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Determine the Association of Thymus and Activation-Regulated Chemokine (TARC)/CCL17 with Asthma Severity

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ABSTRACT

Background*: Asthma is a long-term respiratory disease that results in signs including coughing, chest tightness, wheezing, and shortness of breath due to inflammation and airway narrowing. Th2 cells, a subtype of T helper cells, are particularly attracted to the inflammatory region by novel asthma biomarkers, consisting of thymus and activation-regulated chemokine (TARC)/CCL17, which functions as an indicator of the severity of illnesses and the treatment's efficacy****. Objective:*** *This study assesses the association between serum concentration of (TARC)/CCL17 and asthma severity in adult patients****. Patients and methods****: Respiratory physicians diagnose Asthmatic patients based on the Global Initiative for Asthmatic Patients (GINA)guideline. Classified patients with asthma, according to GINA guidelines, the asthma control test (ACT), and serum biomarkers (TARC) are evaluated using an ELISA kit. Of the 140 participants, ranging in age from 18 to there were 70 cases and 70 controls in this case-control study were matched in age, and six to six cases****.*** ***Result****: Higher serum levels of TARC in the case, but no statistically significant p-values (0.528). Considering the correlation between study indicators' serum levels along with ACT, GINA, and duration, p-value>0.05, these differences were not statistically significant. Patients with a family history of asthma appeared to be significant in asthmatic individuals****. Conclusion:*** *There are differences between the patients and control groups, but no significant apparent differences. Significant variations were not found in correlation with GINA, ACT, or asthma duration, while significant with family history, indicating the biomarker is not correlated with the severity and control of asthma****.***

Keywords: **Asthma, GINA (Global Initiative for Asthmatic Patients), Asthma Control Test (ACT), Thymus and Activation-Regulated Chemokine (TARC)/CCL17**.

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INTRODUCTION

Asthma is a chronic respiratory illness, the most prevalent condition affecting the respiratory system that causes irritation and restriction in the airways in the lungs, causes difficulty breathing, and leads to episodes [1]. **First,** Characteristics by respiratory signs such as wheezing, coughing, chest tightness, shortness of breath, and fluctuating expiratory air flow restriction on spirometry should be the basis for a conclusive diagnosis of asthma [2]. **Second,** the worldwide prevalence of asthma among adults has been calculated to be around 6.6% [3]. According to current research, the frequency of asthma among people in Iraq varies by area. In Nasiriyah, 62% of adult asthma patients were male, with the most afflicted age range being 30-40 years[4], while in Kirkuk, it was found that 91.2% of adult asthma patients had poorly controlled asthma, demonstrating challenges in disease management and control [5]. **Third**, Asthma symptoms are caused by the inflammation of the airways, which sets off reactions such as bronchial hyperresponsiveness (BHR), mucus

production, and airway wall remodeling. Usually starting in childhood, allergic asthma is linked to T helper 2 (Th2) cell responses, which are also observed in other allergic disorders such as allergic rhinitis or atopic dermatitis. Early exposure to environmental allergens like pollen, cockroach, animal dander, or house dust mites (HDM) can cause this type of asthma, but can also be brought on by exposure to a new allergen later in life, such as an occupational one. The production of type 2 cytokines (interleukin IL-4, IL-5, IL-9, and IL-13) by allergen-specific Th2 cells in response to allergen recognition causes an increase in eosinophils in the airway wall, an excess of mucus, and the production of immunoglobulin E (IgE) by allergen specific B cells, which is then found in the serum [6,7].

