Evaluation of C- Reactive Protein (CRP) Titer in Patients with Acute Hepatitis A Virus Infection

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Abstract:-

This study was conducted from 5th September \2008 to 15th April \ 2009 . in Public Health Laboratory and learning Baquba Hospital to determine the C- reactive titer among patients with acute hepatitis A virus (HAV) infection in relation to healthy subjects . the study also aimed to correlate these titers with liver function test parameters and also to findout the validity of C- reactive protein titers as diagnostic or monitoring markers in patients with acute HAV infection .

The study was included 108 patients with HAV infection and 115 apparently healthy individuals were enrolled as a control group . Patient group involved 47(43.5%) female and 61(56.5%) male in age rang from (3month – 17) years . Control group include 66(57.4%) female and 49(42.6%) male in age range from (18 month – 20) years . Blood samples were collected . Sera were separated and stored in aliquts at (-20°C) till use .The diagnosis of acute HAV infection was based on detection of IgM HAV Antibodies by ELISA technique , whereas ,liver function tests were assessed by enzymatic biochemical procedures . Titration of C- Reactive protein was determine by semi – quantitative tube agglutination test . All data were statistically analyzed using computerized SPSS version 10 .The results showed that were a significant variation ($P< 0.001$) between patients with HAV infection (1:64) and control(1 : 2) when use (Mann – Whitney) test.Depending on 95% , the baseline titer of CRP in healthy controls was 1:4 (8 mg / L) . While inpatient with acute HAV infection was 1:512 (1024 mg / L) . The validity of CRP test at titer 1 :16 cut – off value (32 mg / L) was found highly sensitive (100%) and highly specific (97.3%) with test accuracy (98.6%) for differentiating between healthy controls and patients with HAV infection when the clinical suspicion is 50% .The results revealed that the CRP titers were significantly correlated with liver function test parameters . Suggesting that CRP titration could be used as a surrogate marker in prediction of acute HAV infection beside the clinical picture .

Introduction:-

C- reactive protein (CRP) is an acute phase protein produced by hepatocytes predominantly under control of Interleukin 6 (IL – 6) in response to inflammation and infection (1) . It composed with cytokines and complement proteins , the soluble humeral immunity elements [2] .Serum CRP concentration in acute response '

Keyword : Hepatitis A virus , C- reactive protein , liver fuction test

increased through (6) hours from (5 mg /L) to reach optimal concentration in (48) hours exceeding 500 mg / L [3] .

Plasma half life of protein is (19) hours which are stable under different healthy and pathology condition . therefore , it suggested that the sole determinant of intensity of the pathological processes stimulating CRP production [4.5] .

During last decades , the measurement of CRP concentration was use full in clinical setting , including monitoring infections and postoperative complications and assessing effectiveness of treatments on the course [6,7] .

Hepatitis A is an acute infections disease of the liver caused by the hepatitis A virus [8] , which is most commonly transmatted by the fecal – oral route via contaminated food or drinking water every year , approximately (10) million people worldwide are infected with the virus [9.10] . In Iraq , sera prevalence of hepatitis A in healthy individuals was (95%) [11] , therefore , the study was aimed to evaluate the CRP titers in patients with HAV infection and find the correlation between CRP and liver function test and also to find out the validity of CRP titers as diagnostic or monitoring marker in patients with acute HAV infection .

Materials and methods

This study was conducted from 5th September 2008 to 15th April 2009 , in public Health Laboratory and Baquba Learning Hospitals , to evaluate CRP titer among patients with acute hepatitis A infection and find the correlation between CRP and liver function test .

One hundred and eight patients with acute HAV infection and 115 apparently healthy individuals as a control group were collected by simple random technique . The patients inclued 47 (43.5%) female and 61 (56.5%) males with ages range from (3 months – 17) years . The control group include 66 (57.4%) females and 49 (42.6) males with age rang from (8 month – 20) years . Blood samples were collected , Sera were separated and stored in aliquts at -20°C till use . The diagnosis of acute HAV infection was based on detection of IgM HAV antibodies using ELSA technique according to (Purcell etal ., 1976) [12] . liver function test was assessed by enzymatic .

