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PLASMA LEVELS OF 14-3-3H PROTEIN AND ITS CORRELATION WITH ACTIVITY OF RHEUMATOID ARTHRITIS PATIENTS

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ABSTRACT

Back ground: Rheumatoid arthritis (RA) is a chronic inflammatory disease with autoimmune pathogenesis disease that affects about 1.5% of the community. New markers are needed for early diagnosis of rheumatoid arthritis as seronegativity in early rheumatoid arthritis remains a major limitation of both anticitrullinated protein antibodies (ACPA) and rheumatoid factor (RF). The 14-3-3η protein may represent biomarker for the detection of RA. **Objective**: Study aims to investigate about 14-3-3-eta protein as a prospective RA-specific marker that complements both RF and ACPA and increasing their diagnostic value, and their correlation with disease activity and body mass index in the newly diagnosed rheumatoid arthritis. **Material and methods**: Early diagnosis patients with RA groups 103 (56 new early diagnosis without treatment group; 47early diagnosis with treated group), were included according to American College of Rheumatology (ACR) criteria and 25 subjects as healthy control group. All demographic(Age, Gender, Disease duration and Family history), clinical data(Simple Disease Activity Index in 28 joints "SDAI", Body Mass Index "BMI") and serological data(serum ACPA, CRP, RF, plasma 14-3-3-eta investigated by ELISA methods and ESR was estimated by westren methods were recorded. **Results**: Rheumatoid Arthritis new early diagnosis without treatment group divided according to RF results (seropositive 36,

seronegative 20) and early diagnosis with treated group(seropositive 32, seronegative 15), Mean and SE of plasma14-3-3 ηlevels in patients with RA(26.94±2.47 ng/ml), were significantly higher (P<0.05) among both groups, as compared to healthy control individuals (27.42 ± 1.998 ng/ml). Positive Anti-CCP in patients groups 94(91.3%), negative Anti-CCP 9(8.7%) as compare with positive Anti-CCP 0(0.00%) at healthy control group. High significant deference between RF+ve and RF-ve (p<0.01) among 14-3-3-eta and Anti-CCP at both groups except 14-3-3-eta at early treated groups had no significant deference(p=0.077). There was no significant correlation between 14-3-3η levels and SDAI at(P >0.05)in both groups, while significant positive correlation between 14-3-3η levels and BMI in new early diagnosis without treatment group at(P< 0.05). For plasma14-3-3 η diagnostic accuracy in RA; ROC curve analysis comparing patient with RA and control showed no significant AUC(P =0.132)of new early diagnosis without treatment group, the ROC curve yielded a sensitivity of 88.9%, a specificity of 60.0% of new early diagnosis without treatment group. 14-3-3η is an RA key marker that associated with pathogenesis of RA and play an important role for difference between RF+ve and RF-ve among RA patients.

KEYWORDS: Serum14-3-3η; New early RA diagnosis; Disease activity SDAI; Body Mass Index BMI.

INTRODUCTION

Rheumatoid arthritis is a multifactorial disease and its occurrence and severity are related to both genetic and environmental factors(Alamanos and Drosos, 2005). Seronegativity in both early and settled RA remains a major hindrance of both anticitrullinated protein antibodies (ACPA) and rheumatoid factor (RF) highlighting the need for new complementary markers that will improve diagnostic sensitivity (Mjaavatten *et al.*, 2011 and Burr *et al.*, 2012).Currently, the rheumatoid factor "RF"and the anti-cyclic citrullinated peptide "anti-CCP" are present in 50% of patients with early rheumatoid arthritis "RA" therefore its used for RA diagnosis(Umeda *et al.*, 2017). Several studies investigated the differential diagnostic values of ESR and CRP in inflammatory disease, and concluded ESR is a potential meaningful marker for disease differentiation (Liu *et al.*, 2013). The final step when increased levels of these markers lead to elevated of disease activity (The severity of clinical disease activity at a given time point or over a period of time is normally graded as, no disease (remission), low (mild) or minimal disease activity, moderate disease activity and high (severe) disease activity) (Fransen and van Riel, 2005). The thought of early RA and