According to the Global Initiative for Asthma (GINA)Science Committee, there are different classes of asthma: mild, moderate, and severe [2]. A commonly used and indicated test in clinical practice to assess how well a patient's asthma has been managed during the last four weeks is the Asthma Control Test (ACT) [8]. **Fourth,** CCL17 is a chemokine produced by dendritic cells and epithelial cells. IL-4 from immune cells works in provides with other cytokines to promote the synthesis of CCL17. In the bronchi, TNF-α, IFN-γ, and IL-3 work together with alveolar epithelial cells to trigger the manufacture of CCL17 in an NFκB-dependent way. It plays a key role in the pathogenesis of asthma, identifying asthma phenotype, especially allergic and eosinophilic asthma, strongly paired with asthma severity due recruitment of Th2 cells to the inflamed airways. Chemically impacted via CCL17's interaction with CCR4. Therefore, increased tissue and/or plasma levels of CCL17, along with cellular expression of CCR4, may be indicators of disease severity [9,10,11]. This recent study provides a detailed and focused analysis of the role of TARC/CCL17 in asthma, offering insights that go beyond the general observations of previous research. Unlike earlier studies, which often addressed the role of TARC in a broad context, this work explores its relationship with specific clinical indicators, including the number of annual asthma attacks, lung function parameters such as FEV1 and PEF (according to GINA guidelines), and treatment response measured by the Asthma Control Test (ACT). It also highlights the importance of asthma phenotypes and examines variations based on residential areas, especially those that have been understudied in the past. Moreover, this study includes a wide age spectrum, covering both younger and older individuals. Altogether, these findings mark a significant advancement in understanding the immunological mechanisms of asthma and open new directions for more accurate diagnosis and personalized treatment strategies [12,13].

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METHODS

This case control study was conducted on 140 individuals, 70 patients who confirmed the diagnosis with asthma by respiratory physicians according to the Global Initiative for Asthma (GINA) guidelines, aged 18-66 years, consisting of 31 males and 39 females who we're attending the Al-Sadr Teaching Hospital's Allergy Centre in Najaf province in the period from September 2024 and February 2025 and 70 individuals as controls. The serum level of biomarkers (TARC) using an ELISA kit was evaluated for all individuals in this study.

Inclusion Criteria:

Cases: (70) Patients between the ages of 18 and 66 were diagnosed with asthma after a physician's evaluation, lung function testing, and clinical history. The severity of asthma is classified using guidelines from the Global Initiative for Asthma (GINA): Mild: symptoms less than twice a week, FEV1 ≥ 80%. Moderate: everyday symptoms, FEV1 60–80%. Severe: persistent symptoms, FEV1 < 60%.

Exclusion Criteria for Cases:

Acute respiratory infections, additional chronic respiratory conditions, Immunocompromised patients, and women who are pregnant or nursing.

Inclusion Criteria for Controls:

Healthy people of the same age and sex who have never had asthma or any other chronic respiratory conditions.

Exclusion Criteria:

People who have used systemic corticosteroids within the last three months, have had acute respiratory infections during the last four weeks, or are currently smokers or individuals with other long-term respiratory disorders (such as chronic obstructive pulmonary disease).

2.1. Ethical and Scientific Approval:

The AL-Najaf Health Directorate granted ethical approval. Additionally, before collecting the sample, the doctor and the patients verbally agreed. Safety and health precautions were performed during the sampling process.

2.2. Collection of blood samples:

Obtained of three millilitres (3 mL) of venous blood were drawn from every participant, of venous blood samples were received from both asthma sufferers as well as healthy individuals. The whole blood was centrifuged for 15 minutes at 3000 rpm, then the serum was separated and transferred to an Eppendorf tube and stored in a

deep freezer (-70°c) to be employed for immunological assay.

2.3. Laboratory tests And Clinical tests:

Every patient and control serum underwent laboratory tests by an enzyme-linked immunosorbent assay (ELISA) system. The serum of Thymus and activation-regulated chemokine (TARC)/CCL17 levels was measured using ELISA kits (Sunlong/ China), Catalogue number:(SL1697Hu). The processes were done according to the supplier's protocol for the kits.

Also, clinical tests by GINA (Global Initiative for Asthmatic Patients) and the asthma control test (ACT).

2.4. Statistical analysis:

IBM SPSS Statistics Version 21(San Diego, CA, USA) was used to analyze the data. The data was compiled using descriptive statistics, which included frequencies and percentages for categorical variables and means and standard deviations for continuous variables. The ANOVA test was employed to evaluate differences in clinical and laboratory parameters across the groups. Statistical significance was set at P<0.05, with P<0.01 considered highly significant.

RESULTS

TARC levels in asthmatic patients and healthy control subjects were compared; the results are displayed in **Table 1**. Asthma patients' mean TARC levels were 92.23 higher in the case than the control mean (84.42); these differences were not statistically significant (p<0.566). A receiver operator characteristic (ROC) curve evaluation, according to **Figure 1,** was conducted to ascertain the TARC cut-off value and evaluate its predictive power for asthma sickness. With 50 % sensitivity and 34.3% specificity, the cut-off value of TARC was 64.44 pg/ml, and the area under the curve was 0.438.

