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Anti-Carbamylated Protein (Anti-CarP) Antibodies as a Predicting Marker of Disease Activity and Joint Damage in Juvenile Idiopathic Arthritis Patients

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Declaration of interest

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Abstract:

Background: Juvenile Idiopathic Arthritis (JIA) is a heterogeneous group of chronic inflammatory joint disorders that primarily affect children and adolescents. Aim: To evaluate the levels of anti-carbamylated protein (anti-CarP) antibodies in Juvenile Idiopathic Arthritis patients and to determine their association with serological parameters and disease activity. Methods: This Case control study was carried out at pediatric rheumatology unit, Children's Hospital Zagazig University and Elahrar Teaching Hospital in Zagazig on 48 children with Juvenile Idiopathic Arthritis who were admitted to Rheumatology Pediatric Unit and 48 healthy children age and sex matching as a control group. Results: There was a statistically significant positive correlation between anti-carbamylated antibodies and each of CRP [r=0.25, P=0.04], JADAS-10 CRP [r=0.028, P=0.007], CHAQ [r=0.53, P<0.001], JSNS [r=0.79, P<0.001]. The analysis revealed that anti-carbamylated antibodies was a valuable diagnostic marker for JIA with AUC of 0.82. At [12.3] cut off point, anti-carbamylated antibodies had sensitivity of 54.17%, specificity of 100%, PPV of 100% and NPV of 68.57%. there was statistically significant differences among JIA subtypes in anti-carbamylated antibodies as it was higher among poly-articular RF positive group [100%] in comparison to in oligo-articular group [53.3%] and in poly-articular RF negative group [42.9], [p=0.01]. Conclusion: Anti-carbamylated protein (anti-CarP) antibodies can be used as a biomarker for the diagnosis of Juvenile Idiopathic Arthritis (JIA) by measuring differences between their levels in JIA patients and those in the healthy controls, as well as can Anti-carbamylated protein (anti-Carp) antibodies be used in predicting JIA development, disease activity, and joint destruction.

Keywords: Anti-Carbamylated Protein, Anti-CarP, Disease Activity, Joint Damage; Juvenile Idiopathic Arthritis.

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Introduction

A diverse collection of illnesses known as juvenile idiopathic arthritis is defined as arthritis of uncertain aetiology that manifests before the age of sixteen and lasts for at least six weeks after other known causes of arthritis have been ruled out **[1].** Known for its short- and long-term disabilities, juvenile idiopathic arthritis is the most prevalent chronic rheumatic disease in children. Between two and twenty new cases per 100,000 people are reported year **[2].** Though the exact cause of JIA is unknown, genetic and environmental factors are considered to play a role, as immunological dysregulation is assumed to be the primary cause of inflammation. It is an autoimmune condition linked to changes in immunity mediated by cells and humour. T-lymphocytes play a significant part by secreting cytokines that promote inflammation, such as IL-6, IL-1, and TNF-. The increased immunological response to bacterial or mycobacterial heat shock proteins, aberrant levels of reproductive hormones, joint damage, and bacterial and viral infections such as parovirus B 19, rubella, and Epstein-Barr virus are some examples of the environmental triggers. It is possible to identify particular hereditary susceptibility genes, which are generally classified into two categories: HLA genes and non-HLA genes **[3].**

Juvenile idiopathic arthritis appears to have multiple contributing factors. When exposed to an unknown environmental trigger, a genetically vulnerable individual may develop an uncontrollably activated response to a self-antigen. This response results in tissue damage through the self-activation of both innate and adaptive immunity **[4].** The American College of Rheumatology [ACR], the European League against Rheumatism [EULAR], and the International League of Associations for Rheumatology [ILAR] systems are currently used to classify patients under the age of 16 with chronic arthritis; the ILAR criteria are more exact. The JIA classifications as of right now, according ILAR: The conditions listed are: a) oligoarthritis; b) polyarthritis related to rheumatoid factors; c) polyarthritis related to rheumatoid factors; d) arthritis with systemic start; e) psoriatic arthritis; f) arthritis related to enthesitis; and g) undifferentiated arthritis **[1].** Even though rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA) are useful in the diagnosis of juvenile idiopathic arthritis (JIA), dependable serological markers are still desperately needed to assess and forecast joint damage and disease activity in JIA patients. Patients with JIA have been reported to develop antibodies against carbamylated proteins, or anti-CarP antibodies **[5].** The current study set out to assess the anti-

carbamylated protein (anti-CarP) antibody levels in patients with juvenile idiopathic arthritis and ascertain how these levels related to serological markers and disease activity.

Patients and Methods

In this case-control study, 48 children with juvenile idiopathic arthritis who were admitted to the paediatric rheumatology unit at Children's Hospital, Zagazig University and Elahrar Teaching Hospital in Zagazig were compared to 48 healthy children who were age- and sex-matched as the control group. Three criteria must be satisfied for a diagnosis of juvenile idiopathic arthritis (JIA) to be made: the arthritis must be present for more than six weeks; it must also have started before the age of sixteen; and other conditions that are similar to or associated with arthritis must be ruled out. Bacterial arthritis, reactive arthritis, rheumatic fever, psoriatic arthritis, Lyme arthritis, inflammatory conditions, orthopaedic conditions, neoplastic conditions, and growing pains are among the conditions that must be ruled out in order to establish a diagnosis of juvenile idiopathic arthritis (JIA). These conditions can mimic or be associated with arthritis.

