Dickkopf-1: As a Diagnostic and Prognostic Serum Marker for Hepatocellular Carcinoma

Ahmed L Sharaf[1], El-Said G El-Badrawy[1], Naglaa A Khalifa[2], Sameh M Abdel Monem[1], Hosam M Dawod[1]
[1]*Tropical Medicine Department, Faculty of Medicine, Zagazig University, Egypt*
[2]*Clinical Pathology Department, Faculty of Medicine, Zagazig University, Egypt*

Corresponding Author
Ahmed L Sharaf

Mobile:
+201003584402

E mail:
Drahmed_lotfy@hotmail.com

Key words:
Dickkopf-1, alpha-feto protein, hepatocellular carcinoma

Background and study aim: Hepatocellular carcinoma (HCC) accounts for 70 - 80% of all liver cancers and the 5-year survival is only 3 - 5%. This bad prognosis is due to the lack of an effective method for early diagnosis. So, only 30 - 40% of patients with HCC are suitable for curative treatments at the time of diagnosis. Thus, there is a great need for tools to diagnose HCC early especially in cirrhotic patients. The aim of this work is to assess the validity of serum DKK1 as a diagnostic marker for HCC and to assess prognostic value of serum DKK1 in predicting treatment response, complication and survival in HCC patients.

Patients and Methods: This study included 60 Patients divided into two groups. Group A: consisted of 30 patients with post hepatitic C and/or B liver cirrhosis. Group B: consisted of 30 patients with HCC on top of post hepatitic C and/or B liver cirrhosis. Group B patients underwent either radiofrequency ablation or ethanol injection. Clinical assessment, routine laboratory evaluation, CT studies and measurement of serum alpha-fetoprotein (AFP) and DKK1 were performed to all patients and repeated to group B patients 1 and 3 months after treatment.

Results: The optimum cut off value of DKK1 for diagnosis of HCC was 4.3 ng/mL (AUC 0.89, sensitivity 66.7% and specificity 96.6%) (P<0.001). While, the optimum cut off value for AFP was > 101 ng/mL with 90% sensitivity and 75.9% specificity (p<0.001). Testing of both DKK1 and AFP increased the diagnostic accuracy for HCC (AUC 0.901, sensitivity 93.3%, and specificity 75.9) (P<0.001). Serum DKK1 level significantly decreases after HCC treatment with either radio-frequency ablation or ethanol injection (P<0.001).

Conclusion: Testing of both DKK1 and AFP significantly increased the diagnostic accuracy for HCC. Meanwhile, DKK1 can be used alone for HCC diagnosis even in HCC with inconclusive AFP. DKK1 has a promising prognostic value and can be used for follow up of HCC patients who underwent loco-regional treatment.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common malignant disease and the third leading cause of cancer-related death worldwide. HCC is prevalent in Asia and Africa, but recently it raises in the Western world due to an increase in hepatitis C virus (HCV) infection **[1]**. In Egypt, Liver cancer forms 11.75% of the malignancies of all digestive organs and 1.6% of total malignancies **[2,3]**. Risk factors for HCC include chronic hepatitis B virus (HBV) and chronic hepatitis C infections, cirrhosis, chronic alcohol abuse, aflatoxin ingestion, non-alcoholic steatohepatitis and metabolic liver diseases **[4]**. Both HCV and HBV infections are the most common risk factors for HCC among Egyptian patients. 10%-20% of the general Egyptian populations are infected with HCV **[5]**. 80% - 90% of HCC patients have underlying cirrhosis and the remaining 10% - 20% of cases develop HCC without cirrhosis **[6,7].**

HCC is a disease with fast infiltrating growth and poor prognosis **[8]**. The commonly used screening methods for liver cancer are ultrasound examination of the liver and determination of serum AFP level **[9]**. Abdominal ultrasound is a better, simple and easy method for detection of HCC but it is operator dependent and many focal lesions can be missed **[10]**. AFP has approximately 60% specificity and 40% sensitivity for HCC diagnosis, since minor elevations are common in patients with chronic liver disease, cirrhosis, germ cell tumors and in pregnancy **[11].** So, it is necessary to find a specific & sensitive marker for early diagnosis of HCC and for monitoring of treatment response**.**

Dickkopf-1 (DKK1) is a secretory protein which was identified in 1998. DKK1 is an inhibitor of Wnt/β-catenin signalling and a downstream target of β-catenin **[12].** The Wnt/β-catenin signalling pathway plays main role in development of both normal liver and hepatic carcinogenesis **[13].** It is hardly expressed in normal human adult tissues except in placental and embryonic tissues **[14].** DKK1 is up regulated in various cancers including breast, lung, ovarian, prostate cancers and HCC **[15].**

This work aimed to assess validity of serum DKK1 as a diagnostic marker for HCC and to assess prognostic value of serum DKK1 in predicting treatment response, complication and survival in HCC patients.