When comparing TARC amongst patients with varying levels of asthma severity, **Table 2** shows that non-significant (p=0.332) but the serum level of the biomarker among the mild group is higher than moderate and severe (72.97). TARC concentrations are not statistically significant (p=0.712) when compared across patients with varying asthma controller statuses in **Table 3**. TARC is higher at (64.90) in poorly controlled patients than in others. The TARC levels are compared among asthma patients based on the duration in **Table 4**. The TARC's mean in the chronic Group is 121.05 pg/ml, considering a finding that levels of these markers are elevated with prolonged as thma duration.

These variations are not statistically significant (P-values = 0.407). In **Table 5**, immunological parameters and TARC are compared between patients with and without a family history of asthma. The TARC (p-value = 0.015) levels appeared to be significant in asthmatic individuals (p-values < 0.05). In **Table 6**, immunological parameter concentrations in individuals of various sexes are compared. TARC (p=0.528) does not differ significantly across patient sexes, suggesting that sex does not affect these immune parameters. In **Table 7,** the aged 48–57 had the lowest TARC level (62.51± 21.29), whereas those aged 18–27 had the highest (137.95± 193.92). Although there is some variation, these parameters did not differ significantly between age groups, as indicated by the p-values of >0.515.

Table 4.12 compares the levels of immunological parameters in patients with various asthma characteristics. Two patients do not have allergies, while roughly fifty-three people do. Three have mixed allergies, and twelve have exercise-induced allergies. Allergic asthma has greater serum levels of the immunological marker TARC (102.0) than those with other asthma subtypes. They were not statistically significant, though (p-values of 0.582). **Table 9** compares the social and

demographic characteristics of asthmatic patients and controls. the percentage of asthma patients who reside in urban areas was significantly higher (87.1%) than that of healthy controls (72.9%) (p=0.01).

Table 1: Comparison of Immunological Parameters Levels Between patients and controls

Immunological m100.markers		Number	Mean	Std. deviation	*P-value*
TARC pg/ml	Patients	70	92.23	102.63	0.566
	Controls	70	84.42	48.94	

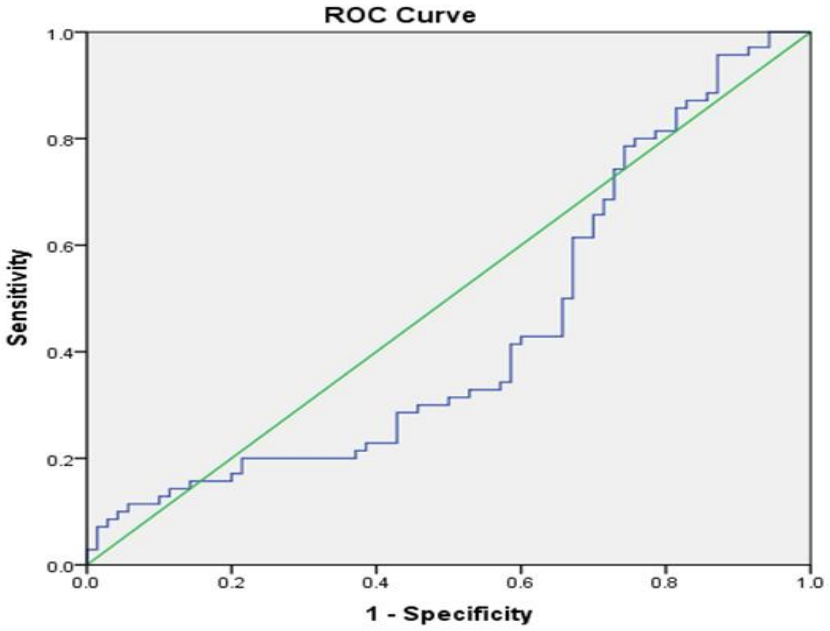

Figure (1): Receiver Operating Characteristic (ROC) Curve for Validity of TARC pg/ml.

Table (2): Comparison of Immunological Parameters Level Among Patients with Different Asthma Severity.