Every case meeting the requirements for juvenile idiopathic arthritis (JIA) was subjected to a comprehensive evaluation, which included a panel of laboratory investigations, a thorough general and local examination, and a full history taking. To help with the diagnosis and rule out other possible underlying conditions, the laboratory tests included a complete blood count, erythrocyte sedimentation rate, C-reactive protein, rheumatoid factor, antinuclear antibody (ANA), and anti-carbamylated protein antibodies (Anti-CarP antibodies). Ethical considerations were adhered to in this study. Informed signed written consent was obtained from all participants, ensuring confidentiality of information. Official permission was acquired from the scientific ethical committee of the department, the building company (with the study's title and objectives explained to ensure cooperation), and the Institutional Reviewing Board (IRB) at the Faculty of Medicine, Zagazig University.

- A childhood health assessment questionnaire (CHAQ) was administered to the patients.

- The Juvenile Arthritis Disease Activity Score (JADAS 10-CRP) was obtained to assess disease activity levels in JIA.

Serum anti-CarP antibody level was measured by enzyme- linked immunosorbent assay (ELISA), using the commercial anti-CarP antibody kits (Novatein biosciences, UK).

Immunoassays: A foetal calf serum-based single-step assay (for research use only, Inova Diagnostics, San Diego, CA, USA) was used to quantify anti-CarP antibodies, as previously described. **(7)**. Using CCP3.1 from Inova Diagnostics, San Diego, CA; rheumatoid factor (RF) from Roche Diagnostics Corporation, Indianapolis, IN; and 14-3-3 eta protein from AugureX, Vancouver, BC, Canada, anti-cyclic citrullinated peptide (CCP) was evaluated. As previously mentioned, anti-CarP antibodies were identified utilising carbamylated foetal calf serum as the antigen. The anti-CCP2 enzyme-linked immunosorbent assay (ELISA; Euro-Diagnostica) was carried out in accordance with the manufacturer's instructions, and IgM-RF was assessed as part of standard care. The mean plus twice the standard deviation (SD) of the healthy controls was used to determine the cut-offs for the anti-CarP antibody ELISA.

Disease activity: JADAS10-CRP was used to evaluate disease activity. Patients scored their pain using the visual analogue scale (VAS), ranging from 0 (no pain) to 100 (the worst pain imaginable). The counts for tender and swollen joints and c-reactive protein (CRP) were also recorded. Patients were categorized according to the JADAS10-CRP results as high disease activity (>5.1), moderate disease activity (3.2–5.1), low disease activity (2.6–3.2), and remission (< 2.6).

Outcome measures: Disease activity was evaluated using the Disease Activity Score (JADAS10-CRP). Patients rated their pain from 0 (indicating no pain) to 100 (indicative of the worst pain). The counts for tender and swollen joints and c-reactive protein (CRP) were recorded. According to the JADAS10-CRP scores, disease activity in the RA patients was categorised as low to moderate, with a cut-off value of 3.2. A self-assessment questionnaire (CHAQ) was administered to the 105 RA patients, who were asked about their ability to perform daily living activities. The disability index scores were determined through responses to questions within the eight categories that covered dressing and grooming, getting up, eating, walking, hygiene, reaching, grip strength and outside activity. According to the CHAQ results, the RA patients in the study were categorised as having mild to moderate disability, with the cut-off value of >1.

STATISTICAL ANALYSIS

The IBM Statistical Package for Social Sciences (SPSS) software was utilised to analyse the data (IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp). The distribution's normality was confirmed using the Kolmogorov-Smirnov test. Categorical data was expressed as numbers and percentage, whereas continuous data was expressed as mean ± standard deviation, median, and IQR. A statistical significance threshold of less than 0.05 was applied. To investigate the relationship between two qualitative variables, the chi-square test was employed. In order to determine whether there was a significant difference between more than two normally distributed groups, continuous data were subjected to analysis of variance (ANOVA or F test). The homogeneity of variances and the assumption of normality in each group were confirmed using the Shapiro-Wilk test and Levine's test, respectively. The Spearman/Pearson method of correlation analysis was employed to evaluate the degree of relationship between two quantitative variables. The degree and direction of the linear link between two variables are defined by the correlation coefficient, symbolically represented by .