PATIENTS AND METHODS

This case control study was conducted in Tropical Medicine and Clinical Pathology Departments, Faculty of Medicine, Zagazig University Hospitals, Egypt during the period from January 2014 till March 2016.

This study included 60 Patients divided into two groups: Group A: consisted of 30 patients with post hepatitic C and/or B liver cirrhosis. Group B: consisted of 30 patients with HCC on top of post hepatitic C and/or B liver cirrhosis.

Inclusion criteria

Group (A) included cirrhotic patients with no evidence of hepatic focal masses in ultrasound evaluation. Cirrhotic patients are child class A or B according to Child Pugh score. Patients with liver cirrhosis were diagnosed by liver biopsy, laboratory and/or imaging evidence including (nodular liver contour, presence of ascites, portal hypertension, varices, enlargement of the caudate lobe, splenomegaly and collateral portal venous anastomoses).

Group (B) included patients with HCC on top of cirrhotic liver. HCC was diagnosed by CT criteria (filling of the dye in arterial phase and rapid fade out in venous and delayed phases) and/or by histopathology according to the American Association for the Study of Liver Diseases guidelines. HCC patients will be Child class A or B according to Child Pugh score for cirrhotic patients.

Exclusion criteria

Patients who had any other tumors or history of other tumors were excluded from the study. Also, patients with Child-Pugh class C, vascular invasion or extra hepatic metastasis were excluded from the study.

All patients were subjected to full history, complete physical examination and laboratory investigation in the form of liver function tests, kidney function tests, complete blood count, AFP, viral markers (HBs Ag and HCV Abs) and serum DKK1. Also, all patients were subjected to abdominal ultrasound. HCC was diagnosed by triphasic CT examination of the abdomen or by liver biopsy (FNAB) (imaging is not conclusive). Group B patients underwent either radio-frequency ablation or ethanol injection according to the Barcelona Clinic Liver Cancer (BCLC) staging system and followed up by laboratory investigations (CBC, LFTs, KFTs, AFP, and DKK1), abdominal ultrasound and triphasic CT scan 1 and 3 months after treatment.

Dickkopf-1 (DKK1)

It was determined by Human Dickkopf-1(DKK1) ELISA Kits provided by WKEA MED SUPPLIES CORP, USA, according to the manufacturer's protocol. This kit allows for the determination of DKK1 concentrations in Human serum, plasma, and other biological fluids.

The kit assay Human DKK1 level in the sample, by using Purified Human DKK1 antibody to coat microtiter plate wells, make solid-phase antibody, then add DKK1 to wells, Combined DKK1 antibody which With enzyme labeled, become antibody - antigen - enzyme-antibody complex, after washing Completely, Add substrate, substrate becomes blue color At HRP enzyme-catalyzed, reaction is terminated by the addition of a sulphuric acid solution and the color change is

measured spectrophotometrically at a wave length of 450 nm.

The concentration of DKK1 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Ethanol injection

All lesions were injected by absolute alcohol; ultrasound guided in multiple sessions, once weekly, under complete aseptic condition and 10 mg midazolam as a sedative agent.

The same operator used spinal needle (20 gauges) to inject ethanol intra-lesionally and leave the needle for 2 minutes in place, then injection of local anesthetic during withdrawal of the needle to minimize the irritant effect of refluxed ethanol to the capsule.

The total amount of ethanol can be calculated according to the following equation:

$$V= 4/3\ \pi(r+0.5)^3$$

Where: V=Volume of ethanol, π= 22/7, r = radius of the tumor by cm plus 0.5 cm as safety margin. The average amount per session was 6.8 cc, with average 5 sessions per lesion and average amount of 35 cc per lesion **[16]**.

Radiofrequency ablation

All patients were fasted before the procedure. Treatment was performed with sedation using midazolam (Dormicum R 10 mg amp; Roche) 0.03-0.1 mg/kg/IV every 30 minutes, propofol (Diprivan R 20 mg amp; Astra) 0.5 mg/kg/IV over 3-5 minutes.