early arthritis clinics was introduced to make an early diagnosis and to plan timely interventions. Early RA patients with undiagnosed or untreated disease may develop persistent inflammation with progressive joint damage (Dixey *et al*., 2004). Development of novel RA markers is required to allow classification of patients into different risk groups properly. The 14-3-3η protein is a biomarker for RA detection(Lee *et al*., 2003). There are seven isforms of the 14-3-3 intracellular proteins family. They share about 50% amino acid similarity among each other and interact with a lot of intracellular proteins, thereby controlling an array of biological processes including protein synthesis, cellular metabolism, protein trafficking, and cytoskeleton transport (Kilani *et al*., 2007). Overall isomers, only 14-3-3η was present in synovial fluid with high levels (at least 5-fold greater than its level in matched sera) implicating the joint as the likely source of 14-3-3η (Chavez-Munoz *et al*., 2009 and Thery *et al*., 2009). levels of 14-3-3η favor to be high in RA patients, but not in another disorder as osteoarthritis, osteoporosis, gout, psoriasis, Crohn's disease, ulcerative colitis, type 1 diabetes, systemic lupus erythematosus, primary Sjogren's syndrome, scleroderma, and multiple sclerosis (Jansen *et al*., 2002 and Maksymowych *et al*., 2011). Early diagnosis of RA can minimize irreversible joint damage (Maksymowych *et al*., 2014). So study aimed to evaluate the diagnostic benefit of plasma 14-3-3-eta as a diagnostic marker for RA and could be a marker for difference between seropositive and seronegative, and their correlation with other markers, disease activity(SDAI)and body mass index(BMI).

MATERIALS AND METHODS

Data collected during the period from March – September 2017. This study includes group of patients with early rheumatoid arthritis (All patients(103) with RA of less than 1 year duration that classify to (new early diagnosis without treatment group 56 patients and early treated group 47 patients)and(25) healthy control subjects and in to two subgroup(seropositive 68 patients and seronegative 35 patients). All patients will be submitted to complete clinical and radiological examination by rheumatologist. Questionnaire form formulated which involved name, age, sex, clinical history, disease duration, family history and BMI by (Kg/m2). **Specimen:** Venous blood specimens (5 ml) were collected from each subject; 3 ml of each sample for serum and plasma separation, and the other part remains of whole blood were collected for ESR test by westregren method. **Study protocols:** Quantitative measurement of 14-3-3eta, Anti-CCP, C-reactive protein (CRP), rheumatoid factor (RF) by Enzyme Linked Immuno-Sorbent Assay (ELISA), according to the manufactures protocol (YWHAH ELISA kit, Chorus Anti-CCP device, AGAPPE and Chorus

RF-G device, respectively). A simple Disease Activity Index (SDAI) = swollen joint count (SJC28) + tender joint count (TJC28) + global assessment of disease activity by the patient (PGA) + global assessment of disease activity by the evaluator (EGA) + c-reactive protein (CRP). Body Mass Index(BMI)=Kg/m^2.

Statistical analysis

Statistical analysis was performed using the SPSS 10 statistical package. Mean, SD and SE were used to express variables. T test was used to compare the difference in mean between two continuous numeric variables, differences were considered statistically significant at $P<0.05$. For comparing more than two groups, the one-way ANOVA method was used to determine if there is statistical significance across the groups or not. Receiver-operating characteristic (ROC) curves were used to evaluate the diagnostic utility of marker as estimated by the area under the curve (AUC). Between-group comparisons were made the Spearman's rank correlation coefficient procedure for non-normally distributed data and Pearson's product-moment correlation for normally distributed variables (Nordness, 2006).

RESULTS

Demographical and serological data of studied groups.

Table(1)shows demographical characteristics variables, patients information such that age, gender, disease duration family history were recorded. Clinical parameter such as body mass index (BMI), and disease activity (SDAI) were also measured. Briefly, the manage patients (42.22±11.23years) while (36.40±11.15years) of healthy controls, gender of patients comprised (female 77(74.8%) and male 26(25.2%), disease duration (46(44.7%), 57(55.3%) for first and second year, respectively, patients with family history were 30(29.1%)less than that without historical family73 (70.9%), body mass index BMI results showed (normal weight 28 (27.2%), over weight 31 (30.1%), obese44(42.7%)patients vs (normal weight 12 (48.0%), over weight 4(16.0%), obese 9(36.0%) in controls and simple disease activity index SDAI data appeared(moderate activity 6(5.8%), high activity 97(94.2) respectively patients vs (no disease[0.00 – 3.33]) of healthy control group.

