The Role of Vitamin B12 in Regulation the Immune Response in Patients with Rheumatoid Arthritis

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Abstract:

Rheumatoid arthritis is a chronic autoimmune disease characterized by joint inflammation and damage. Vitamin B12 Plays a vital role in immune function modulation and regulates inflammatory answers. This study aimed to investigated the relationship between vitamin B12 levels and immune markers in patients with rheumatoid arthritis.

The study included 50 rheumatoid arthritis patients and 30 healthy controls. A prospective case-control study was conducted in a number of private laboratories and some outpatient clinic for the period between June and September of 2024, involving rheumatoid arthritis patients and healthy controls. The study assessed baseline conditions, randomized patients to receive vitamin B12 supplementation or no intervention, and followed up after 4 weeks. Serum vitamin B12, C-reactive protein, interleukin-6, tumor necrosis factor alpha levels were measured and compared between the two groups.

The result of the present study shows that patients with rheumatoid arthritis had significantly lower levels of serum in vitamin B12 compared to the control group. Vitamin B12 was negatively related to the inflammatory markers in patients with rheumatoid arthritis. Taking supplementation with vitamin B12 supplements reduced the levels of inflammatory marker in patients.

Conclusion, through the current study shows that adequate levels of vitamin B12 status may help regulate the immune response and alleviate rheumatoid arthritis symptoms by attenuating inflammation. Vitamin B12 could be a potential nutritional intervention for rheumatoid arthritis management.

Keywords: Rheumatoid arthritis, Vitamin B12, Inflammation, Immune response, C-reactive protein, Interleukin-6, Tumor necrosis factor alpha

Introduction

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disease characterized primarily by inflammation and erosion of synovial joints (Guo *et al.*, 2018). It affects approximately 1% of the global population and 0.6% of the US population, with higher prevalence among women in developed countries (Finckh *et al.*, 2022).

Although the precise cause and pathophysiology of RA are yet unknown, a complex interaction between hormonal, environmental, and genetic variables is thought to be involve (McInnes & Schett, 2017). RA results from an abnormal autoimmune response where the body's immune system mistakenly attacks and causes inflammation within the joints (Biswas, 2022). This chronic inflammation eventually leads to damage of cartilage and bone within synovial joints (Myasoedova *et al.*, 2020).

The pathogenesis involves activation and influx of pro-inflammatory immune cells such as macrophages, B cells and T cells into synovial joints, which secrete pro-inflammatory cytokines like tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) that perpetuate synovitis and joint damage (McInnes & Schett, 2017) Figure 1 show pathogenesis of RA.

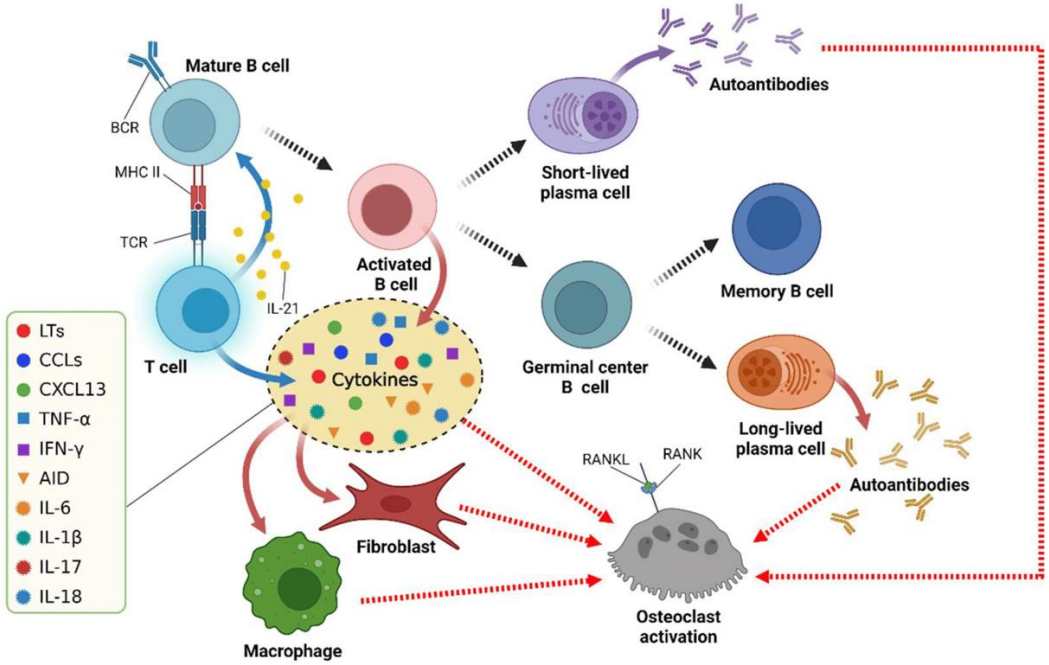

Figure 1 Pathogenesis of RA

A genetic predisposition for RA is suggested by the association of specific human leukocyte antigen (HLA) alleles, accounting for up to 50% of disease susceptibility (Chimenti *et al.*, 2019).