All lesions were ablated by the same operator hands, under complete aseptic condition at Ultra-sonography Unit, Tropical medicine department. Multiple curved, retractable electrodes are kept inside the needle until its tip is positioned within a tumor. When properly positioned, a plunger on the hub of the needle is advanced so that the electrodes extend from the needle tip. Multiple electrode tips of an expanding electrode are active. This results in more homogenous heat distribution within the tumor and creates a reproducible sphere of ablation every time. Patients were observed for 6 hours for blood pressure, pulse, pain and vomiting.

Statistical analysis

All data were collected, tabulated and statistically analyzed using SPSS 20.0 for windows. Quantitative data were expressed as the mean ± SD & median (range), and qualitative data were expressed as an absolute frequencies "number"& relative frequencies (percentage). Independent samples Student's t-test, Mann-Whitney U, Paired t-test and Wilcoxon signed ranks test were used when needed. Percent of categorical variables were compared using the Pearson's Chi-square test or Fisher's exact test when was appropriate. Receiver operating characteristic (ROC) curve analysis was used to identify optimal cut-off values of AFP and DKK1 with maximum sensitivity and specificity for diagnosis HCC and prediction of response, Area Under Curve (AUC) was also calculated. P<0.05 was considered statistically significant (S).

RESULTS

This study showed no statistically significant difference between groups A & B as regard age, sex, viral etiology and Child Pugh score. Most of our patients were males (42 patients) and HCV positive (51). Table (1) showed no statistically significant difference between group A and group B as regard laboratory data except for platelet count, DKK1 and AFP (138.93 ± 37.17 Vs 110.76 ± 38.61 P = 0.006), (2.28 ± 0.90 ng/ml Vs 4.97 ± 2.23 ng/ml P<0.001) and (70.38 ± 80.52 ng/ml Vs 361.93 ± 289.91 P<0.001), respectively. We found that serum DKK1 was more elevated in HCC patients with focal lesions >3 cm than focal lesions < 3cm (6.09 ± 1.77 Vs 2.75 ± 1.09) (P<0.001) (Table 2).

The optimum diagnostic cut off value for DKK1 was >4.3 ng/mL with 66.7% sensitivity and 96.6% specificity while, the cut off value of AFP was >101 ng/mL with 90 % sensitivity and 75% specificity for HCC diagnosis vs. cirrhotic patients (P<0.001). Testing of both DKK1 and AFP increased the diagnostic accuracy for HCC compared with either test alone (AUC 0.901, 95% CI 0.795-0.964, sensitivity 93.3%, and specificity 75.9) (P<0.001) (Table 3; Fig. 3).

Table (4) showed no statistically significant difference among studied group as regard bilirubin, albumin, PT, creatinine and CBC before and after treatment, whereas DKK1, AFP, ALT and AST showed statistically significant improvement in these patients after treatment. DKK1 levels before and after treatment were 4.97 ± 2.23 ng/ml and 2.75 ± 1.52 ng/ml respectively (p<0.001). This study showed highly statistically significant decline of DKK1 level among complete responder's patients (P<0.001) (Table 5). The cut off value of DKK1 (before treatment) for prediction of complete response to treatment was ≤5.67 ng/mL (p<0.001) (Table 6; Fig. 4).

Table (1): Laboratory investigations and tumor markers of both groups

Laboratory findings	Group A (N=30)	Group B (N=30)	P value
AST (U/L)	64.41 ± 18.29	70.46 ± 31.65	0.756 (NS)
ALT (U/L)	64.06 ± 18.54	60.60 ± 24.98	0.391 (NS)
Bilirubin (mg/dl)	1.70 ± 0.76	1.93 ± 0.77	0.140 (NS)
Albumin (g/dl)	3.29 ± 0.49	3.29 ± 0.54	0.769 (NS)
PT (sec)	15.27 ± 1.62	15.83 ± 2.98	0.249 (NS)
Creatinine (mg/dl)	0.93 ± 0.18	1.02 ± 0.27	0.154 (NS)
Hemoglobin (g/dl)	11.46 ± 0.60	11.83 ± 1.55	0.306 (NS)
Plt ($x10^3/mm^3$)	138.93 ± 37.17	110.76 ± 38.61	0.006 (S)
WBCs ($x10^3/mm^3$)	6 ± 1.65	5.81 ± 2.17	0.444 (NS)
AFP (ng/dl)	70.38 ± 80.52	361.93 ± 289.91	<0.001 (HS)
DKK 1 (ng/dl)	2.28 ± 0.90	4.97 ± 2.23	<0.001 (HS)

