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Association Between Interleukin-4 and Chemokine CCL-21 Levels With The Pathogenesis of Rheumatoid Arthritis in Iraqi Women

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Abstract

Background: Approximately 0.5%–1% of the global population is impacted by rheumatoid arthritis, with the prevalence adjusted for age and the rates of disability-adjusted life years showing an increase with age and being higher in female. The susceptibility to RA is notably higher in women, who are two to three times more likely to acquire the condition compared to men. The study highlights the importance of anti-inflammatory and inflammatory levels in treating rheumatoid arthritis in Iraqi women, emphasizing the need for balance in autoimmune disorders.

Objectives: The presented study aimed the anti-inflammatory agents like IL-4 and chemokine CCL-21 marker on the progression and pathogenesis of rheumatoid arthritis in women from Iraq.

Materials and Methods: A case-control study involving 96 female subjects aged 30-70 years, who were divided into two groups: 59 subject with RA patients and 37 healthy individuals as a control. Serum IL-4 and CCL-21 levels were measured using an ELISA technique

Results: The observed results indicated a significant increased in mean ± SD of both IL-4 and CCL-21 levels in sera of Iraqi women with RA as compared with apparently healthy controls group, *P* value ≥ 0.001) respectively. The receiver operating curve (ROC) analysis for IL-4 and CCL-21 showed that the CCL-21 levels indicate a (sensitivity = 89.9 %, specificity 90%) AUC = 66.4205 while IL-4 levels indicate (sensitivity = 94.9%, specificity 93.7%) AUC = 10.6489.

Conclusion: Anti-inflammatory levels play an important role in the pathophysiology of RA, so IL-4 and inflammation can be considered biomarkers for evaluating RA. Maintaining a balance between anti-inflammatory and inflammatory status is essential in treating rheumatoid arthritis and other autoimmune diseases.

العلاقة بين مستويات الانترلوكين-4 والكيموكين CCL-21 في التسبب في التهاب المفاصل الروماتويدي لدى النساء العراقيات

حنين مدلول امانة, فاضل جواد ال-طعمة, اثير حميد عوده

الخلاصة

المقدمة: يتأثر حوالي 0.5% - 1% من سكان العالم بالتهاب المفاصل الروماتويدي، مع معدل الانتشار المعدل حسب العمر ومعدلات سنوات الحياة المعدلة حسب الإعاقة التي تظهر زيادة مع تقدم العمر وتكون أعلى عند الإناث. تكون القابلية للإصابة بالتهاب المفاصل الروماتويدي أعلى بشكل ملحوظ لدى النساء، اللاتي أكثر عرضة للإصابة بالحالة مرتين إلى ثلاث مرات مقارنة بالرجال. تسلط الدراسة الضوء على أهمية المستويات المضادة للالتهابات والالتهابات في علاج التهاب المفاصل الروماتويدي لدى النساء العراقيات، مع التأكيد على ضرورة التوازن في اضطرابات المناعة الذاتية.

الأهداف: استهدفت الدراسة المقدمة العوامل المضادة للالتهابات مثل IL-4 وعلامة chemokine CCL-21 على تطور وإمراض التهاب المفاصل الروماتويدي لدى النساء من العراق

المواد والطرق: دراسة الحالات والشواهد التي شملت 96 امرأة تتراوح أعمارهن بين 30-70 سنة، وتم تقسيمهن إلى مجموعتين: 59 مريضة مصابة بالتهاب المفاصل الروماتويدي و 37 فرداً سليماً كمجموعة تحكم. تم قياس مستويات مصل IL-4 وCCL-21 باستخدام تقنية ELISA

النتائج : أشارت النتائج الملحوظة إلى زيادة معنوية في متوسط ± SD لكل من مستويات IL-4وCCL-21 في أمصال النساء العراقيات المصابات بالتهاب المفاصل الروماتويدي مقارنة بمجموعة السيطرة التي تبدو صحية، قيمة (P ≥ 0.001على التوالي. أظهر تحليل منحنى تشغيل المستقبِل ((ROCلـ IL-4 وCCL-21 أن مستويات CCL-21 تشير إلى (الحساسية = 89.9%، النوعية 90%) AUC = 66.4205 بينما تشير مستويات IL-4 إلى (الحساسية = 94.9%، الخصوصية 93.7%) الجامعة الأمريكية بالقاهرة = 10.6489.