Immunological markers	Asthma Severity			*P-value*
	Mild Median (IQR) N=13	Moderate Median (IQR) N=30	Severe Median (IQR) N=27	
TARC pg/ml	72.97 (99.57)	63.13 (33.69)	65.06 (25.28)	0.332

Table (3): Comparison of Immunological Parameters Concentration Among Patients with Different Asthma Controller Status.

Immunological markers	Asthma Controller Status			*P-value*
	Well-controlled Median (IQR) N=10	Partially controlled Median (IQR) N=21	Poorly controlled Median (IQR) N=39	
TARC pg/ml	61.96 (274.31)	63.79 (30.08)	64.90 (23.56)	0.712

Table 4: Comparing immunological parameters based on the asthma duration.

Duration Groups	Number	Immunological Parameters
		TARC pg/ml Mean ± Std. deviation
Acute/Recent Onset (0–5)	14	56.72± 13.84
Long-Term Duration (10–15 years)	31	91.23± 107.56
Long-Term Duration (16–30 years)	10	101.81± 58.83
Chronic/Severe Duration (31+years)	15	121.05± 150.01
P-value		0.407

Table (5): Comparison of Immunological Markers Level Between Patients with and without a History of Asthma.

Immunological markers	History of Asthma	Number	Mean	Std. deviation	*P-value*
TARC pg/ml	No	21	61.28	19.22	0.015*
	Yes	49	105.49	119.95	

Table (6): Comparison of Immunological Parameters Concentration Between Patient Sexes.

Immunological markers		Number	Mean	Std. deviation	*P-value*
TARC pg/ml	Male	31	101.91	145.43	0.528
	Female	39	84.54	47.86	

Table (7): Comparison of Immunological Parameters Concentration Among Patients with Different Age Groups.

Age Groups	Number	Immunological Parameters
		TARC pg/ml Mean ± Std. deviation
18-27 years	9	137.95± 193.92
28-37 years	19	76.71 ±43.14
38-47 years	23	101.47± 124.31
48-57 years	11	62.51± 21.29
58-66 years	8	91.95 ± 42.92
P-value		0.515

Table (8): Comparison of Immunological Parameters Level Among Patients with Different Asthma Phenotypes.

Immunological markers	Asthma Phenotype				*P-value*
	Allergic Mean ± Std. deviation N=53	Non-Allergic Mean ± Std. deviation N=2	Exercise-induced Mean ± Std. deviation N=12	Mixed Mean ± Std. deviation N=3	
TARC pg/ml	102.0± 115.89	64.63 ± 41.49	59.61± 14.43	68.42± 41.24	0.582

Table 9: Demographic Characteristics of Patients with Asthma and Healthy Controls.

Residence			
Urban , n (%)	61 (87.1%)	51 (72.9%)	0.01*
Rural, n (%)	9 (12.9%)	19 (27.1%)	

DISCUSSION

In the present study, asthmatic patients had a higher TARC level (92.23) than the control group (84.42), but according to the p-value of 0.566, this difference was not statistically. Although we could not identify statistically significant differences in serum TARC between normal and asthmatic patients, current results agree with those of Jo et al. (2018) [12]. Machura [14] found no significant differences in asthmatics' serum chemokine concentrations. Additionally, a contrast study by Luu Quoc [13] found that asthmatics had greater serum TARC levels than controls (P = 0.004). Because CCR4 is extensively expressed on helper T cells, CCL17, a ligand for the C-C chemokine receptor type 4(CCR4), may be involved in T-cell-dependent illnesses. Many autoimmune and inflammatory disorders have been linked to increased amounts of this chemoattractant molecule. Asthma patients' bronchoalveolar fluid contains elevated levels of CCL17, which is believed to drive CCR4+ Th2 cells migration into the lungs. CCL17 or CCR4 inhibition to stop this migration, according to Roufosse and Catherine [10].

According to **Table 2**, which compares the TARC (CCL17) levels among those suffering from mild, moderate, and severe asthma, individuals with mild asthma had higher mean TARC levels (72.97) compared to individuals with moderate to severe asthma. This difference is not statistically significant, according to the p-value of 0.333. Results from earlier research on TARC levels in asthma have been mixed. While some studies have revealed significant correlations identified higher TARC levels have been identified in severe asthma [15]. A limited sample size, significant variation in TARC levels among groups, or the impact of confounding variables such as comorbidities or medication use (e.g., corticosteroids) could all be reasons for this lack of significance.