Table (2): Patients with focal lesion < 3cm and patients with focal lesion 3-5 cm in group (B) as regard tumor markers

	Group B (N=30)		
	<3 cm (N=10)	**3 – 5 cm (N=20)**	**P value**
AFP (ng/dl)	422.30 ± 349.55	331.75 ± 259.86	0.468 (NS)
DKK 1 (ng/dl)	2.75 ± 1.09	6.09 ± 1.77	<0.001 **(HS)**

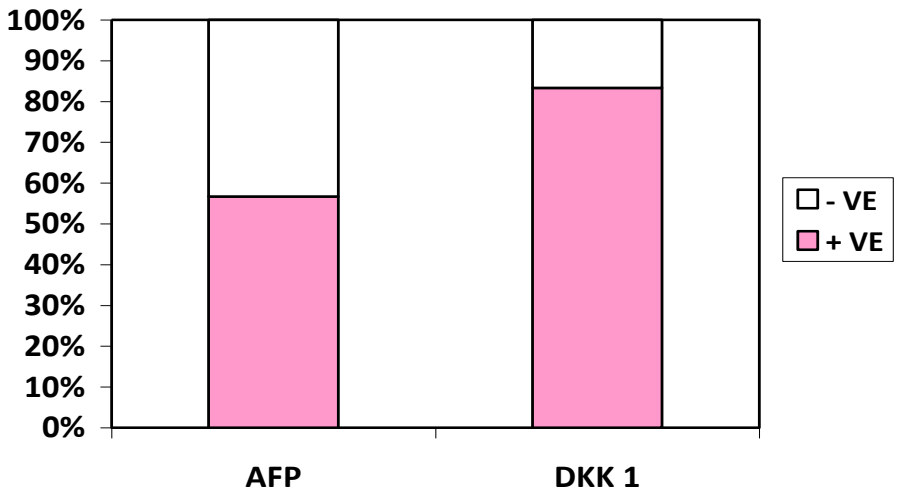

Fig. (1): Percentage of increased level of AFP and DKK 1 among HCC patients (group B)

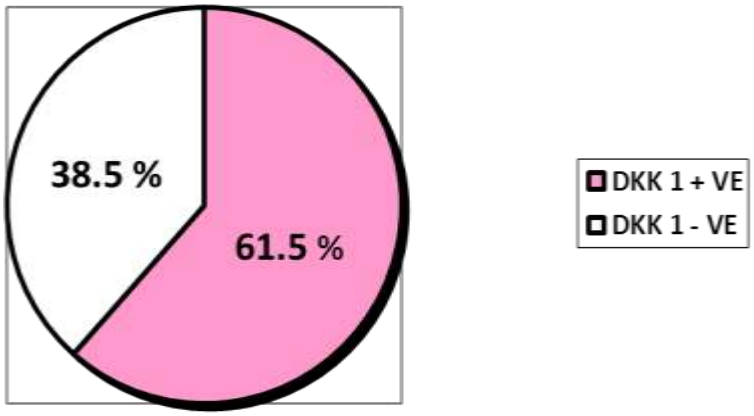

Fig. (2): Percentage of increased level of DKK1 among HCC patients with non-conclusive AFP

Table (3): Validity of DKK1, AFP and DKK1+AFP as diagnostic markers for HCC vs. cirrhotic patient without HCC

Cut-off value	Sens. % (95% CI)	Spec. % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	AUC (95% CI)
DKK1 >4.3 ng/mL	66.7% (47.2—82.7)	96.6% (82.2-99.9)	95.2% (76.2-99.9)	73.7% (56.9-86.6)	0.895 (0.787-0.960)
AFP >101 ng/mL	90% (73.5-97.9)	75.9% (56.5-89.7)	79.4% (62.1-91.3)	88% 968.8-97.5)	0.895 (0.788-0.960)
DKK1+AFP >102.2ng/mL	93.3% (77.9-99.2)	75.9% (56.5-89.7)	80% (63.1-91.6)	91.7% (73-99)	0.901 (0.795-0.964)