الاستنتاج: تلعب مستويات مضادات الالتهاب دورًا مهمًا في الفيزيولوجيا المرضية لمرض RA، لذلك يمكن اعتبار IL-4 والالتهاب مؤشرات حيوية لتقييم التهاب المفاصل الروماتويدي. يعد الحفاظ على التوازن بين الحالة المضادة للالتهابات والالتهابات أمرًا ضروريًا في علاج التهاب المفاصل الروماتويدي وأمراض المناعة الذاتية الأخرى

1. Introduction

Rheumatoid arthritis (RA) stands out as a prevalent chronic autoimmune condition, distinguished by inflammation in the synovial tissues and destruction of joints which result in harm to tissues, limitations in functionality, significant disability, and early mortality (Trivedi, 2024) ; (Turnbull and Perry, 2023) **.** Approximately 0.5%–1% of the global population is impacted by RA, with the prevalence adjusted for age and the rates of disability-adjusted life years showing an increase with age and being higher in females (Shi *et al.*, 2023). The susceptibility to RA is notably higher in women, who are two to three times more likely to acquire the condition compared to men. In Iraq, the prevalence of RA is reported to be 1% (Al-Ghazaly and Jassim, 2022) **.** Among the population, individuals affected by rheumatoid arthritis range from 0.5% to 1.0%, with an annual occurrence of 3 to 5 new cases per 100,000 individuals. This condition displays a female predominance with a ratio of 3:1, which is consistent with other autoimmune conditions (Chancay, Guendsechadze and Blanco, 2019)**.** Research indicates that RA is a intricate condition shaped by various genetic elements, environmental influences (such as gender, infections, and the immune system), and the interplay between genes and the environment (Weyand *et al.*, 2023)**.** The intricate dance among these variables and the precise mechanisms driving the onset and progression of the disease remain shrouded in mystery. Thus, unraveling the genetic and molecular foundations of RA can pave the way for a more solid scientific groundwork for early detection, treatment, and prevention of the illness (Ortíz-Fernández, Martín and Alarcón-Riquelme, 2023) **.** The synovium in RA synovia causes persistent inflammation through the release of chemokines, cytokines, MMPs, and growth factors, stimulating both innate and adaptive immune responses (Iemmolo, Ghersi and Bivona, 2023). and the synovium is crucial for RA development implicated in different processes of RA development including inflammation and angiogenesis (Comerford *et al.*, 2014) **;** (Hu *et al.*, 2024). Studies have recently uncovered a fascinating connection between various variants of cytokine genes and the risk of RA, highlighting the crucial involvement of cytokines in the development of this condition (Yucel *et al.*, 2020)**.** The gene responsible for encoding Interleukin 4, known as IL-4, can be found on chromosome 5 (q31–33) and is predominantly produced by activated Th2 type CD4+ T cells, monocytes, mast cells, and basophilic granulocytes. IL-4, a multifaceted cytokine, plays a pivotal role in stimulating the production of immunoglobulin E (IgE) in B lymphocytes and acts as a key regulator of IgG isotype switching (Huang *et al.*, 2015) (Liu *et al.*, 2024)**.** It orchestrates the differentiation of precursor T helper cells into those of the Th2 subset, which are instrumental in mediating humoral immunity and regulating antibody production. This small yet potent cytokine, IL-4, exerts an immunomodulatory influence on RA**,**(Ferencova *et al.*, 2023)**.** offering promising prospects for RA therapy. Various studies have highlighted the correlation between single nucleotide polymorphisms (SNPs) in IL-4 and the risk of RA (Giri *et al.*, 2021)**.**

The inflammatory factor interleukin-4 (IL-4) has a key role in chronic inflammation, which is a common feature of several autoimmune diseases (Iwaszko, Biały and Bogunia-Kubik, 2021)**.** CCL-21, a chemokine, is crucial in rheumatoid arthritis (RA) by stimulating blood vessel growth, joint inflammation, and bone degradation. It is prevalent in RA synovial tissue and is linked to RA predisposition. CCL-21, along with its receptor CCR7, contributes to joint inflammation and osteoclast formation. Targeting the CCL-21/CCR7 axis could help treat autoimmune conditions like RA. Elevated CCL-21 levels are observed in RA patients (Elemam, Hannawi and Maghazachi, 2020). The impact of Chemokines on Rheumatoid Arthritis Treatment Observations have been made regarding the influence of chemical and biological DMARDs on the modulation of chemokine levels in RA. A plethora of research has demonstrated that NSAIDs, glucocorticoids, and DMARDs impede the generation of multiple chemokines across different clinical scenarios (Szekanecz, Koch and Tak, 2011). In addition,

non-pharmacologic approaches such as patient education, physiotherapy, and nutritional therapy are incorporated into the holistic care (Genel *et al*., 2020)**.**