Results:

There was no significant differences between the studied groups (cases & controls) as regards age or sex Table 1S). There was no significant differences between JIA subtypes as regards age as (P=0.098) while there were significant differences between JIA subtypes as regards sex distribution as (P=0.002) (Table 2S). The most common subtype of JIA patients was oligo-articular among (62.5%) of patients, followed by poly-articular RF negative among (29.2%) of patients, while four patients (8.35) only presented with poly-articular RF positive subtype. Uveitis was present in 10 JIA patients (20.8%) (Table 3S). There was a highly statistically significant difference between the two groups as regards anti-carbamylated antibodies, as in JIA patients anti-carbamylated antibodies ranged from 3.17 to 35.7 with mean 13.72 ± 8.86 SD, while in control group anti-carbamylated antibodies ranged from 0 to 11.8 with mean 5.3 ± 3.32 SD *(P <0.001).* There was 54.2% of the JIA patients were positive for Anti-CarP antibodies versus 0% healthy controls (Table 1). There was a statistically significant positive correlation between anti-carbamylated antibodies and each of CRP (r=0.25, P=0.04), JADAS-10 CRP (r=0.028, P=0.007), CHAQ (r=0.53, P<0.001) and JSNS (r=0.79, P<0.001). Moreover, no statistically significant correlation was detected between anti-carbamylated antibodies and age or ESR among JIA patients (Table 2). There was a statistically significant relation between uveitis

and anti-Carp among JIA patients, as patients with uveitis elicited significantly higher anti-carp level in comparison to those with no uveitis (P=0.001). (Table 4S).

The level of Anti-Carp was found to be significantly increased in patients with positive anti-CCP, RF and ANA compared to those with negative anti-CCP, RF and ANA, respectively (P<0.05) (Table 3). ROC curve analysis (Receiver operation characteristic curve) was conducted to determine the optimal cutoff value of anti-carbamylated antibodies to discriminate JIA patients from control group. The analysis revealed that anti-carbamylated antibodies was a valuable diagnostic marker for JIA with AUC of 0.82. At (12.3) cut off point, anti-carbamylated antibodies had sensitivity of 54.17%, specificity of 100%, PPV of 100% and NPV of 68.57% (Table 4). Both JADAS-10 CRP and CHAQ were significantly higher among poly-articular RF positive JIA group in comparison to oligo-articular and poly-articular RF negative patients (P<0.001). There was statistically significant difference among JIA subtypes in anti-carbamylated antibodies as it was higher among poly-articular RF positive group (100%) in comparison to in oligo-articular group (53.3%) and in poly-articular RF negative group (42.9%), (P=0.01) (Table 5). The proportion of patients receiving biologic treatment among the positive anti-Carp group was (61.5%) which was significantly higher than that among the negative anti-Carp group (27.3%), P=0.004 (Table 6). ROC curve analysis (Receiver operation characteristic curve) was conducted to determine the optimal cutoff value of CRP, ESR and ACCP to determine abnormal Anti Carp in cases group. The analysis revealed that CRP was a valuable diagnostic marker for abnormal Anti Carp with AUC of 0.712. At (36.5) cut off point, CRP had sensitivity of 75%, specificity of 65%, PPV of 30% and NPV of 92.9%. Also, the analysis revealed that ESR was a valuable diagnostic marker for abnormal Anti Carp with AUC of 0.719. At (53.5) cut off point, ESR had sensitivity of 100%, specificity of 45%, PPV of 26.7% and NPV of 100%. Also, the analysis revealed that ACCP was a valuable diagnostic marker for abnormal Anti Carp with AUC of 0.875, ACCP had sensitivity of 75%, specificity of 100%, PPV of 100% and NPV of 95.2% (Table 7).

Tables:

Table (1): Anti-carbamylated antibodies among studied groups

	Group Ⅰ (JIA group)	**Group Ⅱ (Control group)**	**Test**	**P-value***
Anti-carbamylated abs				
mean±SD	13.72±8.86	5.3±3.32		
median (range)	12.48 (3.17-35.7)	4.82 (0-11.8)	U	**<0.001**[a]
Positive	26 (54.2%)	0 (0%)	35.65	**<0.001**[b]
Negative	22 (45.8%)	48 (100%)		

*[a]: Mann-Whitney U test, b: Chi-square test, *: Significant <0.05, Non-Significant >0.05*

Table (2): Correlation between anti-carbamylated antibodies and JIA patients' characteristics

Variables	**Anti-carbamylated antibodies**	
	r	**P***
Age	0.08	0.42
ESR	0.11	0.4
CRP	0.25	**0.04**
JADAS-10 CRP	0.028	**0.007**
CHAQ	0.53	**<0.001**
JSNS	0.79	**<0.001**

***Pearson's/ Spearman's correlation coefficient, Significant <0.05, Non-Significant >0.05*

Table (3): Relation between anti-Carp and (anti-CCP, RF and ANA) among JIA patients

	Anti-Carp	**P value***
	Median (IQR)	
Anti-CCP		
Positive (n=8)	28.21 (25.73 – 30.16)	**<0.001**[a]

Negative (n=40)	8.94 (5.77 – 15.38)	
RF		
Positive (n=4)	27.95 (25.73 – 30.16)	**0.002**[a]
Negative (n=44)	10.74 (6.05 – 15.74)	
ANA		
Positive (n=22)	14.3 (6.67 – 19.65)	**0.047**[a]
Negative (n=26)	8.73 (5.62 – 16.51)	