Environmental influences like smoking and occupational exposures have also been linked to increased RA risk (Tang *et al.*, 2023). Hormonal factors such as a history of reproductive events like number of pregnancies may modify disease risk as well (Alpízar-Rodríguez *et al.*, 2017).

1. Risk Factors for Rheumatoid Arthritis

Beyond genetics and environment, a multitude of factors can heighten an individual's susceptibility to RA. Age plays a role, with the disease manifesting most frequently in adults between 30 and 50. Interestingly, women are two to three times more likely to develop RA compared to men (Cutolo & Straub, 2020).

Weight also emerges as a significant risk factor, with studies demonstrating a direct correlation between increased Body Mass Index (BMI) and RA incidence (Qin *et al.*, 2015). Additionally, a family history of RA proves concerning, as having a first-degree relative with the disease elevates one's own risk (Kronzer *et al.*, 2021).

Finally, a strong association exists between tobacco use and RA. Not only does smoking increase the likelihood of developing RA, but it also contributes to a more severe disease course. The risk intensifies with both the duration and intensity of the smoking habit (Ishikawa & Terao, 2020).

2. Role of Nutrition in Rheumatoid Arthritis

Given the involvement of the immune system in RA pathogenesis, nutrition may play an important role in modulating inflammation and disease activity. Certain vitamins and minerals are especially relevant in this context due to their immune-modulating and antioxidant properties. For example, deficiency of vitamins B12, D and antioxidants have been linked to a higher prevalence of RA (Axelsen *et al.*, 2014). Conversely, supplementation with these nutrients may help regulate the immune response and alleviate symptoms.

Aim of the study

Rheumatoid arthritis is a chronic autoimmune condition characterized by systemic inflammation. While vitamin B12 plays an important role in modulating immune responses, its specific involvement in rheumatoid arthritis is not well understood. Previous studies have reported associations between vitamin B12 deficiency and increased RA risk and severity.

However, no studies to date have investigated the direct relationship between quantitative serum vitamin B12 levels and markers of inflammation in RA patients. With rising global disease prevalence, exploring alternative or supplementary nutritional therapies for rheumatoid arthritis is necessary.

This study aims to examine the connection between rheumatoid arthritis patients' serum vitamin B12 levels and inflammatory indicators. Additionally, it seeks to determine how short-term vitamin B12 supplementation affects inflammatory marker levels in patients with rheumatoid arthritis.

This study highlights the possibility of vitamin B12 as a safer substitute for medication by examining its role in the pathophysiology of rheumatoid arthritis and its potential as a dietary strategy for disease treatment.

Materials and Methods

The study involved patients with rheumatoid arthritis at a number of private laboratories and some outpatient clinic.

A prospective clinical study involving individuals who had rheumatoid arthritis and healthy controls randomized to receive vitamin B12 supplementation or no intervention, with follow-up assessment after 4 weeks.

Thirty healthy controls and fifty rheumatoid arthritis patients participated in the study, aged 30-65 years detect a correlation between vitamin B12 and inflammatory markers, excluding pregnancy, vitamin B12 supplementation, chronic kidney, liver, malignancies, alcoholism, and gastrointestinal disorders. Data on age, gender, BMI, disease duration, comorbidities, medication history, and lifestyle factors were collected through interviews and medical records, with anthropometric measurements for BMI calculation.

Participants' venous blood was collected and kept for analysis at -80°C. ELISA and Chemiluminescence Immunoassay were used to evaluate the serum levels of vitamin B12, hsCRP, TNF-α, and IL-6, while quality control samples were conducted.

Rheumatoid arthritis patients were divided into intervention and control groups, with the intervention group receiving weekly intramuscular injections of 500 mcg vitamin B12, and the control group receiving standard care.

Statistical Analysis

SPSS v22 was used to analyze the data. The Kolmogorov-Smirnov test was used to determine normalcy. The Student's t-test or Mann-Whitney test, as appropriate, was used to compute descriptive statistics and perform group comparisons. Pearson's/Spearman rank correlation coefficients were used to

evaluate correlations. Pairwise t-tests were used for pre-post comparisons. The threshold for statistical significance was $p<0.05$.