2. Materials and Methods

A case-control study with a total of 96 subjects, 59 of them are RA patients with age ranged between 30- 70 year which obtained from each of Al-Hindiyah General / Kerbala Health Directorate and Merjan Teaching Hospital / Babylon Health Directorate during Oct., 2023 to May, 2024. The remaining 37 subjects were obtained from apparently healthy women as controls with matched age range. All of these cases underwent a comprehensive history taking process that included a clinical examination and laboratory investigations in their sera including measurement of IL-4 using an enzyme-linked immunosorbent assay (ELISA), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), (diagnosed by a rheumatologist according to clinical examination and laboratory testing to ensure inclusion in the American College of Rheumatology (ACR). /European League Against Rheumatism (EULAR) 2010 (ACR/EULAR-2010**)** (Aletaha *et al.*, 2010)**.** A questionnaire was developed for the study based on the literature review and discussions between the researcher and the supervisory team. The questionnaire included age, type of treatment, and family history of rheumatoid arthritis.
A blood specimen was extracted from every individual to quantify serum IL-4 and CCL-21 concentrations assessed through the Sandwich-ELISA method (Sino Biological Inc, Beijing, China). CRP and RF levels were determined utilizing a chemical analyzer (DIRUI CS480/China)(DIRUI/ China kit). Furthermore, ESR was gauged employing the Fast Detector(skgmes, China).

2.1. Statistical Analysis

Data analysis was conducted employing IBM's Statistical Package for Social Sciences, version 22.0 (SPSS, Chicago, Illinois, USA). Presenting scale variables for data with a normal distribution was accomplished through the utilization of descriptive statistics in the format of mean ± standard deviation. The normality of the data distribution was assessed utilizing the Box plot test. T-tests and analysis of variance tables were utilized to compare biomarker mean values across various groups statistically. Statistical significance was determined by a *p*-value below 0.05. The determination of the ideal sensitivity and specificity threshold for critical cases was reached by executing receiver operating characteristic (ROC) analysis.

3. Results

Table 1 summarizes the demographic characteristics of the patients (N=59) and control groups (N=37) involved in the study. A total of 96 participants were included in this study 59 patients and 37 control, divided into Tow groups based on age, family history, and. BMI groups. The distribution of age groups is fairly similar across both groups, with a slight trend towards older ages in the patient group. More than half of patients (69.6%) fall within this age range 30-39 years compared to controls (30.4%). Similar distribution for the 40-49 years range, with a slightly higher percentage in the patient group (63.2%) compared to controls (36.8%). A slightly higher percentage of controls (56.5%) are in the age range 50-59 years compared to patients (43.5%). while about of patients (68.4%) were in the age range 60-70 years compared to controls (31.6%), as presented in Fig.1. Results were demonstrated that the patient group appears to have a higher prevalence of overweight and obesity compared to the control group. About (43.9%) patients were classified as normal weight compared to controls (56.1%). A higher percentage of patients (75.6%) were overweight compared to controls (24.4%). All patients (100%) in the obesity category belong to the patient group, with none in the control group, as presented in Fig.2. Regarding

the family history indicate that this characteristic shown a difference between the groups. All patients (100%) reported a family history of the condition, whereas none (0%) in the control group, as presented in Fig.3. The majority of controls (48.5%) do not have a family history, compared to only half (51.5%) of patients.

Table 1: The Difference in The Mean ± SD of Age and Other Parameters

Parameter	RA Mean ± SD	Control Mean ± SD	P-value
	N = 59	N =37	
Age, (year)	48.54 ± 11.26	46.93 ± 10.36	0.164 [NS]
BMI kg/m^2	26.45 ± 3.89	24.45 ± 3.13	≤ 0.001[S]
IL-4, pg/ml	15.39 ± 2.47	7.90 ± 1.39	≤ 0.001[S]
CCL-21, pg/ml	93.15 ± 25.110	40.87 ± 13.20	≤ 0.001[S]
T-test was significant at p ≤ 0.05; SD: standard deviation; S: significant; NS= Non-significant.			

