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ROLE OF PROTEIN KINASE R IN INDUCING DNA DAMAGE IN HCV PATIENTS

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The hepatitis C virus (HCV) may be a major blood borne pathogen of human. There are approximately 120-130 million or 3% of the total world population that are infected with HCV. According to World Health Organization (WHO), there are approximately three to four million new cases of infection every year (Mohd *et al*., 2013). HCV is considered a major community health issue, since the virus is the etiological reason of chronic hepatitis that recurrently evolvement to a cirrhosis and hepatocellular carcinoma (HCC) (Hauri *et al*., 2004). Protein kinase RNA regulating (*PKR*) is a double stranded RNA (ds-RNA) stimulated protein kinase that stimulate cellular apoptosis pathways (Deb *et al.,* 2001; Onuki *et al*., 2004). *PKR* is present in cell as inactive form and exchange to active state by very low concentration of ds-RNAs. Most natural

ds-RNA activators of PKR are created in cell infected with virus as viral replication or transcription products (Terada *et al*., 2004; Garcia *et al*., 2006). PKR is an integral part of mechanism of human innate immune reaction, which is the cell's first line of defense against viral infection (Toroney *et al*., 2010). The molecular mechanisms regulating function of *PKR* in normally cells divided are basically anonymous. PKR is associated with the control of HCV replication (Gale and Foy, 2005). Particular viruses such as hepatitis viruses progress several strategies to *PKR* down-regulation and overawed the host defense mechanism against virus replication (Yan *et al*., 2007). Many DNA tumour viruses promote cellular revolution through inactivating the vital tumor suppressor protein *p53***.** In dissimilarity, it is not known *p53* function is interrupted by HCV, unique, RNA virus oncogene that is the foremost infectious foundation of liver cancer in many world regions (Mitchell *et al*., 2017). *PKR* is *a p53* object gene and shows a vital portion in the tumor-suppressor purpose of *p53*. Stimulation of *p53* by geno-toxic stress changes a significant level of *PKR* expression, resulting in translational inhibition and cell apoptosis. *P53* stimulation convinced by DNA damage assists cell apoptosis by stimulating *PKR* (Agami and Bernards, 2000).

This study aim was to quantify *PKR* and *P53* genes expression in chronic HCV infected patients and indicate the vital significance of these genes in the HCV disease progression.

MATERIALS AND METHODS

Patients

This study was conducted on 50 cases that divided into two groups as follows Group one (GI): included 30 patients with chronic HCV and positive PCR collected from AL-Menoufya liver institutes. Group two (GII): included 20 normal, completely healthy patients considered as the control, they were selected from outpatient clinics of internal department, AL-Menoufya University Hospital. All cases were collected in the period from April to September 2019.

The inclusion criteria of all patients' samples were HCV antibody positive using a second-generation enzyme-linked immune-sorbent assay (ELISA) and positive serum HCV RNA by quantitative polymerase chain reaction (PCR); not under medication and were negative hepatitis B virus infection. Beside investigations needed to fulfill the selection criteria, all cases included in this study were exposed to the following:

1. Full history and clinical examination

2. For complete blood cell count (CBC) and molecular categorization of *PKR* and *P53* genes expression, whole blood sample from each case were used.

3. Serum samples were separated; where one was used for testing liver functions; Alfa Feto-protein (AFP) level and

hepatitis markers which were done using COBAS INTEGRA 800 chemistry auto analyzer (Roche Diagnostics Ltd., CH-6343 Rotkreuz, Switzerland) and therefore the emain aliquots serum sample were stored at -80°C.

4. Quantification of *PKR*-RNA and *P53*-RNA genes was finished using RT- PCR supported SYPR GREEN after mRNA extraction from blood fresh sample. Quantification of mRNA was designed by using the arithmetic formula: ($2^{-\Delta ct.}$), during which ΔCT is the difference between the CT of a given objective complementary DNA (cDNA) and an endogenous reference cDNA. Thus, this value yields the amount of the normalized objective to an endogenous reference.