[a]*: Mann-Whitney U test, *: Significant <0.05, Non-Significant >0.05, IQR: Interquartile range*

Table (4): ROC curve analysis of anti-carbamylated antibodies

Cutoff-point	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC (%)
12.3	54.17%	100%	100%	68.57%	0.82

PPV: Positive predictive value, NPV: Negative predictive value, AUC: Area under the curve

Table (5): comparison between clinical indices, laboratory data and radiographic scores according to subtypes of JIA

	Oligo-art. JIA (n=30)	Poly-art. JIA RF +ve (n=4)	Poly-art. JIA RF –ve (n=14)	P-value
JADAS-10 CRP *mean±SD*	4.52±1.1	7.36±0.4	6.85±1.1	**<0.001**
CHAQ *mean±SD*	0.38±0.1	0.75±0.05	0.56±0.05	**<0.001**
Anti-Carp				
Positive	16 (53.3%)	4 (100%)	6 (42.9%)	
Negative	14 (46.7%)	0 (0%)	8 (57.1)	**0.01**

Table (6): Relation between anti-Carp and biologic treatment among JIA patients

	Anti-Carp		P value*
	Negative (n=24)	Positive (n=24)	
Biologic treatment			
Yes	6 (27.3%)	16 (61.5%)	**0.004**[b]
No	16 (72.7%)	10 (38.5%)	

[b]*: Chi-square test, *: Significant <0.05, Non-Significant >0.05*

Table (7): ROC curve analysis of CRP, ESR and ACCP to determine abnormal Anti Carp (>20 EU/ml) in cases group

Parameter	Cutoff-point	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC (%)
CRP	36.5	75.0%	65.0%	30.0%	92.9%	0.712
ESR	53.5	100.0%	45.0%	26.7%	100.0%	0.719
ACCP	-	75.0%	100.0%	100.0%	95.2%	0.875

PPV: Positive predictive value, NPV: Negative predictive value, AUC: Area under the curve

Table (1S): Demographic data among studied groups.

	Group Ⅰ (JIA group)	Group Ⅱ (Control group)	Test	P-value*
Age (years)				
mean± SD	7.6±2.67	7.17±2.76	0.78	0.43[a]
(range)	(3-12)	(3-12)		
Sex (N. %)				
Female	28 (58.3%)	28 (58.3%)	-	1.0[b]
Male	20 (41.7%)	20 (41.7%)		

[a]*: Independent sample t-test,* [b]*: Chi-square test, *: Significant <0.05, Non-Significant>0.05*

Table (2S): Demographic data among JIA Subtypes.

	Oligo articular n=30	**"Rf+ve Poly articular" n=4**	**"Rf-ve Poly articular" n=14**	**Test**	**P-value***
Age (years) *Mean ±SD* *(range)*	7.19 ±2.57 (3-12)	10.25±2.47 (8.5-12)	7.55±2.69 (3-12)	**(F)** 2.4467	0.0980
Sex (N.%) Female Male	16(53.3%) 14(46.6%)	2(50%) 2(50%)	8(57.1%) 6(42.9%)	**(χ^2)** 12.112	0.002[b]

[b]*: Chi-square test, *: Significant <0.05, Non-Significant>0.05.*

Table (3S): Clinical data among JIA patients

	Group Ⅰ (JIA group) (n=48)
Articular	
Oligo-articular JIA (N. %)	30 (62.5%)
Poly-articular RF +ve (N. %)	4 (8.3%)
Poly-articular RF –ve (N. %)	14 (29.2%)
Extra-articular	
Uveitis	10 (20.8%)

Table (4S): Relation between Uveitis and anti-Carp among JIA patients

	Uveitis		P value*
	No (n=38)	Yes (n=10)	
Anti Carp			
Median (IQR)	8.73 (5.68 – 15.74)	17.28 (15.38 – 23.67)	**0.001**[a]

*[a]: Mann-Whitney U test, [b]: Fisher's exact test, *: Significant <0.05, Non-Significant >0.05, IQR: Interquartile range*

Discussion

Regarding Demographic data among JIA Subtypes, there was no significant differences between the studied groups as regards age (P= 0.098) while there was significant differences between the studied groups as regards sex distribution as (P=0.002). In concordance with our results, **Backström et al. [6]** conducted a multicentre cross-sectional study consisting of 20% of all patients with JIA in Finland. Of the 509 patients studied, 65.8% were females. The median age was 10.8 (1.0-18.0) years. Also, **Muller et al. [7]** reported that the median age was 12.1 (8.4–16.2) years and as regard to gender females were more (67.5%). In contrast to us **Haasnoot et al. [8]** conducted a retrospective cohort study on 358 patients with JIA with younger age than our study. The median age of onset of JIA was 2.7 years in patients with uveitis and 3.1 years in patients without uveitis (P = .029). In our study, the most common subtype of JIA patients was oligo-articular among (62.5%) of patients, followed by poly-articular RF negative among (29.2%) of patients, while four patients (8.35) only presented with poly-articular RF positive subtype. Uveitis was present in 10 JIA patients (20.8%) table (3). **Abou El-Soud et al. [9]** conducted a population-based, multicenter study among rheumatologists and pediatricians in Sharkia Governorate to identify patients with JIA less than 15 years. According to subgroup distribution, they reported that patients with oligoarthritis were the largest group, with 69 cases, representing 52.2 % of the total population, 21 patients of this group had uveitis (30.4 %), and 43 of them had positive tests of ANA (62.3 %). **Khawaja et al. [10]** illustrated that with regards to subtype distribution; 66 patients had oligoarticular JIA (48%). Nine had extended oligoarticular JIA (7%). Thirty-five patients had polyarticular (25%), 7 patients had systemic JIA.