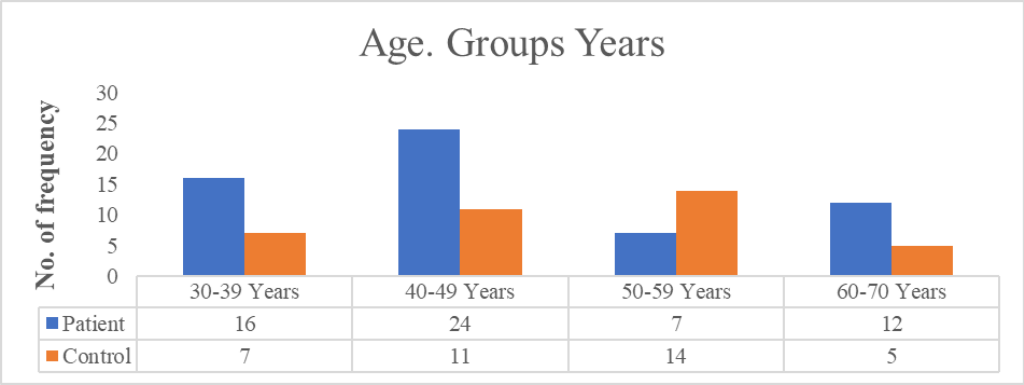

Figure 1: Baseline Characteristics of Age in RA Disease and Control Groups Studied

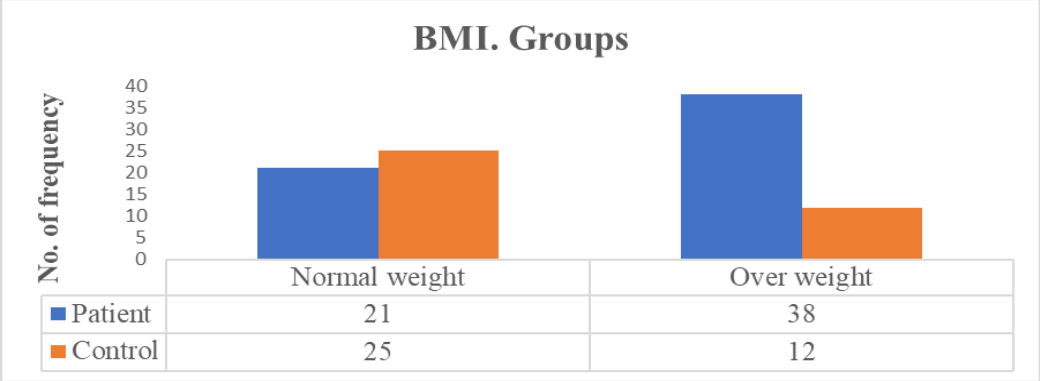

Figure 2: Baseline Characteristics of BMI in RA Disease and Control Groups

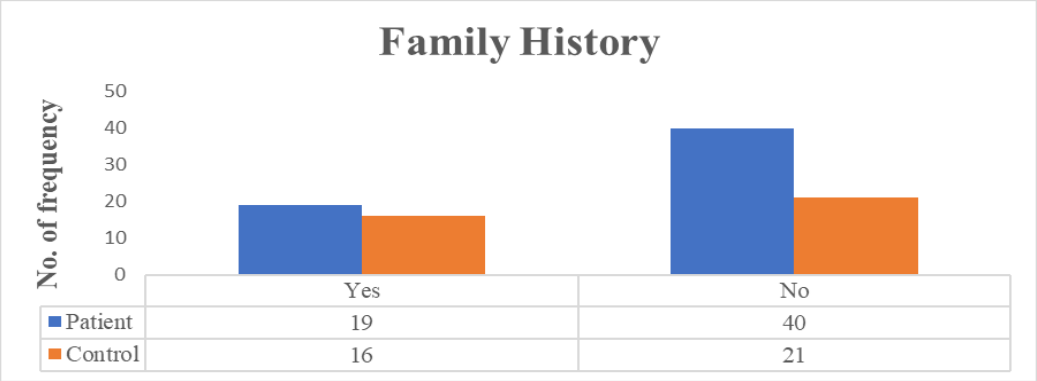

Figure 3: Baseline Characteristics of Family History in RA Disease and Control Groups