The Asthma Control Test (ACT) revealed that the well-controlled group had a greater level of the immunological marker TARC (64.90) than the partially-controlled and poorly-controlled groups. However, the difference was not significant(p=0.712). While there have been no direct studies investigating the association between ACT scores and (TARC) levels, there has been a study into the relationship between ACT scores and other biomarkers of asthma inflammation. For example, one study looked at the relationship between ACT score and fractional exhaled nitric oxide (FeNO) level in asthmatic patients. The findings demonstrated a strong negative connection between ACT score and FeNO levels, showing that greater FeNO levels, which represent airway inflammation, were linked with poor asthma control as determined by the ACT [16].

In addition, studies have looked at the link between ACT score and oxidative stress markers in asthmatic patients. The study discovered that individuals with poor asthma control, as evidenced by lower ACT scores, had greater levels of oxidative stress indicators. These studies demonstrate that the ACT score can connect with a variety of indicators of asthma inflammation and oxidative stress. Investigating the link between ACT score and TARC level may provide useful insights into asthma pathogenesis and influence therapeutic methods [17].

Although the outcome is not statistically significant, the trend may still be relevant. One possible reason is that people with well-controlled asthma may still have underlying immunological activity, even when their symptoms are mild. This implies that asthma control, as evaluated by clinical symptoms or ACT scores, may not always capture the entire picture of immune system activity. The role of drugs should also be considered. Patients in the well-controlled group are more likely to be taking frequent medications, such as inhaled corticosteroids, which might affect immunological markers such as TARC. Instead of entirely suppressing it, the medication may stabilize the immune response at a specific point. Asthma is also a complicated and varied illness.

Based on the length of their disease, asthmatic patients' TARCs are compared. Considering the result that levels of these markers are higher with extended asthma duration, the TARC mean in the acute group was 56.72 pg/ml, whereas evaluated it was 121.05 pg/ml among those who had chronic. This difference, nevertheless, was not statistically significant (p=0.407). In contrast, a study by Amin [18] discovered that the length of asthmatic illness and the blood TARC level were positively correlated (p<0.001). The duration of asthma was longer in patients with T2-high asthma, while the onset of asthma was notably later, according to Imoto [19]. Raising blood eosinophils in T2-high asthma suggests a higher degree of airway inflammation, which may have caused this.

The diagnostic accuracy of TARC, whose diagnostic performance is evaluated, is sensitivity (50%) and specificity (34.3%), with AUC values of 0.438. However, according to a researcher by Luu Quoc et al. 2022[13]. Additionally, Yamada et al. (2024) [20] studied. The study concludes that no activity is a reliable indicator for diagnosing asthma. When comparing TRAC levels in patients with and without a history of asthma, those with a family history have significantly higher TARC levels (p-value = 0.015) than those without. TARC levels are inconsistent with our results with Yamada [20]; a history of asthma had no significant effect on TARC levels. A p-value of 0.555 indicates no significant variation in TARC values among those with a history of asthma and those without. Conflicting data exists about the direct link between Th2-mediated inflammation, airway remodelling, despite strong evidence linking high serum TARC levels to asthma and associated inflammatory processes. These underlying mechanisms are present in poor response to corticosteroid therapy, and adult-onset is characterized by Th2-low asthma, and no history of childhood allergies [20]. According to a study of TRAC concentrations between patient sexes, females had a higher TRAC concentration (101.91) than males (84.54). This difference is not statistically significant because there are more females with asthma than males (p=0.528) in the current study. These findings contradict another study by Yamada[20] that found significant variation between men's and women's serum TARC levels. TARC levels were significantly lower in women than in men (p-value = 0.0023).

The TARC levels for the five age groups of patients with asthma included in the present study were compared. TARC levels were lowest among those aged 48–57 (62.51± 21.29) and greatest among those aged 18–27 (137.95± 193.92). The p-values of >0.515 show that these parameters do not

differ significantly between age groups, as a result of variations in the number of participants in each age group. Conversely, Jo *et al* [12] found that the old adult group's serum TARC level was significantly higher than that of the young adult and middle-aged adult groups. According to *Jo et* al [12], there is no significant difference in TARC between patients with asthma and those without the condition in any group. A study by Yamada [20] supports the findings that there was no significant difference in TARC serum levels between age groups. These variations in results may be due to the variation number in each group. IgE levels in the older adult group were positively impacted by TARC in serum.