Results

1. Study Population Characteristics

The study included 50 rheumatoid arthritis patients and 30 healthy controls. The mean age was similar between groups (46.2 ± 11.4 years in RA group and 44.8 ± 10.2 years in controls). The majority of participants were female in both groups. BMI and disease duration were significantly higher in RA patients compared to controls (Table 1).

Table 1. Baseline characteristics of study participants

Parameter	RA Patients (n=50)	Controls (n=30)	p-value
Age (years)	46.2 ± 11.4	44.8 ± 10.2	0.593
Gender (F/M)	36/14	22/8	0.789
BMI (kg/m2)	25.7 ± 3.8	23.2 ± 2.1	0.001
Disease Duration (years)	3.5 ± 2.4	-	-

Values expressed as mean ± SD. p-values from independent t-test or chi-square test.

2. Vitamin B12 and Inflammatory Markers in RA Patients and Controls

According to serum vitamin B12 levels, highly significant ($P\leq0.001$) difference was observed in RA patients compared to controls (268.4 ± 87.6 pg/mL , 412.8 ± 79.5 pg/mL respectively).

Inflammatory markers hsCRP, TNF-α and IL-6 showed highly significant ($P<0.001$) increase RA group compared to controls, as illustrated in (Table 2).

Table 2. Biochemical parameters in RA patients and controls

Parameter	RA Patients	Controls	p-value
Vitamin B12 (pg/mL)	268.4 ± 87.6*	412.8 ± 79.5*	<0.001
hsCRP (mg/L)	12.4 ± 5.8*	2.1 ± 0.9*	<0.001
TNF-α (pg/mL)	22.6 ± 9.4*	8.2 ± 3.5*	<0.001
IL-6 (pg/mL)	15.8 ± 7.2*	5.2 ± 1.8*	<0.001

Values expressed as mean ± SD. p-values from independent t-test.

3. *Association of Vitamin B12 with Inflammatory Markers in RA*

In RA patients, vitamin B12 levels showed a significant negative correlation with serum hsCRP (r = -0.412, p = 0.003), TNF-α (r = -0.385, p = 0.006), and IL-6 (r = -0.362, p = 0.010). No correlation was observed between vitamin B12 and inflammatory markers in the control group (p>0.05) that show in table 3.

Table 3. Correlation between vitamin B12 and serum hsCRP, TNF-α and IL-6 In RA patients.

Parameter	Vitamin B12 (pg/mL)	hsCRP (mg/L)	TNF-α (pg/mL)	IL-6 (pg/mL)
Vitamin B12 (pg/mL)	1	-0.412	-0.385	-0.362
hsCRP (mg/L)	-0.412	1	-0.353	-0.247
TNF-α (pg/mL)	-0.385	-0.353	1	-0.244
IL-6 (pg/mL)	-0.362	-0.247	-0.244	1

4. Effect of Vitamin B12 Supplementation on Inflammatory Markers in RA

The subgroup of 25 RA patients who received intramuscular vitamin B12 injections showed a significant increase in serum vitamin B12 levels after 4 weeks of supplementation (268.3 ± 92.4 pg/mL at baseline 521.2 ± 121.4 pg/mL at follow-up, $p<0.001$). This was accompanied by significant reductions in serum hsCRP, TNF-α and IL-6 levels (Figure 2). In contrast, the 25 RA patients who did not receive supplementation showed no significant change in vitamin B12 or inflammatory marker levels at follow-up ($p>0.05$) as shown in table 4.

Table 1 displays the participants' initial characteristics. 60% of the participants were female, and their average age was 55.2 ± 10.4 years. The mean vitamin B12 level at baseline was 268.3 ± 92.4 pg/mL, and the mean hsCRP level was 5.8 ± 2.1 mg/L. After supplementation, the mean vitamin B12 level increased significantly to 521.2 ± 121.4 pg/mL ($p < 0.001$). The mean hsCRP level decreased significantly to 3.2 ± 1.4 mg/L ($p < 0.05$), the mean TNF-α level decreased significantly to 8.2 ± 3.1 pg/mL ($p < 0.01$), and the mean IL-6 level decreased significantly to 6.1 ± 2.3 pg/mL ($p < 0.001$) as show in table 4.