In our study, There was a highly statistically significant difference between the two groups as regards anti-carbamylated antibodies, as in JIA patients anti-carbamylated antibodies was 54.2%, while in healthy control group anti-carbamylated antibodies was 0% *(P <0.001).* Also there was statistically significant differences among JIA subtypes in anti-carbamylated antibodies as it was higher among poly-articular RF positive group (100%) in comparison to in oligo-articular group (53.3%) and in poly-articular RF negative group (42.9%). (p=0.01). **Moore et al. [11]** observed that 20% [28/140] of the JIA patients were positive for α-CarP antibodies versus 0% [0/37] healthy controls . The highest percent positive was found in the oligoarticular-onset patients at 31% , 21% of the RF positive polyarticular-onst patients and 13% of RF negative polyarticular-onset patients, this is slightly different than our results . Overall, **Moore et al. [11]** study shows a higher percentage of α-CarP antibodiesin JIA than previously shown by **Muller et al. [7]**, especially in the oligoarticular-onset group. These studies show the originality of the α-CarP antibodies with no clear correlation with other biomarkers, but confirm the presence of α-CarP antibodies in JIA. Anti-CarP antibodies were demonstrated to be present in RA patients in **Othman et al. [12]** study, consistent with the findings of **Shi et al. [13]** in which it was reported that anti-CarP immunoglobulin (Ig)G (45%) and IgA antibodies (43%) were present in the serum of RA patients." Elsewhere, the percentage of individuals who were positive for anti-CarP antibodies was reported to be 44.9% for RA patients and 2.3% for the controls **[14].** Elevated anti-CarP antibodies levels were observed (46.1%) in a recent study by **Challener et al. [15]** on seropositive patients with established RA (n=167), consistent with the fndings of yet another study by **Scinocca et al. [16]** in which 40-55% anti-CarP antibody positivity was seen in RA patients. In **Yee et al. [17]** study, 36 of 120 RA patients (30%) were positive for anti-CarP antibodies. Similarly, of the 105 RA patients in **Othman et al. [12]** study, only 44 (41.9%) RA patients were positive for anti-CarP antibodies, with a corresponding figure of 11 (22%) for the control group. It has been reported that anti-CarP antibodies can be present in healthy individuals several years before the development of clinical RA symptoms. This is consistent with the findings of a **Verheul et al. [14]** study, in which significantly elevated levels of anti-CarP antibodies in RA patients were reported, in comparison with those for the control group (p<0.001). Also this finding is similar to that in the study performed by **Verheul et al. [18]** in which significantly varied anti-CarP levels were observed in a Japanese RA cohort (p<0.001). A significant diff erence in anti-CarP antibodies between the healthy controls and RA patients was also reported in **Shi et al. [13].** Above studies except **Moore et al. [11]** were conducted on adult patients as **Moore et al. [11]** conducted their study on 140 JIA patients and the mean age of

patients was 9.5±6.2, while **Hafez et al. [19]** conducted their study on Sixty rheumatoid arthritis patients and the mean age of patients was 47.3±9.5 and **Othman et al. [12]** illustrated that serum samples were collected from 105 patients with RA with a mean age of 55.0±14.0 years.

In the current study we found that there was a statistically significant positive correlation between anti-carbamylated antibodies and each of CRP (r=0.25, P=0.04), JADAS-10 CRP (r=0.028, P=0.007), CHAQ (r=0.53, P<0.001), JSNS (r=0.79, P<0.001). Moreover, no statistically significant correlation was detected between anti-carbamylated antibodies and age or ESR among JIA patients. **Hafez et al. [19]** results have shown that RA patients with sero positivity of antiCarp antibodies have more active disease and they were more disabled compared with sero negative patients. they reported a significant association between anti-CarP positivity and DAS-ESR levels and HAQ results in agreement with **truchete and his colleagues [20]** on 2017 who find a significant association between the anti-CarP anti-bodies and severe disease clinical activity. Similar data could be recorded from a study by **Othman et al. [12]** who reported a significant association between anti-CarP level and HAQ and CRP. Also similar results were reported by **Humphreys et al. [21]**. There was a statistically significant relation between uveitis and each of anti-Carp and ANA among JIA patients, as patients with uveitis elicited significiantly higher anti-carp level in comparison to those with no uveitis (p=0.001). **Haasnoot et al. [8]** reported that patients with uveitis were significantly more often ANA-positive compared to patients without uveitis (P ¼ .007). Presence of HLA-B27 did not entail a significant difference between the 2 groups.