Immune markers, such as TARC, may behave differently depending on individual disease phenotype, age, or genetic background. This may explain why the levels were greater in the well-controlled group, despite predictions (Ma, 2022; Lorente-Sorolla et al., 2023) [21,22]. When comparing the TRAC levels across patients with different asthma traits. Though not statistically significant (p-values of 0.582), those with allergic asthma had higher serum levels of TARC (102.0) than those without the condition. Although the research's allergic component was substantial, there were no significant variations between allergic and non-allergic asthmatics concerning TARC levels (P > 0.050 for both), which is consistent with another study by Luu Quoc *et al*. (2022) [13].

As a result of the TARC's membership in the C-C chemokine family, which is crucial to immune-inflammatory responses. It has been shown that people with a variety of allergic illnesses had higher serum concentrations of TARC. Serum TARC levels showed a substantial correlation with the intensity of atopic dermatitis (AD) [14]. Due to its role in attracting Th2 cells to inflammatory areas, the interaction between TARC and CCR4 is especially relevant in allergic disorders. Th2 cells release cytokines including IL-4, IL-5, and IL-13, which promote the generation of IgE, raise eosinophil counts, improve mucus secretion, and heighten airway sensitivity, all of which lead to the development of asthma. According to Yamada [20], serum TARC levels are linked to these pathophysiological processes and may serve as a biomarker of Th2-mediated inflammation in asthmatic patients. A state of systemic allergic inflammatory response is linked to increased TARC synthesis.

This study found that a larger proportion of patients with asthma were female (55.7%) compared to male patients (44.3%). This study found no statistically significant difference between the patients and the control group (P = 0.388). According to a recent study by Trivedi and Denton (2019)[23], women in middle age are more atopic than men, and their disorders are more severe and begin earlier. However, according to a study by Alem et al. (2020)[24], 33.48% of patients with severe asthma were men and 27.51% were women. 33.91% of those with mild asthma were men, and 28.04% were women. After puberty, females are more predisposed than males to acquire asthma for many different kinds of hormonal, immunologic, and occupational/environmental causes. Women had lower asthma control and more severe flare-ups that led to hospitalization than males[25]. Women are more likely than men to have adult asthma, and this sex difference in prevalence reverses after adolescence, indicating that sex hormones may contribute to the genesis of asthma and that women may be more vulnerable [26].

The proportion of patients in urban regions was higher than that in rural areas in this study (87.1% vs. 12.9%). Al-Ghizzi *et al*. (2020)[27] state that asthma is more prevalent in developing nations, and urbanization may have a greater environmental influence than in rural areas. The fact that the study was conducted in a city explains this. Guo *et al.* (2022)[28] documented that although the prevalence of current asthma varied significantly by urban-rural classification among adults in 19 states and among children in 7 states, the majority of states did not show such a variation. On the other hand, Fuhlbrigge *et al*. (2002)[29] reported that asthma rates were higher in rural areas 54.3%, 984) than in urban ones 23.5%, 395). Asthma rates are rising in less developed Asian and African nations. According to several studies, asthma rates are rising in less-developed Asian and African nations. Factors such as smoking, urbanization, population growth, seafood consumption, and the presence of moist 2air, which leads to the production of mold and mildew, have been proposed as mechanisms explaining these developments (Enilari & Sinha, 2019) [30]. Another factor contributing to the gap is the so-called hygiene theory, which explains how growing up in a rural area, with its associated greater exposure to microbial agents and endotoxin's crucial role in the development and regulation of the immune system, protecting against allergic disorders like asthma [31].

CONCLUSION

Although there are visible variations in serum TARC concentration among the patient and control categories. These differences are not statistically significant. There is no statistically significant difference in the relationship between these biomarkers and asthma duration, severity, and control, but a significant relationship with family history.

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Ethical approval

The present study, which was conducted by the authors (Duaa Salim Bakhit), was approved by the local Department of Medical Microbiology, Faculty of Medicine, University of Kufa, Najaf, Iraq committee.

Statement of Permission and Conflict of Interests

The others declare that there is no conflict of interest associated with the manuscript.

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