Table 2 demonstrates the mean ± SD levels of serum IL-4 and CCL-21 between study groups RA and control. The IL-4 levels was significantly elevated in sera of RA patients (15.39 ± 2.47 pg/ml) as compared with the control group (7.90 ± 1.39pg/ml), , *P* value ≤ 0.0001, also, an increased mean ± SD levels of serum chemokine CCL-21 was observed in sera of RA patients and reached to (93.15 ± 25.11pg/ml) as compared with healthy control group (40.87 ± 13.20pg/ml), $P \leq 0.000$. The receiver operating curve (ROC) and area under the curve (AUC) study for the CCL-21and IL-4 were analyzed for the RA patient and control group. CCL-21and IL-4 showed a good performance in predicting RA patients compared with a control group; data is shown in Table-2. CCL-21 levels indicate a (sensitivity = 89.9 %, specificity 90.7%) , AUC = 66.4205 while, IL-4 levels indicate (sensitivity = 94.9%, specificity 93.7%) AUC = 10.6489. The p-values of the AUC were <0.05 and highly statistically significant, as shown in Table (2). Youden's J statistics of the parameters in Fig.3 and Fig.4.

Table 2: AUC, Optimal Threshold, Sensitivity, and Specificity For (CCL-21and IL-4)

Variable	AUC	Sensitivity %	Specificity %	Cut-off points	Youden index	CI (95%)
CCL-21, pg/ml	95.9%	89.9%	90.7%	66.4205	0.888	(0.92-0.997)
IL-4, (pg/ml)	99.7%	94.9%	93.7	10.6489	0.946	(0.991-1.00)

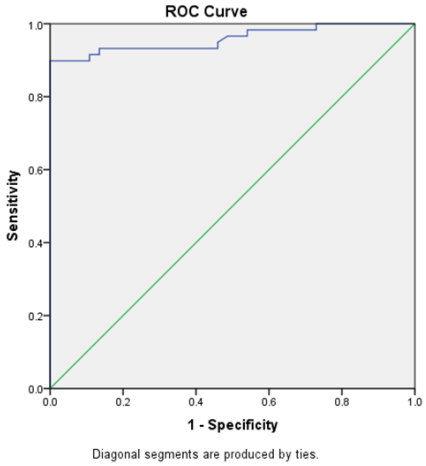

Figure 4: ROC Curves For IL-4 Pg/Ml in RA Patients as Compared to The Control Group, P-Value <0.05

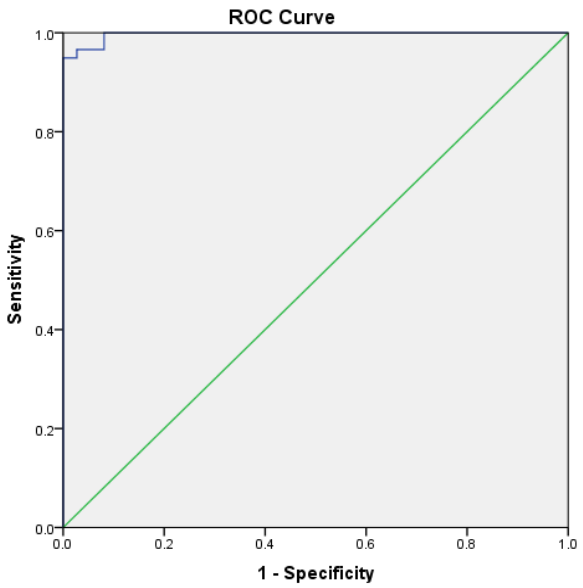

Figure 5: ROC Curves For CCL-21 Pg/Ml In RA Patients as Compared to The Control Group, P-Value <0.05