The level of Anti-Carp was found to be significantly increased in patients with positive anti-CCP, RF and ANA compared to those with negative anti-CCP, RF and ANA, respectively (P<0.05). **Moore et al. [11]** showed that the overall presence of α-CarP antibodies was significantly higher in the RF positive, polyarticular-onset, RF negative, polyarticular-onset,, and oligoarticular-onset groups compared to healthy controls [p<0.05]. There were no statistical correlations with the presence of anti-nuclear antibodies or RF and α-CCP antibody isotypes. There was evidence of correlation with disease activity and duration of disease but did not reach statistical significance. **Othman et al. [12]** reported that the presence of anti-CarP antibodies was not significantly associated with the DAS28 results. This supports the findings of the study by **Yee et al. [17]** who reported that no individual marker (anti-CCP , RF and anti-CarP) correlated with disease activity (as measured by the DAS28) but a significant correlation was found when anti-CCP and anti-CarP antibodies positivity were present (p=0.026). In addition, it was

demonstrated in the study by **Humphreys et al. [21]** that the DAS28 score at baseline diff ered between anti-CarP-positive and negative patients with inflammatory polyarthritis ($p<0.001$). Elsewhere, **Albers et al. [22]** reported that an association was not observed between the presence of anti-CarP antibodies and disease activity. Although RF is considered to be the main serum marker to use to diagnose RA, it was shown in **Šenolt et al. [23]** study that it did not correlate with disease activity and anti-CarP status was not independently associated with remission**.** More studies are needed to determine the utility of anti-CarP antibodies with regard to any correlation with disease activity. ROC curve analysis (Receiver operation characteristic curve) was conducted to determine the optimal cutoff value of anti-carbamylated antibodies to discriminate JIA patients from control group. The analysis revealed that anti-carbamylated antibodies was a valuable diagnostic marker for JIA with AUC of 0.82. At (12.3) cut off point, anti-carbamylated antibodies had sensitivity of 54.17%, specificity of 100%, PPV of 100% and NPV of 68.57%. **Moore et al. [11]** observed that 20% [28/140] of the JIA patients were positive for α-CarP antibodies versus 0% [0/37] healthy controls . The highest percent positive was found in the oligoarticular-onset patients at 31% , 21% of the RF positive polyarticular-onst patients and 13% of RF negative polyarticular-onset patients, this is slightly different than our results . Overall, **Moore et al. [11]** study shows a higher percentage of α-CarP antibodiesin JIA than previously shown by **Muller et al. [7]**, especially in the oligoarticular-onset group. These studies show the originality of the α-CarP antibodies with no clear correlation with other biomarkers, but confirm the presence of α-CarP antibodies in JIA. Anti-CarP antibodies were demonstrated to be present in RA patients in **Othman et al. [12]** study, consistent with the findings of **Shi et al. [13]** in which it was reported that anti-CarP immunoglobulin (Ig)G (45%) and IgA antibodies (43%) were present in the serum of RA patients." Elsewhere, the percentage of individuals who were positive for anti-CarP antibodies was reported to be 44.9% for RA patients and 2.3% for the controls **[14].** Elevated anti-CarP antibodies levels were observed (46.1%) in a recent study by **Challener et al. [15]** on seropositive patients with established RA (n=167), consistent with the fndings of yet another study by **Scinocca et al. [16]** in which 40-55% anti-CarP antibody positivity was seen in RA patients. In **Yee et al. [17]** study, 36 of 120 RA patients (30%) were positive for anti-CarP antibodies. Similarly, of the 105 RA patients in **Othman et al. [12]** study, only 44 (41.9%) RA patients were positive for anti-CarP antibodies, with a corresponding figure of 11 (22%) for the control group. It has been reported that anti-CarP antibodies can be present in healthy individuals several years before the development of clinical RA symptoms. This is consistent with the findings of a **Verheul et al. [14]** study, in which significantly elevated levels

of anti-CarP antibodies in RA patients were reported, in comparison with those for the control group ($p<0.001$). Also this finding is similar to that in the study performed by **Verheul et al. [18]** in which significantly varied anti-CarP levels were observed in a Japanese RA cohort ($p<0.001$). A significant diff erence in anti-CarP antibodies between the healthy controls and RA patients was also reported in **Shi et al. [13].** Above studies except **Moore et al. [11]** were conducted on adult patients as **Moore et al. [11]** conducted their study on 140 JIA patients and the mean age of patients was 9.5±6.2, while **Hafez et al. [19]** conducted their study on Sixty rheumatoid arthritis patients and the mean age of patients was 47.3±9.5 and **Othman et al. [12]** illustrated that serum samples were collected from 105 patients with RA with a mean age of 55.0±14.0 years.