The study showed that the number and percentage of studied groups according to positively results of ESR, RF, CRP and ACPA. Positive ESR patients 82(79.6%), while healthy control group2(8.00%), positivity. RF, CRP and Anti-CCP of patients results showed 67(65.0%), 89(86.4), and 94(91.3%), respectively.

Parameter which represents biomarker such as 14-3-3-eta was measured in patients and compared with controls. Briefly the results showed that mean and SE of 14-3-3-eta was (26.94 ± 2.47 ng/ml)vs (27.42 ± 1.998 ng/ml) of patients with RA and healthy control group, respectively.

Table 1: Demographical and serological data of study groups.

Parameters		**Patients (No. = 103)**	**Control (No. = 25)**
Age (Yrs.)	Mean± SD	42.22± 11.23	36.40± 11.15
Gender No. and %	Female	77 (74.8%)	22 (88.0%)
	Male	26 (25.2%)	3 (12.0%)
Disease Duration (Months) No. and %	(1 - 6)	46 (44.7%)	Non Exist
	(7 - 12)	57 (55.3%)	
Family History No. (no/yes)	No	73 (70.9%)	25 (100%)
	Yes	30 (29.1%)	0 (0.00%)
BMI No. and %	Normal weight	28 (27.2%)	12 (48.0%)
	Over weight	31 (30.1%)	4 (16.0%)
	Obese	44 (42.7%)	9 (36.0%)
SDAI No. and %	11.1-26.0 (mod. activ.)	6 (05.8%)	No disease (0.00 - 3.33)
	26.1-86.0 (high activ.)	97 (94.2%)	
ESR No. and %	Elevated	82 (79.62%)	2 (0.00 %)
	Normal	21 (20.40%)	23 (92.0%)
CRP No. and %	Positive	89 (86.4%)	0 (0.00%)
	Negative	14 (13.6%)	25 (100%)
Anti-CCP No. and %	Positive	94 (91.3%)	0 (0.00%)
	Negative	09 (08.7%)	25 (100%)
RF No. and %	Positive	67 (65.0%)	0 (0.00%)
	Negative	36 (35.0%)	25 (100%)
14 3 3 ETA(ng/ml)	Mean±SE	26.94±2.47	27.42±1.998

Concentration parameters in studied groups

Studied different concentrations parameter's readings along all different of RA groups, as well as controlled proved that having normal distribution function, since probability levels of significant through testing goodness of fit are accounted $P<0.05$, which reflects validity of using the conventional methods, either for descriptive, or for inferential statistics.

Table 2: Descriptive statistic of markers concentrations among studied groups.

Marker	Groups	No.	Mean	SE	Range	ANOVA test (F)	
						Statistics	P-value
Anti CCP	RF-ve (new e.untreated)	20	22.61	2.045	25.00	F= 13.580	0.000 (HS)
	RF+ve (new e.untreated)	36	102.82	16.697	279.9		
	RF-ve (early Treated)	15	24.11	1.304	12.00		
	RF+ve (early Treated)	32	42.81	6.419	180.9		
	Control	25	6.19	0.547	7.900		
14.3.3.eta	RF-ve (new e.untreated)	20	20.733	2.985	35.67	F= 4.231	0.003 (HS)
	RF+ve (new e.untreated)	36	32.000	1.821	40.10		
	RF-ve (early Treated)	15	21.208	2.909	30.56		
	RF+ve (early Treated)	32	27.826	2.095	46.38		
	Control	25	27.422	1.998	38.42		

(*) HS: Highly Sig. at P<0.01; NS: Non Sig. at P>0.05

Regarding to Anti-CCP concentration recorded highly significant different are accounted among studied groups at (p< 0.05 of two groups), about to 14.3.3.eta concentration, Plasma 14-3-3-eta level recorded highly significant different are accounted among studied groups at (p= 0.003 of both groups), and that for testing equality of mean values. Figure (1) represent graphically stem-leaf plots for the distribution of (Anti-CCP and 14-3-3-eta) markers among studied groups.