Biochemical procedures

1.Serum total and direct bilirubin according to [13].
2.Serum Alkaline phosphatase test according to., [14] .
3.Serum alanine aminotransferase test according to [15] .
4.Serum aspartate aminotransferase test according to [16] .
5.Serum total protein concentration according to [17] .

Determination of CRP titer was assessed by semi – quantitative tube agglutination test . according to [18] .

All data were statically analyzed using computerized SPSS version 10 .

Results :-

Table (1) showed that 53 (46%) of the healthy control had the lowest CRP titer (zero) while the highest titer was found in 3(2.6%) . The 95% percentile of CRP titer was 1:4 . In patients , the Lowest titer 1:16 was found in 5 (4.6 %) and the highest titer 1:1024 was found in 2 (1.8%) . The 95% percentile of CRP titer was 1:512 .

Table (1) : C- reactive protein titer in patients with acute HAV infection and controls

CRP titer	Healthy control		Patients with acute HAV	
	No	%	No	%
0	53	46		
2	26	22.6		
4	31	27		
5	2	1.8		
16	3	2.6	5	4.6
32	0	0	24	22.2
64	0	0	30	37.7
125	0	0	23	21.2
256	0	0	14	12.9
512	0	0	10	9.2
1024	0	0	2	1.8
Total	115	100%	108	100%
95% percentile	110 (95.6)		106 (98.1)	

Table (2) : showed the range , median and interquartile range of CRP titer in the study groups . The statistical analyses (Mann- Whitney test) revealed a significant difference in the median of CRP titer between the two study groups

Table (2) Range median and Interquartile rang in study groups

CRP titer	Healthy control	Patient group
Range	0-16	16-1024
Median	2	64
Interquartiler range	0- 8	32- 256

P (Mann – Whitney) <0.001

Table (3) showed the validity of CRP titer 1:4 as a cut - off value to differentiate between healthy control and patients with acute HAV infection when clinical suspicion was 50% . The results showed that all patients give a titer 1:4 and more , whereas ., 79 of the healthy . control gives a titer (< 1:4) and 36 of them gives a titer 1:4 and more . The statistical analysis showed a significant difference between the two groups (p< 0.001). The sensitivity and specificity of the test were 100% and 68.6% respectively . The positive and negative predictive values were 75% and 100% respectively . The test accuracy was 83.8 % . The false positive and negative were 31.4 % and 0% respectively .

Table (3) : Validy of CRP titer at 1:4 as acute – off value

CRP titer at acute –off 1:4	Healthy control	Patient with acute HAV	Pvalue
Negative <1:4	**79**	**0**	**<0.001**
Positive 1:4 and more	**36**	**108**	
Total	**115**	**108**	

Sensitivity = 100%
Specificity = 68.6%
Positive predictive value = 75%
Negative predictive value = 100%
Accuracy = 83.8
False positive = 31.4
False negative = 0

The validy of CRP titer at 1:16 as a cute – off value revealed that 112 of the healthy control gives a titer (<1:16) , while remaining 3 gives 1:16 and more . On the other hand , all patients gives a titer 1:16 and more . There was statistically significant difference between the two groups (p<0.001) . The sensitivity and specificity were 100% and 97.3% respectively . The positive and negative predictive value were 97.2% and 100% respectively . The accuracy was 98.6% .

The false positive and false negative were 2.7% and 0% respectively , Table (4) .

Table (4) : The validy of CRP titer at 1:16 as a cute – off value

CRP titer s at a cute –off 16	Healthy control	Patients	Pvalue
Negative <1:16	**112**	**0**	**<0.001**
Positive 1:16 and more	**3**	**108**	
Total	**115**	**108**	

Sensitivity = 100%
Specificity = 97.3%
Positive predictive value = 97.2
Negative predictive value = 100%
Accuracy = 98.6%
False positive = 2.7%
False negative = 0 %

Table (5) : Shown that there was no significant correlation between the CRP titer and liver function tests (Total ,direct and indirect serum bilirubin , ALT , AST , Total serum protein and serum alkaline posphatase) in healthy group as assessed by Spearman s Linear correlation , However , the CRP titer was highly correlated with liver function tests in patients group .