In the current study we found among studied JIA patients, 16 patients (33.3%) had radiographic progression over one year in Joint space narrowing score (JSNS). **Yee et al. [17]** illustrated that the median JES (Joint Erosion Score) of the patients with available data (n = 39) was 14.1 with a standard deviation of 11.5. The JES was significantly higher in anti-CarP antibody-positive (JES = 20) versus anti-CarP antibody-negative patients (JES = 10). In addition, anti-CarP antibodies were correlated with JES (q = 0.34, 95 % CI 0.03–0.59; p = 0.0332). They observed no correlation between ACPA and JES with any of the CCP assays. Due to the small cohort size, the correlation analysis could not be performed in ACPA-positive versus ACPA-negative patients. **Othman et al. [12]** reported that anti-CarP antibodies were associated with more severe radiological damage, measured using the Sharp-van der Heijde method, when X-rays of the hands and feet of RA patients were taken **[13],** also showed that the presence of anti-CarP antibodies were associated with more severe joint damage in the anti-CCP2-negative subgroup. The presence of anti-CarP antibodies correlated with joint erosion scores ($p=0.033$), whereas a correlation was not observed between the anti-CCP2 and the joint erosion scores in the RA patients (n=40).

Our study showed that disease characteristics among subtypes of JIA among studied patients, both JADAS-10 CRP and CHAQ were significantly higher among poly-articular RF positive JIA group in comparison to oligo-articular and poly-articular RF negative patients ($P<0.001$).

Hafez et al. [19] found a significant moderate correlation between Anti-CarP and DAS 28 similar to data reported by **Yee et al. [17]**; they found a significant mild correlation with DAS28. Another study by **Elsayed et al. [24]** who found same moderate significant correlation. In **Hafez et al. [19]** study, they reported that patients with anti-CarP-antibodies were more disabled than those with anti-CarPnegative. This was explained by the significant difference between patients

with positive and patients with negative anti-CarP antibodies and the HAQ results. This finding is almost in agreement with other previously reported studies by **Kumar et al. [25]**.

ROC curve analysis (Receiver operation characteristic curve) was conducted to determine the optimal cutoff value of CRP, ESR and ACCP to determine abnormal Anti Carp in cases group. The analysis revealed that CRP was a valuable diagnostic marker for abnormal Anti Carp with AUC of 0.712. At (36.5) cut off point, CRP had sensitivity of 75%, specificity of 65%, PPV of 30% and NPV of 92.9%. Also, the analysis revealed that ESR was a valuable diagnostic marker for abnormal Anti Carp with AUC of 0.719. At (53.5) cut off point, ESR had sensitivity of 100%, specificity of 45%, PPV of 26.7% and NPV of 100%. Also, the analysis revealed that ACCP was a valuable diagnostic marker for abnormal Anti Carp with AUC of 0.875, ACCP had sensitivity of 75%, specificity of 100%, PPV of 100% and NPV of 95.2%.

Conclusion:

By comparing the levels of anti-carbamylated protein (anti-CarP) antibodies in JIA patients and healthy controls, it is possible to use these antibodies as a biomarker for the diagnosis of juvenile idiopathic arthritis (JIA). Anti-carbamylated protein (anti-Carp) antibodies can also be used to predict the onset, course, and degeneration of the disease as well as joint destruction.

References:

1. Martini A, Ravelli A, Avcin T, Beresford MW, Burgos-Vargas R, Cuttica R, et al. Toward New Classification Criteria for Juvenile Idiopathic Arthritis: First Steps, Pediatric Rheumatology International Trials Organization International Consensus. J Rheumatol. 2019;46(2):190-197.

2. Giancane G, Alongi A, Ravelli A. Update on the pathogenesis and treatment of juvenile idiopathic arthritis. Curr Opin Rheumatol. 2017;29(5):523-529.

3. Horton DB, Shenoi S. Review of environmental factors and juvenile idiopathic arthritis. Open Access Rheumatol. 2019;11:253-267.

4. Brown RA, Henderlight M, Do T, Yasin S, Grom AA, DeLay M, et al. Neutrophils From Children With Systemic Juvenile Idiopathic Arthritis Exhibit Persistent Proinflammatory Activation Despite Long-Standing Clinically Inactive Disease. Front Immunol. 2018;9:2995.

5. Shi J, van de Stadt LA, Levarht EN, Huizinga TW, Hamann D, van Schaardenburg D, et al. Anti-carbamylated protein (anti-CarP) antibodies precede the onset of rheumatoid arthritis. Ann Rheum Dis. 2014; 73:780–783.