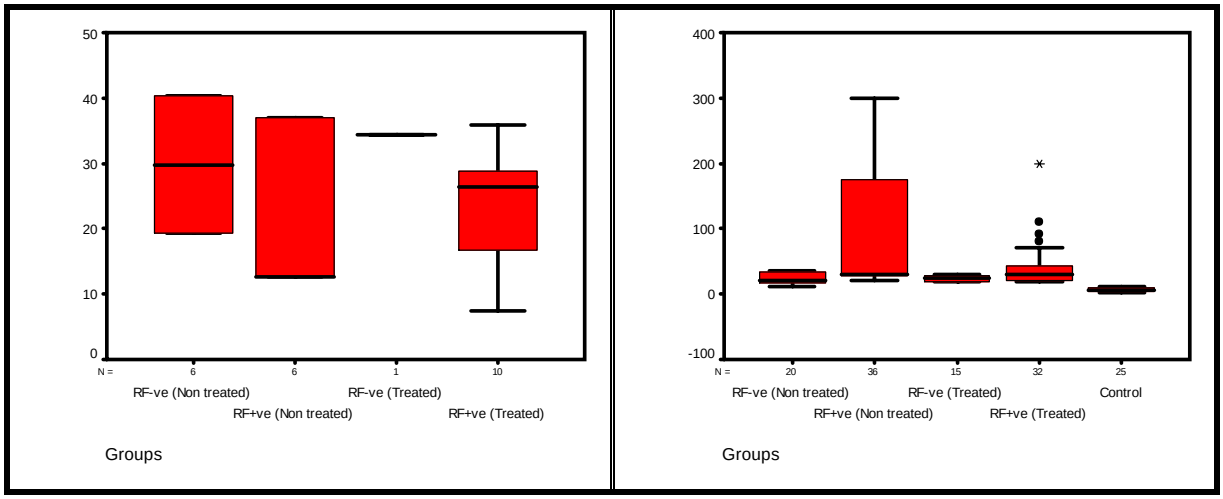

Figure (1): Stem - Leaf Plots for (Anti CCP & 14-3-3-ETA) Markers in the studied groups.

Concentration parameters in patients with RA subgroups(RF+ve and RF-ve)

Plasma 14-3-3-eta level recorded higher concentration in RF+ve patients compare to RF-ve patients among studied groups, but new early diagnosis patients group showed significantly higher 14-3-3-eta in RF+ve than RF-ve while not significantly higher in treated groups.

Higher plasma levels 14-3-3-eta and sera Anti-CCP level appeared in RF-ve patients with early diagnosis RA compared to RF-ve patients with significant differences at($p<0.05$) and so level of 14-3-3-eta and Anti-CCP of RF+ve in patients with treatment showed higher than RF-ve patients but only Anti-CCP appeared significant differences at ($p<0.05$).

Table 3: Statistic of markers concentrations between subgroups(RF+ve and RF-ve)of RA patients.

Groups	Markers	Groups	No.	Mean	SE	t-test	df	P-value (*)
New early diagnosis without treated	Concentration 14.3.3.eta	RF+ve	36	32.0	1.8	3.413	54	0.001 HS
		RF-ve	20	20.7	3.0			
	Anti CCP	RF+ve	36	102.8	16.7	4.768	36	0.000 HS
		RF-ve	20	22.6	2.0			
Earlydiagnosis (treated)	Concentration 14.3.3.eta	RF+ve	32	27.8	2.1	1.812	45	0.077 NS
		RF-ve	15	21.2	2.9			
	Anti CCP	RF+ve	32	42.8	6.4	2.855	33	0.007 HS
		RF-ve	15	24.1	1.3			

(*) HS: Highly Sig. at P<0.01; NS: Non Sig. at P>0.05

Correlation of studied biomarkers with BMI and SDAI indicators

Table (5) shows Person's correlation coefficients among different studied groups of RA disease and Controlled with comparisons significant.

Table 5: Correlation ships among biomarkers for studied groups of RA disease and Controlled with BMI and SDAI with comparisons significant.