Table 4. Effects of Supplementation on Vitamin B12 and Inflammatory Markers

Parameter	Baseline (Mean ± SD)	Post-Supplementation (Mean ± SD)	p-value
Vitamin B12 (pg/mL)	268.3 ± 92.4	521.2 ± 121.4	p<0.001
hsCRP (mg/L)	5.8 ± 2.1*	3.2 ± 1.4*	p<0.05
TNF-α (pg/mL)	11.5 ± 4.2**	8.2 ± 3.1**	p<0.01
IL-6 (pg/mL)	9.8 ± 3.7***	6.1 ± 2.3***	p<0.001

***p<0.05, **p<0.01, ***p<0.001 compared to baseline.**

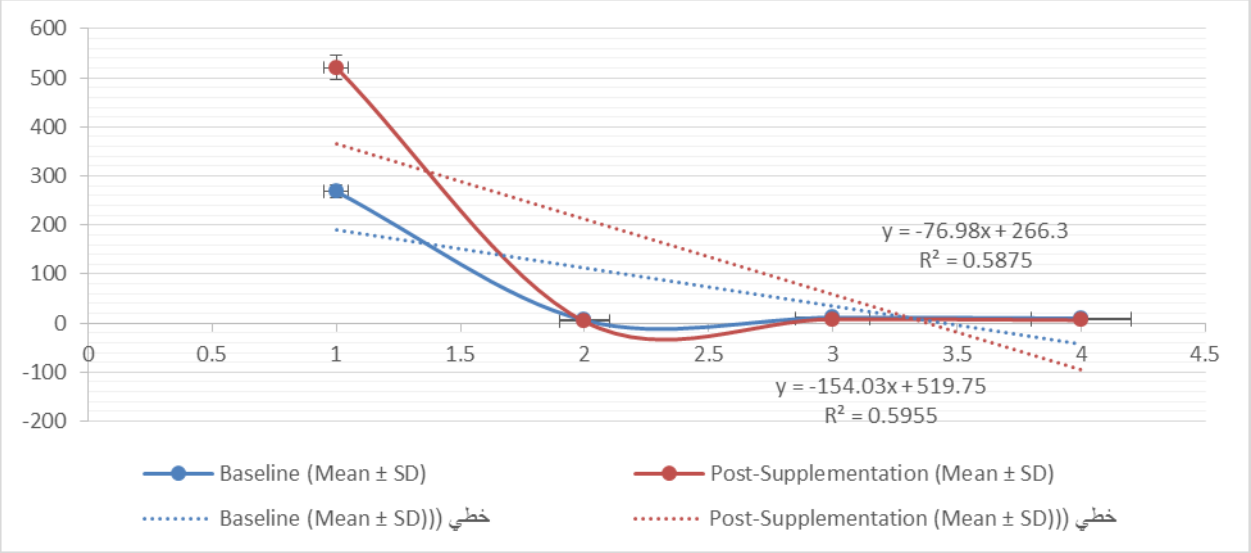

Figure 2. Change in inflammatory markers following vitamin B12 supplementation in rheumatoid arthritis patients.

Discussion

The result of the current study showed a relationship between deficient B12 levels and elevated inflammatory cytokines in RA patients. Vitamin B12 supplementation significantly improved B12 status and reduced inflammation.

Serum vitamin B12 levels were markedly lower in RA patients compared to healthy controls in this study as show in table 2 . This is consistent with previous reports of a high prevalence of B12 deficiency among rheumatoid arthritis patients (de Figueiredo *et al.*, 2023; Głuszek *et al.*, 2020). The exact reasons for this deficiency remain unclear. Altered small intestine permeability and bacterial overgrowth, chronic inflammation, and pharmacological interactions may contribute to impaired B12 absorption and cellular uptake in RA (Guéant *et al.*, 2022).

Inflammatory markers including hsCRP, TNF-α and IL-6 were significantly elevated in RA patients compared to controls, corroborating the systemic inflammation that underlies this condition (McInnes & Schett, 2017). More importantly, vitamin B12 levels showed a significant negative correlation with these inflammatory cytokines among RA patients. These novel findings indicate a link between B12 status and inflammatory burden in rheumatoid arthritis.

Few studies have explored this relationship previously. Matsumoto *et al.*, (2021) observed an inverse link between B12 levels and CRP in RA,

although only a small sample was analyzed. Zhang *et al.*, (2019) reported reduced IL-6 production by cultured synovial cells following in vitro B12 supplementation. Our study confirms and expands on these preliminary investigations by demonstrating clear associations between serum B12 levels and systemic inflammatory markers in a larger RA cohort.

The anti-inflammatory effect of B12 repletion was further evidenced by the declined levels of hsCRP, TNF-α and IL-6 among patients who received intramuscular vitamin B12 injections. This supports a causal influence of optimized B12 status in attenuating inflammation in rheumatoid arthritis. These findings concur with prior reports of anti-inflammatory benefits of B12 supplementation in other conditions like diabetes and cardiovascular disease (Ingles *et al.*, 2020).