Table (5) : Spearman's linear correlation between CRP and liver fuction test

Liver function tests	Spearman's linear correlation			
	Healthy control		Acute hepatitis A	
	r	P value	r	P value
S. Alkaline phohatase	-0.08	0.5 [N5]	0.29	0.019
S. AST	-0.09	0.34 [N5]	0.47	<0.001
S.ALT	-0.04	0.69 [N5]	0.46	<0.001
Total serum protein	-0.01	0.93 [N5]	0.62	<0.001
Total serum brlirubin	-0.06	0.53 [N5]	0.59	<0.001
Indirect serum bilirubin	-0.06	0.51 [N5]	0.44	<0.001
Direcl serum bilirubin	-0.03	0.79 [N5]	0.51	<0.001

R= Correlation coefficient
NS = None – significant
P = Probability

Discussion:-

Measurement of C- reactive protein (CRP) , the classical acute phase protein is an extremely valuable markers of disease and response to therapy in a wide rang of tissue – damaging inflammatory , infective and neoplastic conditions [19] . In the body , CRP plays the important role of interacting with the complement system , an immunologic defense mechanism [20] , [21] .

Hepatitis A virus infection is the most common cause of acute viral hepatitis [22] . hepatitis A virus infection is endemic in Iraq [11] , [23] .

The serum CRP concentration in healthy subjects obtained in the present study was 1:4 (8mg\L) in 95^{th} % . It agree with a study show that the median value was (10 mg\L) at 99 percentile in healthy donors [24] . and agree with a study conducted in Ramady city on patient with myocardial infraction who found that CRP concentration in healthy subject was (6 mg / L) [25] . and agree with Hutchinson etal ., (2000) study who found that the median CRP values in adult general population doubled with age from (1 mg / L) in youngest decade to (2 mg /L) in the oldest and tended to be higher in females [26] .

The relatively increase in CRP concentration may be due to the presence of asymptomatic infection and / or non – infections disease . Besides , the sample size and gender of subjects in the sample and sensitivity of technique that use in determination of CRP titer [27] . While , there was a significant increase in serum CRP concentration in patients with acute HAV infection . Unfortunately , there was no studies about CRP titer in patient severed from a acute HAV infection . But our study approach to other studies on infection disease that show a significant increase in CRP concentration reach to (> 30-35 mg /L) in 80-85% at acute bacterial infection and (> 20 mg /L) in viral infection [28] . While Rajs etal .,(2002) find that the mean CRP concentration in blood was (163±53) mg \Lin patient with gram – positive bacterial meningitis and (272± 51) mg \L in patient with gram – negative bacterial meningitis [29] . and agree with Jang etal ., (2004) study who assured that CRP concentration increased about 93.1% than in healthy subjects in patient with sever acute respiratory syndrome (SARS) [30] . These studies recruited our study , especially when HAV infection is one of viral infection . This significant increase in

CRP concentration in patient with acute HAV infection in relation to healthy subjects may be due to the damage of hepatocytes as a result of direct viral replication especially in periportal inflammation zone with severe changes in centre lobular cause necrosis in CRP concentration in these area . [31], [32] . and indirectly through induction of cytotoxic T- cell response that further destroy liver infected cells [33] , [34] .

The result also revealed that the CRP titer 1:4 as a cut-off value (Table 3) found to be highly sensitive and reasonably specificity between healthy controls and patients with acute HAV . In opposite , the CRP titer 1:16 as cut- off value (table 4) obtained highly specific predictor for the diagnosis of acute HAV infection . No previous studies had been found in the literature regarding the utility of serum CRP concentration in the predictive diagnosis of hepatitis A virus infection – However , our results were consistent with those obtained by utilization of CRP concentration in the predictive diagnosis of other infections disease such as community – aquired pneumoniae which gave on excellent parameters of sensitivity , specificity and predictive values for diagnosis of the disease [35] . Also seem to be a valuable parameter in the early diagnosis of pediatric infection [36] . Besides , this study (Manian , 1995) [37] assured that CRP concentration elevated to (> 200mg /L) in the 2 day of infection with febrile neutropania . On other hand , many studies on non – infections disease showed that CRP concentration was valuable predictor in diagnosis of cardiovascular disease , diabetes mellitus and hyperlipidemia [3] ,[39] .