Groups	Parameter	Pearson Correlation	14.3.3.eta	Anti CCP	RF	CRP	ESR
RF-(new e.RAgroup)	BMI	Corr.	-0.45	0.225	0.430	-0.24	-0.01
		Sig.	*0.045	0.341	0.058	0.313	0.983
RF+(newe.RAgroup)	BMI	Corr.	0.459	-0.14	-0.10	0.080	-0.09
		Sig.	*0.005	0.425	0.551	0.644	0.611
RF-(e.treated group)	BMI	Corr.	-0.19	-0.26	0.026	-0.18	-0.17
		Sig.	0.510	0.344	0.927	0.530	0.558
RF+(e.treated group)	BMI	Corr.	0.261	-0.01	-0.04	-0.15	-0.11
		Sig.	0.149	0.943	0.824	0.403	0.561
RF-(new e.RAgroup)	SDAI	Corr.	0.040	-0.20	0.430	0.429	0.482
		Sig.	0.868	0.401	0.071	0.059	*0.031
RF+(new e.RAgroup)	SDAI	Corr.	-0.31	0.321	0.450	0.543	0.439
		Sig.	0.064	0.056	*0.006	*0.001	*0.007
RF-(e.treated group)	SDAI	Corr.	0.389	0.604	0.314	0.342	-0.14

		Sig.	0.152	*0.017	0.254	0.212	0.612
RF+(e.treated group)	SDAI	Corr.	-0.25	0.234	0.283	0.462	0.487
		Sig.	0.177	0.197	0.117	*0.008	*0.005

(*) HS: Highly Sig. at P<0.01; S: Sig. at P<0.05; NS: Non Sig. at P>0.05.

Respect to subject of BMI and patient's groups, some studies markers has accounted strong and significant relationships in at least at p< 0.05 concerning early RA groups, either with RF+ve, and with RF-ve, but negatively relationships between RF-ve and BMI, while with the leftover groups (i.e. Treated RF+ve, and RF-ve). Briefly in early RA group who have RF-ve, the results showed significant correlation between BMI and level of 14-3-3-eta (p= 0.045), but in RF+ in the same group BMI with 14-3-3-eta (p= 0.005).

ROC Curve of 14.3.3.eta, Anti CCP&RF Markers

Table (4) shows estimation area of trade - off between sensitivity and specificity through plotting sensitivity against complement values of specificity to examine that trade - off, which is called a receiver operating characteristic or ROC curve, as well as significant levels for testing area parameter under fifty percent, with 95% confidence interval of area parameter in each status (early diagnosis RA without treatment and treated) groups.

Table 4: Statistics of ROC curve concerning studied of markers according to studied groups of RA patients.

Status	Con.	Cut off Point	Area	SE	Asymp. Sig. (*)	Asymptotic 95% C.I.		Sensitivity	Specificity
						L.b.	U.b.		
New early RA without Treated	Con. 14.3.3.eta	20.38	0.638	0.090	0.132	0.461	0.814	0.889	0.600
	Anti CCP	23.60	0.746	0.074	0.007	0.602	0.890	0.944	0.650
	RF	18.50	1.000	0.000	0.000	1.000	1.000	0.972	1.000

(*) HS: Highly Sig. at P<0.01; S: Sig. at P<0.05; NS: Non Sig. at P>0.05

Results shows at table(4) that area under the ROC curve is often quoted (0.638, 0.746 and 1.000) for concentration 14.3.3.eta, Anti CCP and RF markers respectively in light of new early diagnosis RA without treatment patients. Anti CCP and RF markers concerning area under the ROC curve are accounted highly effect with highly significant discrimination at P<0.01, while concentration 14.3.3. eta marker has no significant effect at P>0.05, and it could be conclude that Anti CCP and RF markers good indicator for diagnosing RA and patients with early diagnosis RA without treatment. Figures (2) show ROC curve concerning

concentration 14.3.3. eta, Anti-CCP and RF markers according to (new early diagnosis RA without treatment) of RA patients.