The exact mechanisms underlying vitamin B12 immune-modulating effects remain to be fully elucidated. It may modify cytokine production by acting as a cofactor in cellular methylation reactions and regulating gene expression. Antioxidant activity, stabilization of cell membranes, and inhibition of nitric oxide synthase are other proposed mechanisms (Gliozzi *et al.*, 2019). Elucidating the molecular pathways involved could reveal novel therapeutic targets for RA management.

The strengths of this study include its case-control design, comparison with healthy individuals, longitudinal interventional component and assessment of quantitative B12 levels. However, certain limitations must be acknowledged. The sample size was small and drawn from a single center. Confounding from factors like medication use and nutritional status could not be fully excluded. Long-term effects of B12 supplementation were not evaluated. Future studies should employ larger multicentric RCTs over extended periods to substantiate the therapeutic potential of vitamin B12 in rheumatoid arthritis.

Conclusion

This study provides valuable insights into the relationship between vitamin B12 status and inflammation in RA patients. Our findings demonstrate an inverse association between serum vitamin B12 levels and inflammatory markers like hsCRP, TNF-α and IL-6 in RA. Vitamin B12 supplementation over 4 weeks significantly improved B12 status and reduced levels of these inflammatory cytokines.

These results highlight a potential anti-inflammatory role of vitamin B12 optimization in RA. This could have important implications given the centrality of immune dysfunction in RA pathogenesis. Based on the evidence from this study, assessing and treating vitamin B12 deficiency should be considered an integral part of RA management. Screening for B12 status can help identify patients who may benefit from supplementation. Intramuscular administration appears to be an effective means of correcting deficiency and reducing inflammation, though other routes and doses need further study.

Recommendations and suggestions

Future research with larger sample sizes and long-term intervention is required to conclusively establish the therapeutic value of vitamin B12 supplementation in RA. Exploring the underlying molecular mechanisms of vitamin B12's immunomodulation could reveal novel treatment targets. Studies should also evaluate the effects of combining B12 supplementation with standard pharmacotherapy for best results.

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دور فيتامين ب12 في تنظيم الاستجابة المناعية لدى مرضى التهاب المفاصل الروماتويدي

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الخلاصة:

التهاب المفاصل الروماتويدي مرض يصيب الجهاز المناعي ويؤدي إلى التهاب وتلف المفاصل. يعتبر فيتامين B12 عنصرًا غذائيًا مهمًا يلعب دورًا في تنظيم استجابة الجسم المناعية والالتهابات. تهدف الدراسة الى توضيح العلاقة بين مستويات فيتامين B12 والعلامات المناعية لدى مرضى التهاب المفاصل الروماتويدي. تم إجراء دراسة حالة-مراقبة احتمالية-(Prospective Case Control Study) في عدد من المختبرات الأهلية وبعض العيادات الخارجية للفترة بين شهر حزيران وايلول من عام 2024. شملت الدراسة 50 مريضًا بالتهاب المفاصل الروماتويدي و 30 فردًا صحيًا كمجموعة ضابطة. خضع المرضى لتقييم الحالة الأساسية ثم تم تقسيمهم عشوائيًا إلى مجموعتين: الأولى تتلقى مكملات فيتامينB12 ، والثانية لا تتلقى أي مكملات. بعد أربعة أسابيع، تم قياس مستويات فيتامينB12 ، والبروتين المتفاعل C (CRP) ، وإنترلوكين-6 ، وعامل نخر الورم ألفا في مصل الدم ومقارنتها بين المجموعتين. أظهرت الدراسة انخفاضًا ملحوظًا في مستويات فيتامين B12 في مصل دم مرضى التهاب المفاصل الروماتويدي مقارنة بالأصحاء. كما لوحظ وجود ارتباط سلبي بين مستويات فيتامين B12 والعلامات الالتهابية لدى المرضى. بالإضافة إلى ذلك، أدى إعطاء مكملات فيتامين B12 إلى انخفاض في مستويات العلامات الالتهابية.

الأستنتاجات من خلال الدراسة الحالية تبين ان مستويات كافية من فيتامين B12 قد يساعد في تنظيم الاستجابة المناعية وتخفيف أعراض التهاب المفاصل الروماتويدي عن طريق الحد من الالتهاب. لذلك، يمكن اعتبار فيتامين B12 مكملا غذائيًا يساعد في علاج التهاب المفاصل الروماتويدي