6. Backström M, Tynjälä P, Ylijoki H, Aalto K, Kärki J, Pohjankoski H, et al. Finding specific 10-joint Juvenile Arthritis Disease Activity Score (JADAS10) and clinical JADAS10 cut-off values for disease activity levels in non-systemic juvenile idiopathic arthritis: a Finnish multicentre study. Rheumatology, 2015; 55(4), 615-623.
7. Muller PH, Anink J, Shi J, Levarht EWN, Reinards HCM, Otten MH, et al. Anticarbamylated protein (anti-CarP) antibodies are present in sera of juvenile idiopathic arthritis (JIA) patients. Ann Rheum Dis, 2013; 72(12), 2053-2055.
8. Haasnoot AJ, van Tent-Hoeve M, Wulffraat NM, Schalij-Delfos NE, Los LI, Armbrust W, et al. Erythrocyte sedimentation rate as baseline predictor for the development of uveitis in children with juvenile idiopathic arthritis. Am. J. Ophthalmol., 2015; 159(2), 372-377.
9. Abou El-Soud AM, El-Najjar AR, El-Shahawy EE, Amar HA, Hassan TH, Abd-Allaha SH, et al. Prevalence of juvenile idiopathic arthritis in Sharkia Governorate, Egypt: epidemiological study. Rheumatol. Int., 2013; 33, 2315-2322.
10. Khawaja K, Kalas R, Almasri N. Subtype frequency, demographic features, treatment and outcome of Juvenile Arthritis in one Centre in Abu Dhabi in the United Arab Emirates. Pediatr. Rheumatol., 2023; 21(1), 14.
11. Moore TL, Bhardwaj P, Pepmueller PH. Anti-carbamylated protein antibodies in juvenile idiopathic arthritis: A new biomarker. Autoimmun. Rev, 2017; 4, 1022-1029.
12. Othman MA, Ghazali SW, Hamid WZW, Wong KK, Yahya NK. Anti-carbamylated protein antibodies in rheumatoid arthritis patients and their association with rheumatoid factor. Saudi Med. J., 2017; 38(9), 934.
13. Shi J, Knevel R, Suwannalai P, van der Linden MP, Janssen GM, van Veelen PA, et al. Autoantibodies recognizing carbamylated proteins are present in sera of patients with rheumatoid arthritis and predict joint damage. Proc Natl Acad Sci U S A, 2011; 108(42), 17372-17377.
14. Verheul MK, Van Erp SJH, Van Der Woude D, Levarht EWN, Mallat MJK, Verspaget HW, et al. Anti-carbamylated protein antibodies: a specific hallmark for rheumatoid arthritis. Comparison to conditions known for enhanced carbamylation; renal failure, smoking and chronic inflammation. Ann Rheum Dis, 2016; 75(8), 1575-1576.

15. Challener GJ, Jones JD, Pelzek AJ, Hamilton BJ, Boire G, de Brum-Fernandes AJ, et al. Anti-carbamylated protein antibody levels correlate with anti-Sa (citrullinated vimentin) antibody levels in rheumatoid arthritis. J. Rheumatol., 2016; 43(2), 273-281.
16. Scinocca M, Bell DA, Racapé M, Joseph R, Shaw G, McCormick JK, et al. Antihomocitrullinated fibrinogen antibodies are specific to rheumatoid arthritis and frequently bind citrullinated proteins/peptides. J. Rheumatol., 2014; 41(2), 270-279.
17. Yee A, Webb T, Seaman A, Infantino M, Meacci F, Manfredi M, et al. Anti-CarP antibodies as promising marker to measure joint damage and disease activity in patients with rheumatoid arthritis. Immunol. Res. 2015; 61:24–30.
18. Verheul MK, Shiozawa K, Levarht EN, Huizinga TW, Toes RE, Trouw LA, et al. Anti-carbamylated protein antibodies in rheumatoid arthritis patients of Asian descent. Rheumatology, 2015; 54(10), 1930-1932.
19. Hafez AE, Abdelazeem AH, Darwesh AF. Anti-Carbamylated antibodies as a marker of activity and disability in Rheumatoid Arthritis. MJMP, 2020; 31(4), 6-12.
20. Truchetet ME, Barnetche T. Anti-carbamylated protein antibodies presence in early arthritis with a poorer clinical and radiological outcome: data from the French ESPOIR cohort.
21. Humphreys JH, Verheul MK, Barton A, MacGregor AJ, Lunt M, Toes RE, et al. Anticarbamylated protein antibodies are associated with long-term disability and increased disease activity in patients with early inflammatory arthritis: results from the Norfolk Arthritis Register. J. Rheum. Dis., 2015; annrheumdis-2015.
22. Albers HM, Brinkman DMC, Kamphuis SSM, van Suijlekom- Smit LWA, Van Rossum MAJ, Hoppenreijs AH, et al. Clinical course and prognostic value of disease activity in the first two years in different subtypes of juvenile idiopathic arthritis. Arthritis Care & Research: ACR, 2010; 62(2), 204-212.
23. Šenolt L, Grassi W, Szodoray P. Laboratory biomarkers or imaging in the diagnostics of rheumatoid arthritis? BMC Med. 2014;12:49.
24. Elsayed SA, Esmail MA, Ali RM, Mohafez OM. Diagnostic and prognostic value of anti-CarP antibodies in a sample of Egyptian rheumatoid arthritis patients. Clin. Rheumatol., 2019; 38, 2683-2689.
25. Kumar S, Pangtey G, Gupta R, Rehan HS, Gupta LK. Assessment of anti-CarP antibodies, disease activity and quality of life in rheumatoid arthritis patients on

conventional and biological disease-modifying antirheumatic drugs. Reumatologia, 2017; 55(1), 12-17.