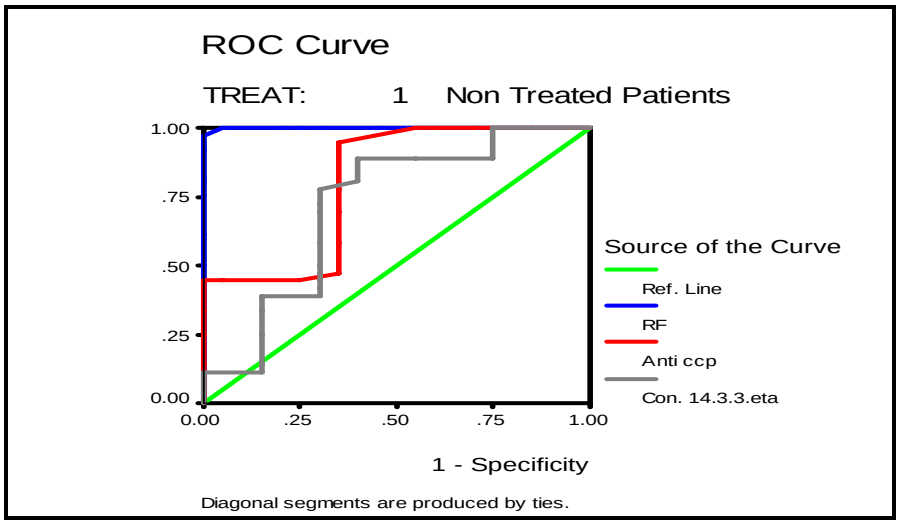

Figure (2): ROC Curve for concentration 14.3.3.eta, Anti CCP, and RF markers distributed with new early diagnosis RA group without treated.

DISCUSSION

A study showed aging is associated with the development of RA is unclear, but recent research suggests that increase aging can lead to chronic inflammation and immune mediated tissue damage (Weyand *et al*., 2014). The present study revealed that prevalence of RA was more in female patients than male patients similar to result in 2014 showed (75.25% vs 24.75%, respectively) (Weyand *et al*., 2014). Few studies have shown opposite result more frequent in men than in women (72% versus 55%, respectively)(Weyand *et al*., 1998).

In present study the percentage of patients had 7-12 mounths of disease duration more than the 1-6 mounths of patients groups, while study reported the patients fulfilling the American College of Rheumatology (ACR) criteria at presentation, 53% with disease duration of <or =3 months, compared with 94% of patients who presented with disease duration of >12 weeks, therefore the strongest predictor of persistent disease was a disease duration of >3 months(Green *et al*., 1999). Although family history of RA is an old concept, the degree of familial aggregation of RA (Frisell *et al*., 2016), present data appeared patients had family history less than non historical family this whether the small sample that taken in this study and due to it differs by age, sex or serology. Somers and his staff noted about family history who reported a high rate RA in female offspring with a maternal history of RA (Somers *et al*., 2013).

Obesity comprised(42.7%) of patients RA and this agree with study recorded about 66% of patients with RA are obese (Versini *et al*., 2014), this result due to apart from the destructive effect of excessive weight that lead to damage joints, fat affects the disease process, excessive fat leads to greater production of inflammatory proteins that increase the joint inflammation due to the disease itself (Versini *et al*., 2014). Therefore, many factors associated with RA may be likely consequences of disease rather than related to disease risk, however, obese individuals had a significantly increased risk of RA disease(Qin *et al*., 2015).