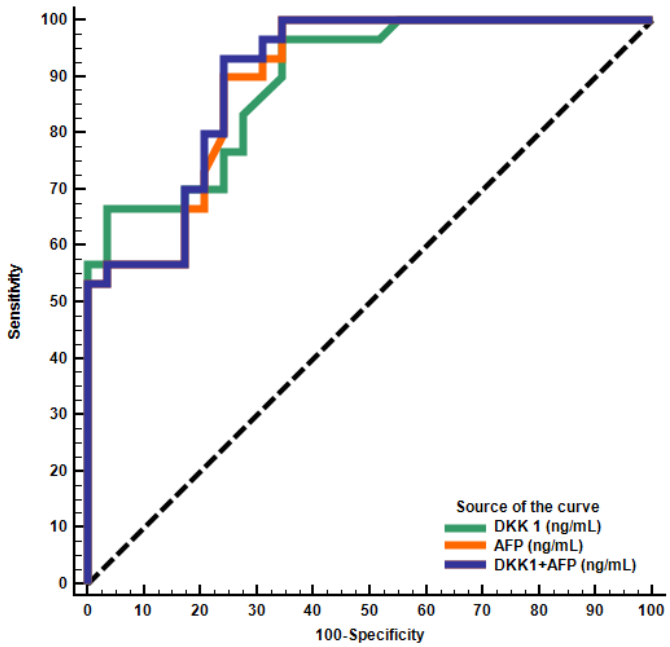

Fig. (3): ROC curve of DKK1, AFP and DKK1+AFP as diagnostic markers for HCC vs. cirrhotic patients without HCC

Table (4): Laboratory findings before and after treatment in Group B

Laboratory findings	Before treatment (N=30)	After treatment (N=30)	P value
AST (U/L)	70.46 ± 31.65	55.26 ± 25.12	0.003(S)
ALT (U/L)	60.60 ± 24.98	55.86 ± 26.17	0.004(S)
Bilirubin (mg/dl)	1.93 ± 0.77	2.05 ± 0.74	0.131(NS)
Albumin (g/dl)	3.29 ± 0.54	3.08 ± 0.45	0.129(NS)
PT (sec)	15.83 ± 2.98	17.20 ± 3.99	0.057(NS)
Creatinine (mg/dl)	1.02 ± 0.27	1.07 ± 0.24	0.07(NS)
Hemoglobin (g/dl)	11.83 ± 1.55	11.23 ± 1.45	0.16(NS)
Plt ($x10^3/mm^3$)	110.76 ± 38.61	109.5 ± 36.62	0.231(NS)
WBCs($x10^3/mm^3$)	5.81 ± 2.17	5.10 ± 1.99	0.18(NS)
AFP (ng/dl)	361.93 ± 289.91	286.93 ± 241.30	<0.001(HS)
DKK 1 (ng/dl)	4.97 ± 2.23	2.75 ± 1.52	<0.001(HS)

Table (5): Tumor markers 1 month after treatment among partial and complete responder's patients

Tumor markers	Partial responder (N=9)	Complete responder (N=21)	P value
AFP before	402.11 ± 264.37	344.71 ± 304.75	0.402(NS)
AFP after	253.22 ± 135.39	301.38 ± 276.34	0.751(NS)
DKK 1 before	7.52 ± 1.44	3.88 ± 1.49	<0.001**(HS)**
DKK 1 after	3.36 ± 1.57	2.49 ± 1.45	0.167(NS)

Table (6): Validity of DKK1 (before treatment) in prediction of complete response to treatment; ROC curve Analysis

Cut-off value	Sens. % (95% CI)	Spec. % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	AUC (95% CI)
DKK1 before ≤ 5.67 ng/mL	100% (83.9-100)	100% (66.4-100)	100% (83.9-100)	100% (66.4-100)	100% (78.7-100)

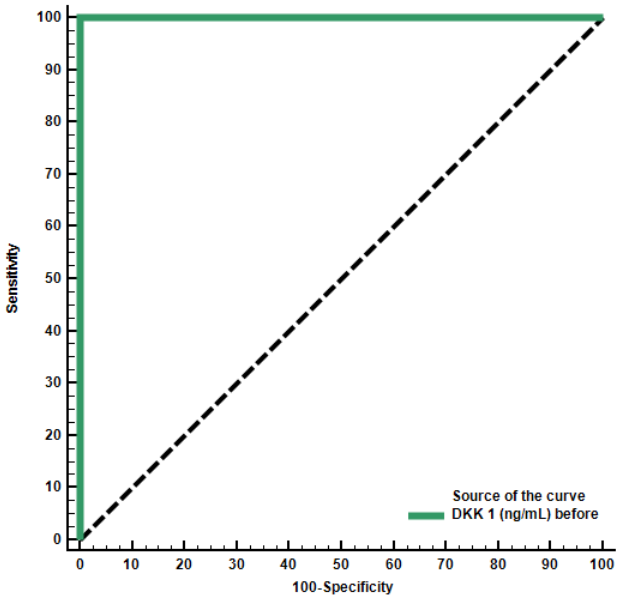

Fig. (4): ROC curve of DKK1 (before treatment) in prediction of complete response to treatment