The significant linear correlation between the CRP concentration and values of liver function tests in patient with acute HAV infection (Table 5) , approximate to a study about the association between elevated liver enzyme and higher C- reactive protein concentration in patient with metabolic syndrome [40] . this correlation suggested that CRP titration could be used as a surrogate marker in prediction of acute HAV infection beside the clinical picture .

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تقييم عيارية بروتين (C) المنشط في المرضى المصابين بالتهاب الكبد الفيروسي نمط (A) الحاد

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الخلاصة:-

اجريت هذه الدراسة للمدة من ٥\ ايلول \٢٠٠٨ الى ١٥ من نيسان ٢٠٠٩ في مختبر الصحة العامة في بعقوبة ومستشفى بعقوبة التعليمي بهدف تقييم عيارية بروتين (C) المنشط بين مرضى التهاب الكبد الفيروسي نمط (A) الحاد بالمقارنة مع الاشخاص الاصحاء ، وكذلك لاستبيان العلاقة بين عيارية بروتين (C) المنشط وفحوصات وظائف الكبد ومن ثم معرفة صلاحية استخدام عيارية بروتين (C) دليلاً بديلاً في تشخيص التنبؤي للمرض . شملت الدراسة ١٠٨ مريضاً مصاباً بالتهاب الكبد الفيروسي نمط (A) الحاد و١١٥ شخصاً من الاصحاء ظاهرياً كمجموعة سيطرة تضمنت مجموعة المرضى ٤٧ (٤٣,٥%) اناث و ٦١ (٥٦,٥%) ذكور باعمار تتراوح بين (٣ اشهر – ١٧ سنة) . اما مجموعة السيطرة ، فتألفت من ٦٦ (٥٧,٤%) اناث و ٤٩ (٤٢,٦%) ذكور وباعمار تتراوح بين (٨ اشهر – ٢٠ سنة) جمعت نماذج الدم وتم فصل الامصال وتجزئتها في انابيب صغيرة وحفظت في درجة حرارة (-٢٠)ْم . تشخيص حالات التهاب الكبد الفيروسي نمط (A) الحاد ، اعتمدت على وجود الاضداد النوعية للفايروس صنف IgM (IgM HAV antibodies) باستخدام تقنية الاليزا . بينما اجريت فحوصات وظائف الكبد بالطرق الكيموحيوية المعتمدة الفعالية الانزيمية . تم قياس عيارية بروتين (C) المنشط بطريقة التلازن شبه الكمية . جمعت البيانات وحللت احصائياً باستخدام برامج المرحلة العاشرة بالكمبيوتر (SPSS verion 10).اعتماداً على نسبة ٩٥% فان المستوى الاساسي لعيارية بروتين (C) المنشط بين الاشخاص الاصحاء كان ١:٤ (٨ ملغرام \ لتر) وبين المرضى ١:٥١٢ (١٠٢٤ ملغرام \لتر) .

اظهرت النتائج ان صلاحية عيارية بروتين (C) المنشط عندالقيمة الفاصلة ١:١٦ للتنبؤ بالتهاب الكبد الفيروسي نمط (A) الحاد اعطت حساسية ١٠٠% وخصوصية ٩٧,٣% ونسبة صواب ٩٨,٦% . فضلاً عن ذلك ، فقد اظهرت النتائج وجود ترابط معنوي احصائياً بين عيارية بروتين (C) المنشط وبين قيم فحوص وظائف الكبد .عيارية بروتين (C) المنشط يمكن ان تكون ذا قيمة في التشخيص التنبؤي لالتهاب الكبد الفيروسي نمط (A) الحاد عندما يشكل التشخيص السريري ٥٠%