Early diagnosis of rheumatoid arthritis is important for preventing joint damage via treatment. For patients having typical symptoms, the disease could be easily diagnosed, often in the first year of disease onset. For many patients with atypical symptoms, it could take more time to diagnose. Therefore, specific and sensitive serological tests are required for diagnosis. Among most common symptoms of RA in present study was observed considerably SDAI score was calculated to obtain the severity of the disease, farther more, ESR and CRP with high positive results, although ESR and CRP are nonspecific for the diagnosis of RA, but they are important auxiliary markers for the diagnosis of RA. Simultaneous detection of RF, Anti-CCP, CRP and ESR is helpful for the confirmed diagnosis of RA (Shen *et al*., 2015). Patients that had positive RF and Anti-CCP more than with negative test results, recently the new criteria for diagnosis of RA have been introduced in addetion Anti-CCP, together with RF(Alethera *et al*., 2010). Thus, these parameters could be used as specific serologic markers for RA. A data suggested that Anti-CCP have the power to predict the development of RA in patients with early arthritis, and the possibility of future onset of RA in certain high–risk populations(Bizzaro *et al*., 2013). Forslind and his workers reported that Anti-CCP was a stronger marker in the diagnosis of RA disease (Forslind *et al*., 2004), and study investigated that the Anti-CCP antibody assay has a similar diagnostic sensitivity to that of RF in early RA, but is better as a predictor of the disease course over 3 years(Kastbom *et al*., 2004), and also agree with result reported that Anti-CCP conceder prognostic factors of rapidly progressive course and could be used for the disease prognosis at its early stage (Shilkina *et al*., 2011).In present study found the strength associations of Anti-CCP and RF, indeed, results of present study in antibody discordant patients suggest that RF may be more closely associated with increased level of Anti-CCP in RA patients, agree with study showed RF and Anti-CCP had highest autoantibody concentrations in patients with RA(Miriovsky *et al*., 2010). Level of Anti-CCP in early diagnosis RA treated group less than in new early diagnosis RA without treatment of present data, according to

other investigations study have shown that Anti-CCP and RF concentrations can be influenced by treatment(Bos *et al*., 2008). Anti-CCP was superior to other laboratory tests by ROC analysis. RF and Anti-CCP had higher sensitivity of 66.7%, 61.5%, respectively, therefore, Anti-CCP better for the diagnosis of early RA than RF. Anti-CCP was also reported to associate with the progression of joint damage and may be also used as a prognostic test (Hayashi *et al*., 2007).

Plasma level of 14-3-3-eta biomarker of RA patients higher than healthy controls, serum 14-3-3η, which was first described in 2007, data reported that 14-3-3-eta is expressed at higher levels than the other isoforms in patients of RA disease(Kilani *et al*., 2007). In present study of 103 plasma patients reported high significant of 14-3-3-eta between RF+ve and RF-ve subgroups within new early diagnosis without treatment group of RA patients, 14-3-3-eta protein complements to RF marker, so like which study demonstrated that 14-3-3η is an RA-specific marker that complements both RF and ACPA, increasing their diagnostic value(Marotta *et al*., 2011). Study by Maksymowych and his colleague who investigated whether 14-3-3η is associated with more severe disease in both early and established RA(Maksymowych *et al*., 2014). 14-3-3η is expressed at higher levels than the other isoforms in the synovial fluid of patients with arthritis and that these levels were three to five times higher than corresponding levels in the serum of matched donors, citing the joint as the likely source of serum 14-3-3η(Kilani *et al*., 2007). Serum 14-3-3η positivity is denoted by two studies(Marotta *et al*., 2011 and Maksymowych *et al*., 2011). Positive 14-3-3η status is also significantly associated with early RA at years 1, 3 and 5 indicating prognostic utility(Maksymowych and Marotta, 2014). Patients who reverted to negative 14-3-3-eta levels had better clinical response than patients who remained positive at 1 year or became positive (Hirata *et al*., 2015).

The sensitivity and specificity of 14-3-3-eta less than sensitivity and specificity of RF and Anti-CCP, present study results agree with research which showed that Anti-CCP has similar sensitivity to RF but better specificity (Steuer *et al*., 2008). Other research reported the rate of sensitivity 50% and specificity 100% for Anti-CCP reactivity for the diagnosis RA were measured in RA (Sedaroglu *et al*., 2008). Different studies for RF and Anti-CCP, reported pooled sensitivity for RF 69% and specificity 85%. Pooled sensitivity of Anti-CCP was estimated to be 67% and specificity 95% in RA patients. In 2014 recorded that 14-3-3-eta used a new ELISA based assay has diagnosis utility for RA with sensitivity of 63.6% and

specificity of 92.6% using the optimal cut-off from ROC analysis, adding 14-3-3-eta to Anti-CCP resulted in an identification rate of 72% compared to 59% for Anti-CCP alone, adding RF to Anti-CCP increased diagnostic capture from 59% to 72% and this increased further to 78% when 14-3-3-eta was added (Maksymowych and Marotta, 2014). In a project conducted on 619 subjects, 14-3-3-eta protein sensitivity and specificity for RA was 77% and 93%, respectively, in the early stages of the disease, the determination of protein 14-3-3-eta along with RF and Anti-CCP increases the diagnostic rate from 72%(RF + Anti-CCP)to 78% (RF + Anti-CCP + 14-3-3-eta) (Maksymowych *et al*., 2014). Results showed that Anti-CCP sensitivity and specificity of 54% and 96% or RF with 59% and 91%, respectively(Shi *et al*., 2015).