DISCUSSION

HCC is usually asymptomatic in early stages and tends to be invasive. Most HCC patients are presented with non-operable disease and this makes its early diagnosis critical for a good prognosis. Early HCC detection gives the opportunity to employ curative treatments such as liver transplantation, resection or local ablative therapy, which are the best way to prolong survival **[17]**. So, continuous researches are ongoing worldwide to find and evaluate an early sensitive and specific marker for HCC diagnosis **[18].**

Protein markers that measured in serum are the most applicable tests for clinical assessments and population studies **[19,20].** DKK1 is a secretory protein, specifically over expressed in cancer cells and is hardly detectable in human adult normal tissues except in placenta and embryonic tissues. Therefore, this protein might have potential as a cancer-specific serum biomarker for various human cancers including HCC **[21].**

In the present study, there was a statistically significant difference between the mean value of DKK1 in patients with HCC compared to patients with liver cirrhosis with mean values of 4.97±2.23 ng/ml and 2.28±0.90 ng/mL respectively. These results were in agreement with those of Shen et al., 2012 and Zhang et al., 2014 who showed that serum DKK1 level was higher in patients with HCC than cirrhotic patients, chronic hepatitis B and healthy control **[22,23].**

In our study, serum DKK1 level was more elevated in Child B cirrhotic patients than Child A patients (in group A) with mean level 9.95 ± 1.04 and 1.87±0.49 respectively. So, DKK1 levels increase with hepatic dysfunction. Also, serum DKK1 was more elevated in HCC patients with focal lesions >3 cm than focal lesions <3 cm (6.09±1.77 and 2.75±1.09 respectively) in group B. This indicated that DKK1 level increase with disease progression from cirrhosis to small focal lesion then large focal mass. These results agreed with those of Tung et al., (2011) who reported a stepwise increase in serum DKK1 from cirrhosis group to early HCC then to advanced HCC group **[24]**.

In this study, ROC curves revealed that the optimum diagnostic cut off value of DKK1 is 4.3 ng/mL for diagnosis of HCC (AUC 0.895, 95% CI 0.787-0.960, sensitivity 66.7%, specificity 96.6%). This result is in agreement with that of Shen et al., (2012) and Zhang et al., (2014) who reported AUC (0.848 & 0.84), sensitivity (69.1% & 65%), specificity (90.6% & 94%) for HCC diagnosis versus cirrhosis control **[22,23]**. In contrast, Yang et al., (2013) showed that the DKK1 AUC (0.717) for HCC diagnosis was lower than the AUC in our study (0.895) **[26]**.

In the present study, diagnostic cut off value for AFP was > 101 ng/mL for HCC in cirrhotic patients with 90% sensitivity, 75.9% specificity and 0.895 AUC. Serum DKK1 had similar AUC as AFP, higher specificity and lower sensitivity than AFP and this could be due to small sample size and only cirrhotic patients included as a control group not healthy control. This was in agreement with Nakamura et al., 2006 who showed that the cut off value of AFP for HCC diagnosis was 100 ng/ml with (33%) sensitivity and (99%) specificity **[27]**. In contrast, Farinati et al., 2006; and Debruyne and Delanghe, 2008 reported other sensitivity, specificity and cut off value for AFP for HCC diagnosis **[28,29]**.