- ELISA for HBcAb and HBsAg was finished for all cases to exclude the presence of hepatitis B viral etiology of liver disease.

Statistical analysis

Statistical analysis was completed by means of the Statistical Package for the Social Sciences (SPSS software version 25, Chicago, Illinois). The devices recycled for statistical comparisons were analyzed by the Student's t-test for parametric data and Mann-Whitney test for non-parametric data. Regression and correlation were finished by spearman's coefficient method. Receiver Operating Characteristic (ROC) curve was used for detecting the cut off value, Kappa agreement to test the agreement. The cut-off value for significance was at a P-value less than 0.05.

RESULTS AND DISCUSSION

This study was conducted on the samples from 50 patients who were categorized into two groups. The age of the studied testers ranged from 21-75 years with a mean age of 47.8±14.3. Sex distribution in studied patients (GI) was 11 (36.7%) females and 19 (63.3%) males, while in the control cases (GII) number of females was six (30%) and number of males was 14 (70%). No significant difference was observed between the two studied groups as regards the distribution of age and sex.

There wasn't significant difference between the two studied groups regards the CBC results (Table 1), this approve with (El-dahshan *et al*., 2018). But the other parameters such as alkaline phosphatase (ALP); Gamma-glutamyl transferase (GGT) and also AFP showed highly significant difference between the two groups (Table 2). This disagreed with McPherson *et al*., (2011).This may due to the difference in the HCV genotype while they used samples collected from patients infected with HCV genotype 1 and other infected with HCV genotype 3. While There is a highly significant difference in levels of ALP in GI compared with G2 (P-value less than 0.001), while the value of ALP concentration in GI ranged from 34-70 IU/L with mean ±SD (52.4±9.15) but it ranged from 25-45 IU/L with mean ±SD (31.6±6.05) in group II.

Furthermore a highly significant

difference in GGT levels was observed in GI compared with GII (P-value less than 0.001), while the median value of GGT concentration was 50 IU/L with Inter-Quartile range (IQR) of 45-75 IU/L in group I, but it was 31.5 IU/L with IQR of 25-35 IU/L in group II.

The median value of serum level of AFP was 3.0 ng/ml with IQR of 2.20-9.00 ng/ml in group I but it was 0.07 mg/dl with IQR of 0.01-0.20 ng/ml in group II.

The liver functions show highly significant difference in G1 compared with G2 that was shown in Table (3). This disagreed with El-dahshan *et al.,* (2018), who compared between 2 groups infected with chronic HCV before treatment and during follow up. The results before treatment showed no significant difference but the results during follow up showed significant value for several parameters (p value <0.001), namely Alanine aminotransferase (ALT), but different parameters showed no significant difference such as Aspartate aminotransferase (AST); total bilirubin (T BIL), direct bilirubin (D BIL), albumin (ALB) and AFP. In all HCV cases compared with the controls, very highly significant difference was observed in levels of T BIL (p-value less than 0.001) but ALB level show significant result (p equal to 0.007).

A highly significant difference in levels of ALT in group I was observed as compared with group II (P-value less than 0.001), while in group I the median value of ALT concentration was 55.0 IU/L with (IQR) of 45.0-65.0 IU/L, but it was 24.00 IU/L with IQR of 20.0-25.0 IU/L in group II.

Moreover a highly significant difference in median AST levels was observed in G1 compared with G2 (P-value less than 0.001),while the median value of AST concentration was 55.0 IU/L with IQR of 48.0-65.0 IU/L in GI, but it was 23.00 IU/L with IQR of 20-25 IU/L in GII (Table 3).

In group I, the median value of T BIL was 1.15 mg/dl with IQR of 1.0-1.3 mg/dl, and it was 0.4 mg/dl with IQR of 0.3-0.55 mg/dl in group II.