The association of body mass index (BMI) with biomarkers levels in RA patients, observed significant associations between measures of BMI significant with 14-3-3-eta in early RA patients of RF+/-. But not showed any correlation between BMI and others markers (CRP, ESR, Anti-CCP and RF). Jon and his co-workers reported significant associations between all measures of adiposity, particularly truncal fat, and CRP levels in women (Jon *et al*., 2008). Other study was observation that higher CRP or ESR association with a greater risk of weight loss, supports the hypothesis that a history of more severe disease and greater systemic inflammation in RA is an important factor in determining an individuals BMI, a CRP above the normal range (>1.1 mg/dl) was associated with about a 32% increased risk of significant weight loss, (Joshua *et al*., 2015) this study like the present study in BMI had negative correlation with CRP and ESR.

Results appeared correlation between (SDAI with ESR, CRP, Anti-CCP and RF), but no correlation with (14-3-3-eta), corresponds with other study which showed no correlation between 14-3-3-eta and the Disease Activity Score in 28 joints in early RA (Maksymowych *et al*., 2014). SDAI score were significantly higher (p= 0.002) across 14-3-3-eta positive patients (Carrier *et al*., 2016). Study observed that serum 14-3-3-eta did not correlate with disease activity (Maksymowych *et al*., 2014). Levels of 14-3-3-eta at baseline were associated with lower likelihoods of ever reaching SDAI remission(Carrier *et al*., 2016). Data recorded 14-3-3-eta positive patients had more severs disease before the initiation of treatment, when combined with C-reactive protein (CRP), 14-3-3-eta positivity added significantly and incrementally to the identification of patients with high disease activity, this association with disease activity is further corroborated by the fact that 88% of 14-3-3-eta

negative patients at 1year were in remission or in a low disease activity state compared with 66% of 14-3-3-eta positive patients(Hirata *et al.*, 2015).

The fact that Anti-CCP positive patients have a more severe disease course with greater joint destruction has also fueled the hypothesis that Anti-CCP themselves may be pathogenic and had positive correlation with SDAI(Toes and van der Woude., 2011). The high prevalence of Anti-CCP in RA patients with extensive disease activity and severe radiological change, and even more impressively in RA patients who are RF-ve, suggests that Anti-CCP is more useful than the RF alone in the early predication of disease outcome and disease activity (Serdaroglu *et al.*, 2008). Anti-CCP is good predictor of disease activity and better than RF in predicting disease activity over 3 years after the diagnosis of recent onset of RA (Kastbom *et al.*, 2004).Other study showed an association of RF and Anti-CCP with clinical signs of disease activity (Bas *et al.*, 2002 and Bas *et al.*, 2003). Opposite result in study that showed an interesting observation that RF correlate with the RA disease activity score, but Anti-CCP not correlated with disease activity (Egerer *et al.*, 2009). Approximately 60-70% patients with RA have a positive RF, which is predictive of disease severity, but an association was not found between disease activity and bone erosions with RF isotopes (Jonsson *et al.*, 1992). SDAI value are highly dependent on the patients pain perception and its effect by patients age, gender, disease duration, Anti-CCP and RF were inconclusive with respect to the value of the respective disease activity index.

CONCLUSION

Elevated level of 14-3-3-eta protein may induces factors associated with the pathogenesis of RA at concentrations found in patients with RA. Either RF or Anti-CCP antibody was positive in a considerable proportion of RA patients. Therefore, Anti-CCP antibody are important in the diagnosis of RF negative patients who present with clinical findings of RA, therefore, all three markers were very important for diagnosis of RA disease especially in early stage, and suggested that obesity may increase the risk of developing of RA. The SDAI is a valid and sensitive assessment of disease activity.

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