A greater proportion of HCC patients in our study were positive for DKK1 than for AFP. Furthermore, 8 of 13 AFP negative HCC patients had positive DKK1 result and all AFP-positive patients had + ve DKK1 results (Fig. 1; Fig. 2). The ROC curves for DKK1 indicated the diagnosis of HCC irrespective of AFP status. This finding was in agreement with that of Shen et al., (2012) and Yang et al., (2013) **[22,26].**

In this study, testing of both DKK1 and AFP increased the diagnostic accuracy for HCC compared with either test alone (AUC 0.901, 95% CI, 0.795-0.964, sensitivity 93.3%, and specificity 75.9).This was in agreement with Ge et al., 2015 who showed that testing of both AFP and DKK1 had AUC 0.93, sensitivity 88.8%, and specificity 88.12% **[30]**. In contrast, Eun et al., 2016 reported that testing of AFP and DKK1 had AUC 0.76, sensitivity 78 %, and specificity 73% **[31].**

Group B patients underwent either radiofrequency ablation (12 patients) or ethanol injection (18 patients) according to the Barcelona Clinic Liver Cancer (BCLC) staging system **[32].** Percutaneous ablation is the preferred treatment option for patients in this study. Both radiofrequency ablation and percutaneous injection therapy have a well-documented loco-regional antitumor effect and are the most two commonly employed methods for HCC treatment **[33,34]**. Liver transplantation and surgical resection are the standard treatment modality to achieve a long-term survival.

However, both of them are major surgery with many complications and have negative impact on patients' especially cirrhotic **[35,36].**

This study showed no statistically significant difference between patients treated with either radiofrequency ablation or ethanol injection as regards AFP and DKK1. There was decrease in mean level of both markers after treatment, with mean level of DKK1 4.97±2.23 ng/ml pre-treatment and 2.75±1.52 ng/ml post-treatment. These findings agreed with those of Tung et al. **[24]** and Shen et al. **[22]** who reported that serum DKK1 levels dropped in HCC patients following surgery. Also, Yamabuki et al., (2007) reported reduced DKK1 serum levels following surgical resection of primary tumors in esophageal squamous cell carcinoma and lung cancer patients **[37].**

After 1 and 3 months of treatment, there was no statistically significant difference between both groups regarding procedure success, stationary ablation, recurrence, decompensation and survival. Both techniques were successful (83.3% with radiofrequency and 61.1% with ethanol injection).

We found that level of DKK1 was significantly decreased after treatment. DKK1 before treatment was 7.52±1.44 in patients with partial response and 3.88±1.49 in patients with complete response and this suggest that DKK1 may have a prognostic role in predicting treatment response. DKK1 was assessed only one month after treatment where no recurrence is detected during this period with CT. Therefore, we couldn't emphasize that level of DKK1 elevated again with tumor recurrence.

No studies have been done before to put a cut off value for DKK1 for prediction of treatment response even after surgical resection. In this study, we have a cut off value for DKK1 for prediction of complete response to treatment. This value was ≤5.67 ng/mL (AUC 100%, 95% CI 78.7-100, sensitivity 100% and specificity 100%) and this value need more studies to be confirmed and to prove prognostic role of DKK1 in prediction of treatment response, recurrence and survival.

From this study and its results, serum DKK1 is a secretory protein, it can be easily detected in circulation and it is elevated in HCC cells and not in normal cells. Serum DKK1 could be used to diagnose HCC, especially with inconclusive AFP. Furthermore, serum DKK1 can complement AFP levels to improve the diagnostic accuracy of HCC. DKK1 could predict treatment response and may be a promising prognostic marker for HCC.

Funding: None.
Conflicts of interest: None.
Ethical approval: The protocol of the study was approved by the ethical committee of Faculty of Medicine, Zagazig University. Informed consents were obtained from all patients.

REFERENCES

1. Altekruse SF, McGlynn KA, Reichman ME. Hepatocellular carcinoma incidence, mortality, and survival trends in the United States from 1975 to 2005. *J Clin Oncol*. 2009; 27: 1485-1491.
2. Mokhtar N, Gouda I, Adel I. Cancer Pathology registry (2003-2004) and time trend analysis. *Department of pathology, NCI*. 2007.
3. Omar A, Abou-Alfa GK, Khairy A, Omar H. Risk factors for developing hepato-cellular carcinoma in Egypt. *Chin Clin Oncol*. 2013; 2 (4): 43.
4. Severi T, van Malenstein H, Verslype C, van Pelt JF. Tumor initiation and progression in hepatocellular carcinoma: risk factors, classification, and therapeutic targets. *Acta Pharmacol Sin*. 2010; 31:1409-1420.
5. Habib M, Mohamed MK, Abdel Aziz F, Magder LS, Abdel-Hamid M, Gamil F, et al. Hepatitis C Virus in a community in the Nile Delta: risk factors for Seropositivity. *Hepatology* 2001; 33 (1): 2485.
6. Nguyen VT, Law MG, Dore GJ. Hepatitis B-related hepatocellular carcinoma: epidemiological characteristics and disease burden. *J Viral Hepat*. 2009; 16:453-463.
7. Kanwal F, Hoang T, Kramer JR, Asch SM, Goetz MB, Zeringue A, et al. Increasing prevalence of HCC and cirrhosis in patients with chronic hepatitis C virus infection. *Gastroenterology* 2011; 140:1182-1188.e1.
8. Yao DF, Dong ZZ, Yao M. Specific molecular markers in hepatocellular carcinoma. *Hepatobiliary Pancreat Dis Int*. 2007; 6: 241-247.
9. Daniele B, Bencivenga A, Megna AS, Tinessa V. α-fetoprotein and ultrasonography screening for hepatocellular carcinoma. *Gastroenterol*. 2004; 127: S108-12.
10. Sheu JC, Sung JL, Chen DS, Lai MY, Wang TH, Yu JY, et al. Early detection of hepatocellular carcinoma by real-time ultrasonography. A prospective study. *Cancer* 1985; 56, 660-666.
11. Nguyen MH, Garcia RT, Simpson PW, Wright TL, Keeffe EB. Racial differences in effectiveness of alpha-fetoprotein for diagnosis of