Also a significant elevated level of ALB in G1 as compared with G2 was observed but not as in other liver functions, in which the mean ±SD of ALB concentration in G1 was 3.54±0.73, while in G2 mean ±SD was 3.96±0.28.

No significant differences were observed between the two studied patients for *p53* gene expression. This finding was concordant to that found by Loguercio *et al.,* (2003).

No significant differences were observed between the two studied groups according to *PKR* gene expression (p value 0.094) as that showed in Table (5). This is in concordance with other study (Chen *et al*., 2004).

When correlation occurred between two markers and different parameters in two groups, the

values showed that ALB was high significantly correlated with two markers (*PKR* and *P53*) (p value <0.001*).

There was a negative correlation between *PKR* gene expression and *P53* gene expression and other parameters (Age, ALT, AST, BIL, AFP, PLT, Hemoglobin Hb, GGT and ALP) (p value ≥0.05) (Table 6), this finding agreed with Mohamed *et al.,* (2012). But this differs from that the recent studies observed that a positive correlation was found between AST but not ALT and the degree of inflammation in chronic hepatitis patients. This difference is due to the degree of inflammatory activity, while higher AST level was related with higher grades histology activity index (HAI) of HCV related chronic hepatitis (Hung *et al.,* 2008).

The results revealed that the significance of *PKR* wasn't detected in chronic HCV patients. Others recounted a significant increase after the HCC progression indicating the involvement of HCV in the process of hepato-carcinogenesis (De-Mitri *et al.,* 2007; Koike 2007).

There was a negative relationship between *PKR* gene expression for predict cases and the control. ROC curve appeared in Fig. (1).

Also there was a significant negative correlation between *P53* gene expression for predict cases and the control. ROC curve appeared in Fig. (2). *P53* considered critical in cell-cycle arrest and apoptosis after DNA damage; alterations in its function may accelerate the progression from chronic liver disease to HCC (Kumar *et al*., 2011). The infection with HCV may cause the loss of normal *p53*-mediated DNA damage responses and may have relevance to HCC origination in persons infected with chronic HCV. But this was dependent on the viral impact and also *PKR* expression, this was agreement with Mitchell *et al*., (2017).

Though, there wasn't a significantly *PKR* and *p53* genes expression in patients with chronic liver disease and normal cases in this current study. But Fig. (3) show highly sensitivity for the two genes level.

Moreover, there was a sensitive cut-off for *PKR* level which can be used in exclusion of HCV patients into low risk and high risk groups for progress of tumour. Some authors support our findings as they suggested that *PKR* was not acting as a classical tumour suppressor protein but a potential growth stimulus (Hiasa *et al.,* 2003**).**

Furthermore other studies revealed that expression of *PKR* gene was lower in HCC cases with HBV than in HCV infection (Tamada *et al*., 2002). These conclusions may reflect the difference in viral effect on *PKR* gene expression and recommend that *PKR* might have a tumour promoting action in some cancer cells.

SUMMERY

PKR is a *p53* target gene and acts an imperative role in the tumor-suppressor role of *p53*. Initiation of *p53* by genotoxic tension prompts a significant level of *PKR* expression, that ensuing in translational embarrassment and cell apoptosis. The current study aims to evaluate prognostic influence of *PKR* gene expression in chronic HCV patients; Correlate *PKR* and *P53* gene expression to liver function tests and also shows the role of *PKR* in patients infected with HCV. We concluded that *PKR* was established to be an independent prognostic issue indicating the vital biological significance of this gene in the HCV disease process. In spite of the restriction of this study related to sample size, it paved the way for further future studies using more samples. Further investigations on a larger scale *via* well-standardized performances and more samples are recommended to validate these results of ROC curve or define a suitable one.

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Table (1): Comparison between the two studied groups according to CBC.

