.47	39 (%)	5 (%)
< 4505.47	1 (%)	35 (%)
Sensitivity %	97.5 %	
Specificity %	87.5 %	
PPV %	88.6%	
NPV %	97.2 %	
AUC (95% CI)	0.988 (0.972- 1.000)	
CI: Confidence interval, AUC: Area under curve.		

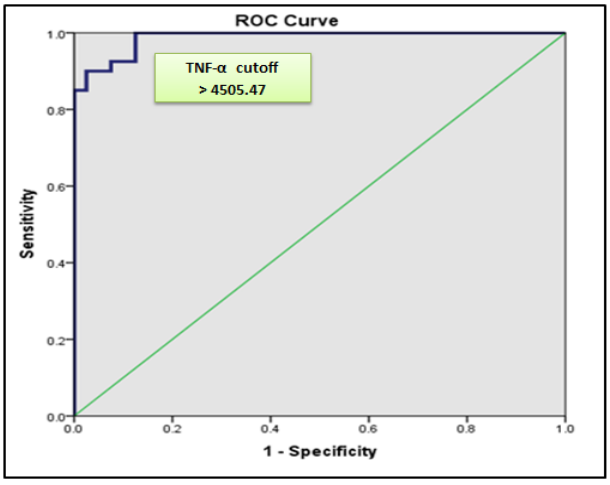

Figure (4): Receiver operator characteristic curve analysis of TNF-α for the calculation of possible diagnostic cut-off value.

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