4. Discussion

The findings of this research reveal that the age does not have a significant impact on RA and control groups. These results are consistent with previous studies (Mititelu *et al.*, 2020). Aging increases autoimmune disorders like RA, affecting immune-related conditions. Premature immunosenescence in RA involves reduced thymic functionality, late-differentiated effector T cell proliferation, telomeric erosion, and increased pro-inflammatory cytokine generation (Bauer, 2020)**.** The study focused on female participants due to higher rheumatoid arthritis incidence, possibly due to estrogen levels irregularities, making women more susceptible to autoimmune disorders (Sciarra *et al.*, 2023)**.** In Table 1 and Fig.2, the correlation between BMI and RA risk revealed a favorable connection between obesity and RA. Furthermore, there are other research works that echo this finding, such as **.**(Yahya, AL-Jarash and Omran, 2022). Numerous inquiries have delved into the link between obesity and RA onset; nevertheless, the exact nature of this association remains enigmatic (Qin *et al.*, 2015). Prior investigations have indicated that over 60% of RA sufferers belong to the obese or overweight group (BMI≥25 kg/m2). Moreover, obesity represents a substantial and increasingly prevalent coexisting condition, even during the initial phases of RA manifestation (Challener *et al.*, 2022)**.** Fig.3, the ancestral background of an individual with RA historyis closely linked to other autoimmune conditions, increasing the likelihood of developing the disease. This is influenced by both genetic and environmental factors (Martu *et al.*, 2021). RA has been observed to be more prevalent among immediate family members, with a potential risk increase of up to 15 times. Those with compromised immunity are at a higher risk of acquiring RA, possibly due to shared genetic or environmental causes (Murata *et al.*, 2020). In the current study, lower levels of IL-4 were demonstrated among RA patients compared to the healthy group Th-2-mediated immunity and the pathophysiology of allergic inflammation are significantly influenced by IL-4. Different cell types secrete them (Liang *et al.*, 2012), and the STAT6 signaling pathway is how they work. They have an impact on many different types of cells,

including monocytes, fibroblasts, eosinophils, basophils, and B cells. Promising effects were obtained when blocking IL4 in Th-2-induced illnesses such atopic dermatitis and asthma (Dong *et al.*, 2018)**.** Nonetheless, a growing body of research suggests their involvement in the etiology of autoimmune-mediated illnesses such inflammatory arthritis (Ciesielski *et al.*, 2022)**.** Findings from both in vivo and animal models of arthritis indicate that the anti-inflammatory characteristics of IL-4 may be helpful for treating inflammatory arthritis. Induction of the IL-4 signaling pathway may provide a new and effective treatment strategy for inflammatory arthritis (Iwaszko, Biały and Bogunia-Kubik, 2021)**.** Thus, additional. Numerous chemokines and their corresponding receptors have been found to play a significant role in the pathophysiology of RA.by aiding in the recruitment of immune cells to arthritic joints (Elemam, Hannawi and Maghazachi, 2020). As a result, chemokine targeting may be a useful therapeutic strategy for treating RA. However, when these research were put into clinical trials, many of the antagonist and antibody investigations against chemokines and chemokine receptors ended in failure (Murayama *et al.*, 2023)**.** As more potent chemokine treatments are developed, additional clinical trial possibilities for the treatment of RA and related autoimmune disorders will become available (Miao, De Clercq and Li, 2020). With this post, we hope to clarify some of the key roles that chemokines and chemokine receptors play in RA illness. This data ought to offer a strong foundation for creating novel medications or other therapeutic approaches that target chemokines (Harvanová, Duranková and Bernasovská, 2023)**.** It is proposed that diminishing levels of oxidative stress and boosting anti-inflammatory capacities may prove advantageous in addressing rheumatoid arthritis. The discoveries emphasize the significance of comprehending and addressing oxidative stress as a component of the therapeutic strategy for rheumatoid arthritis (Nawaz *et al.*, 2021). We encountered a challenge due to the limited time and scarcity of samples. Had there been ample time available, the outcomes would have been more precise and thorough.

5. Conclusion

There is a significant relationship between inflammatory and anti-inflammatory conditions through the diagnosis of RA. It is necessary to address these factors effectively in the treatment of arthritis in women. In the investigation, it was observed that the levels of inflammatory(IL-4) and anti-inflammatory(CCL-21) indicators were elevated, thus indicating their potential as reliable biomarkers.

6. Ethical approval

Ethical endorsement for the research was formally granted by the esteemed institutions of Kerbala College of Medicine, Kerbala University, and Kerbala Health Directorate (Ethics Board, 24 on 5/11/2023).

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