	Patients (n = 30)	Normal (n = 20)	p
PLT($\times 10^3$) Min. – Max.	120.0 – 460.0	160.0 – 340.0	0.211
Median (IQR)	222.50(190.0 – 320.0)	194.0(185.0 – 226.50)	
Hb Min. – Max.	9.90 – 16.0	10.40 – 16.0	0.338
Mean ± SD.	13.41 ± 1.81	13.94 ± 1.95	
TLC ($\times 10^3$) Min. – Max.	4.0 – 9.0	4.0 – 9.0	0.658
Mean ± SD.	6.60 ± 1.59	6.40 ± 1.50	

P: p value for relating between the studied groups. PLT: platelets;

Hb: Hemoglobin; TLC: T lymphocyte cell.

Table (2): Comparison between the two studied groups according to different parameters.

	Patients (n = 30)	Normal (n = 20)	Test of Sig.	P
ALP Min. – Max.	34.0 – 70.0	25.0 – 45.0	t=8.931*	<0.001*
Mean ± SD.	52.40 ± 9.15	31.60 ± 6.05		
Median (IQR)	50.0(45.0 – 59.0)	30.0(26.50 – 34.50)		
AFP Min. – Max.	1.0 – 15.0	0.01 – 0.40	U=0.0*	<0.001*
Mean ± SD.	4.91 ± 4.08	0.10 ± 0.11		
Median (IQR)	3.0(2.20 – 9.0)	0.07(0.01 – 0.20)		
GGT Min. – Max.	35.0 – 194.0	20.0 – 45.0	U=22.0*	<0.001*
Mean ± SD.	64.23 ± 33.94	32.15 ± 7.33		
Median (IQR)	50.0(45.0 – 75.0)	31.50(25.0 – 35.0)		

t: Student t-test. U: Mann Whitney test.

P; p value for relating between the studied groups. *: Statistically significant at $p \leq 0.05$.

Table (3): Comparison between the two studied groups according to liver functions.

	Patients (n = 30)	Normal (n = 20)	p
Direct bilirubin Min. – Max. Median (IQR)	0.20 – 2.20 0.50(0.40 – 0.80)	0.03 – 0.30 0.10(0.10 – 0.20)	<0.001*
Total bilirubin Min. – Max. Median (IQR)	0.80 – 3.0 1.15(1.0 – 1.30)	0.10 – 0.90 0.40(0.30 – 0.55)	<0.001*
ALB Min. – Max. Mean ± SD.	2.10 – 4.90 3.54 ± 0.73	3.50 – 4.40 3.96 ± 0.28	0.007*
AST Min. – Max. Median (IQR)	35.0 – 90.0 55.0(48.0 – 65.0)	16.0 – 40.0 23.0(20.0 – 25.0)	<0.001*
ALT Min. – Max. Mean ± SD.	35.0 – 89.0 57.07 ± 14.20	16.0 – 34.0 23.50 ± 4.78	<0.001*

P: p value for relating between the studied groups; *: Statistically significant at $p \leq 0.05$.

Table (4): Comparison between the two studied groups according to *P53* gene.

P53	Patients (n = 30)	Normal (n = 20)	U	p
Min. – Max.	0.13 – 1.87	0.05 – 5.66		
Mean ± SD.	0.59 ± 0.46	1.11 ± 1.29	217.0	0.100
Median (IQR)	0.44(0.22 – 0.81)	0.71(0.47 – 1.23)		

U: Mann Whitney test. P: p value for matching between the studied groups.

Table (5): Comparison between the two studied groups according to *PKR* gene.

PKR	Patients (n = 30)	Normal (n = 20)	U	p
Min. – Max.	0.08 – 2.14	0.0 – 2.0		
Mean ± SD.	0.66 ± 0.52	0.48 ± 0.56	215.50	0.094
Median (IQR)	0.56(0.25 – 1.0)	0.32(0.02 – 0.71)		

U: Mann Whitney test. P: p value for relating between the studied groups.

Table (6): Correlation between the two markers (*PKR* and *P53*) and different parameters in patients (n= 30).

	PKR		P53	
	r_s	p	r_s	P
Age (years)	0.288	0.123	0.334	0.071
PLT	0.077	0.685	0.195	0.302
Hb	-0.200	0.289	-0.158	0.404
TLC	0.058	0.761	0.167	0.378
ALP	0.181	0.337	0.068	0.720
AFP	0.332	0.073	0.253	0.177
GGT	0.138	0.468	-0.076	0.688
Direct bilirubin	0.234	0.213	0.044	0.816
Total bilirubin	0.105	0.580	-0.222	0.238
ALB	-0.675	<0.001*	-0.639	<0.001*
AST	0.208	0.270	0.166	0.381
ALT	0.348	0.059	0.230	0.222

r_s: Spearman coefficient; n: number of patients; *: Statistically significant at $p \leq 0.05$.

Table (7): Agreement (sensitivity, specificity) for *PKR* to predict cases (vs control)

	AUC	P	95% C.I	Cut off	Sensitivity	Specificity	PPV	NPV
PKR	0.641	0.094	76 – 0.806	0.125	90.0	35.0	67.50	70.0

AUC: Area Under a Curve; p value: Probability value; CI: Confidence Intervals;
NPV: value of Negative predictive PPV: value of Positive predictive.

Table (8): Agreement (sensitivity, specificity) for *P53* to predict cases (vs control)

	AUC	P	95% C.I	Cut off	Sensitivity	Specificity	PPV	NPV
P53	0.638	0.100	0.471–0.806	≤1.072	90.0	40.0	69.2	72.7

AUC: Area Under a Curve; p value: Probability value; CI: Confidence Intervals;
NPV: value of Negative predictive PPV: value of Positive predictive.

Table (9): Agreement (sensitivity, specificity) for *P53* and *PKR* to predict cases (vs control).

	AUC	P	95% C.I	Sensitivity	Specificity	PPV	NPV
P53+ PKR	0.635	0.109	0.462 – 0.808	93.33	50.0	73.7	83.3

AUC: Area Under a Curve; p value: Probability value; CI: Confidence Intervals;
NPV: value of Negative predictive PPV: value of Positive predictive.

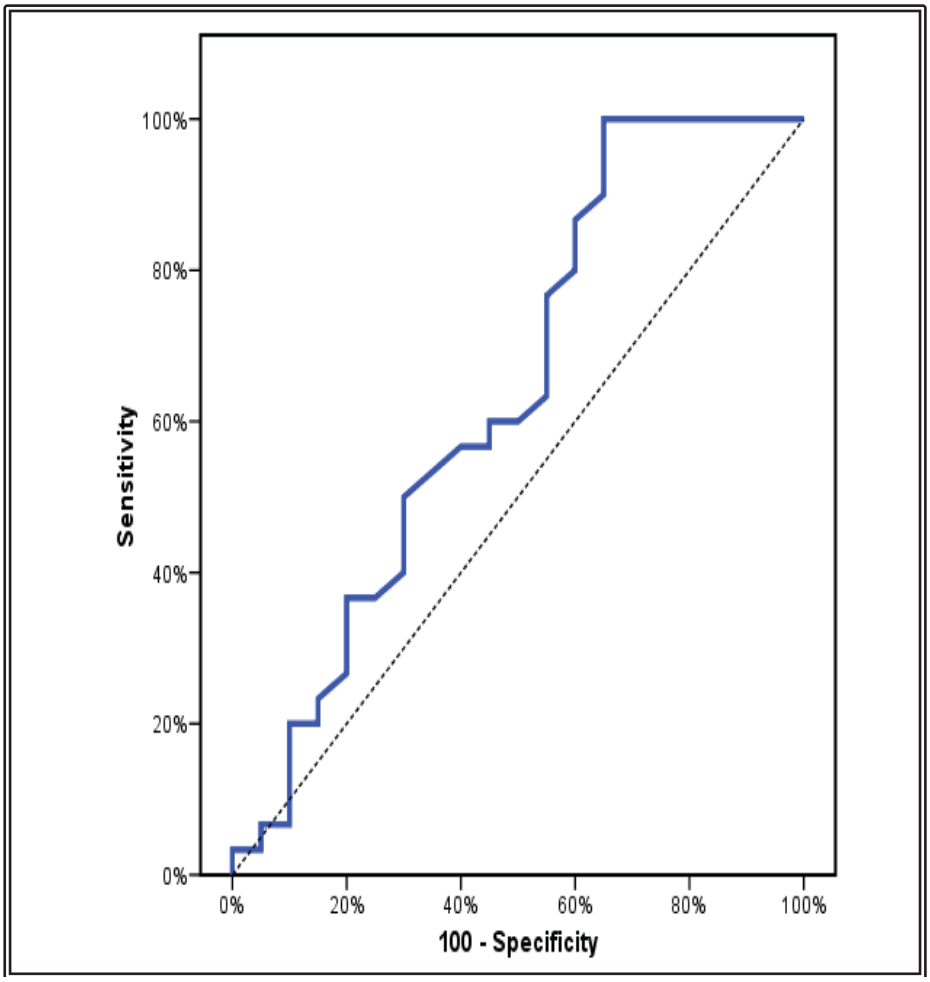

Fig. (1): ROC curve for *PKR* gene expression to predict cases (vs control).

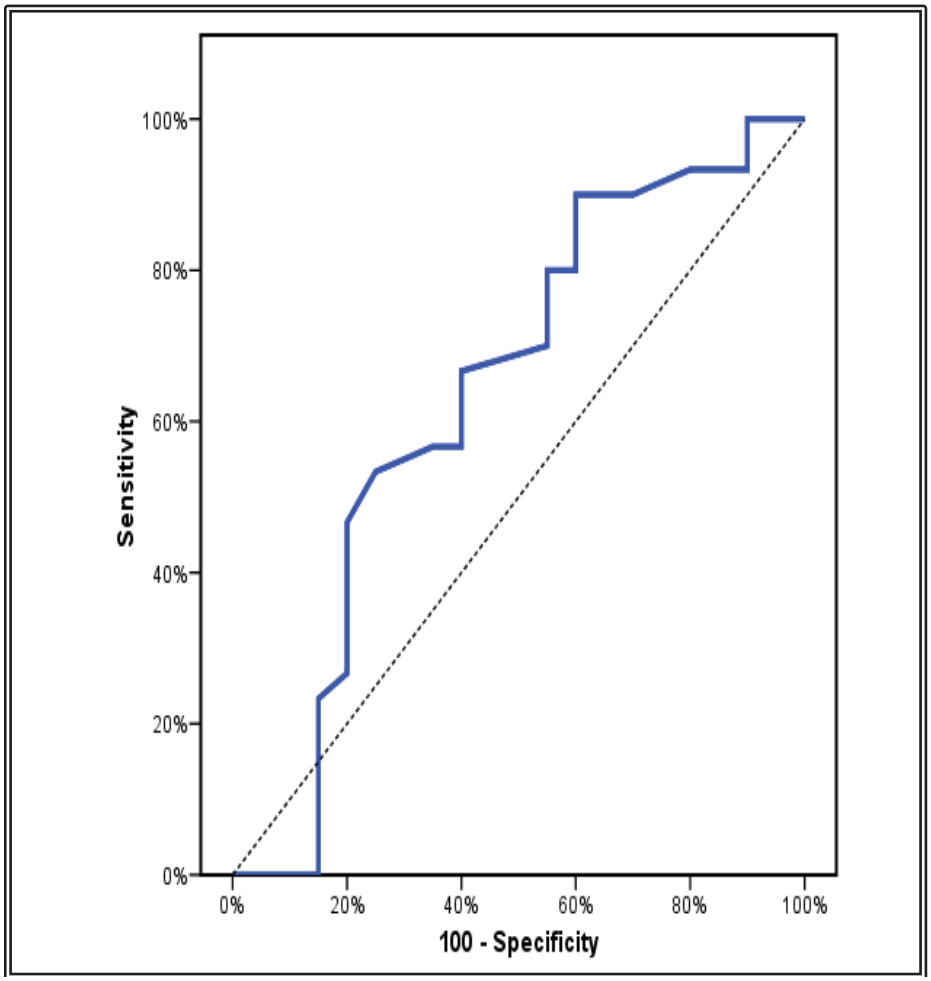

Fig. (2): ROC curve for *P53* gene expression to predict cases (vs control).

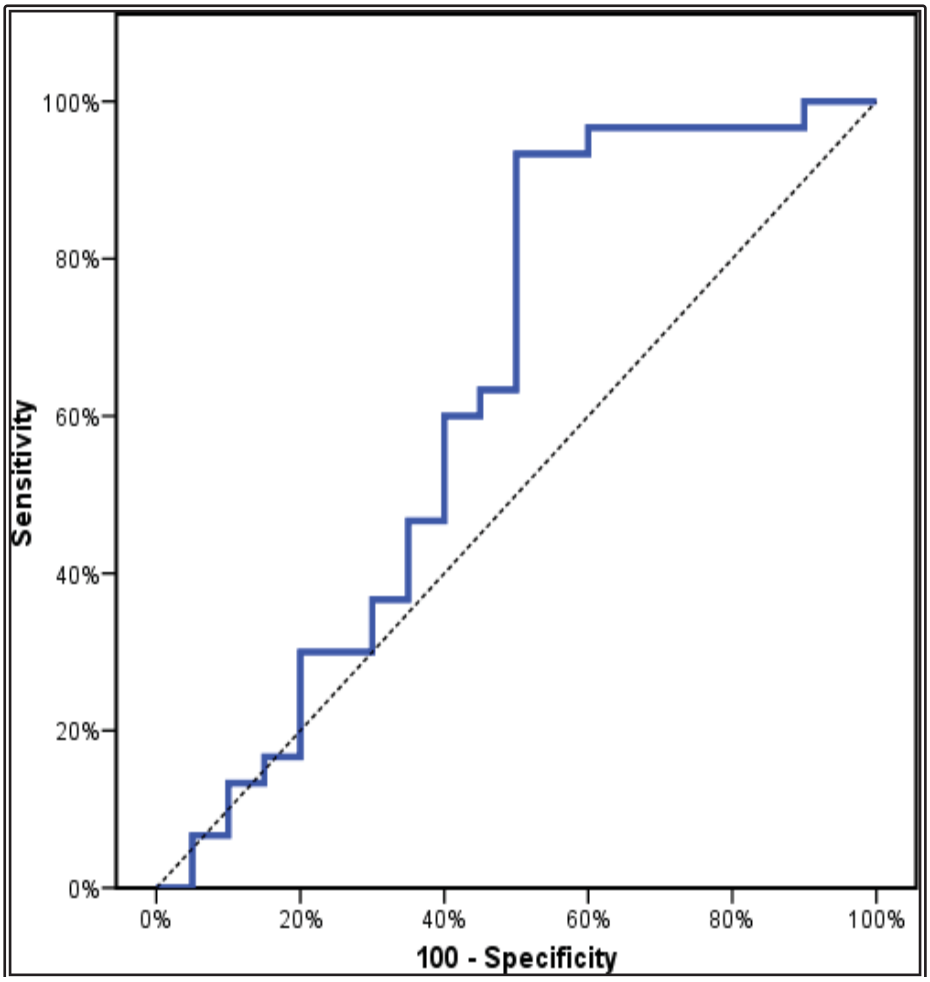

Fig. (3): ROC curve for *P53* and *PKR* genes expression to predict cases vs. control.