hepatocellular carcinoma in hepatitis C virus cirrhosis. *Hepatology* 2002; 36, 410-417.
12. Fatima S, Lee NP, Luk JM. Dickkopfs and Wnt/β-catenin signalling in liver cancer. *World J Clin Oncol.* 2011; 2:311-25.
13. MacDonald BT, Tamai K, He X. Wnt/β-catenin signaling: components, mechanisms, and diseases. *Dev Cell*. 2009; 17: 9-26.
14. Fedi P, Bafico A, Soria AN, Burgess WH, Miki T, Bottaro DP, et al. Isolation and biochemical characterization of the human Dkk-1 homologue, a novel inhibitor of mammalian Wnt signaling. *J Biol Chem*. 1999; 274: 19465-72.
15. Hall CL, Daignault SD, Shah RB, Pienta kJ, Keller ET. Dickkopf-1 expression increases early in prostate cancer development and decreases during progression from primary tumor to metastasis. *Prostate* 2008; 68: 1396-404.
16. Shiina S, Tagawa K, Unuma T, Fujino H, Uta Y, Niwa Y, et al. Percutaneous ethanol injection therapy of HCC: analysis of 77 patients. *AJR* 1990; 155: 1221.
17. Forner A, Llovet JM, Bruix J. Hepatocellular carcinoma. *Lancet* 2004; 379:1245-55.
18. Filmus J, Capurro M. Glypican 3 and alpha-fetoprotein as diagnostic tests for hepatocellular carcinoma as diagnostic tests. *Mol. Diagn*. 2004; 8: 207.
19. Brawley O, Ankerst D, Thompson I. Screening for prostate cancer. *CA Cancer J Clin* 2009; 59: 264-73.
20. Malaguarnera M, Vacante M, Fichera R, Cappellani A, Cristaldi E, Motta M, et al. Chromogranin A (CgA) serum level as a marker of progression in hepatocellular carcinoma (HCC) of elderly patients. *Arch Gerontol Geriatr* 2010; 51:81-5.
21. Le Sheng S, Huang G, Yu B, Qin WX. Clinical significance and prognostic value of serum Dickkopf-1 concentrations in patients with lung cancer. *Clin Chem.* 2009; 55:1656 - 64.
22. Shen Q, Fan J, Yang XR, Tan Y, Zhao W, Xu Y, et al. Serum DKK1 as a protein biomarker for the diagnosis of hepatocellular carcinoma: a large-scale, multicentre study. *Lancet Oncol*. 2012; 13(8): 817 - 826.
23. Zhang J, Zhao Y, Yang Q. Sensitivity and specificity of Dickkopf-1 protein in serum for diagnosing hepatocellular carcinoma: a meta-analysis. *Int J Biol Markers* 2014; 29(4):e403 - e410.
24. Tung EK, Mak CK, Fatima S, Lo RC, Zhao H, Zhang C, et al. Clinicopathological and prognostic significance of serum and tissue Dickkopf-1 levels in human hepatocellular carcinoma. *Liver Int.* 2011; 31: 1494-1504.
25. Kim SU, Park JH, Kim HS, Lee JM, Lee HG, Kim H, et al. Serum Dickkopf-1 as a Biomarker for the Diagnosis of Hepatocellular Carcinoma. *Yonsei Med J*. 2015; 56(5):1296-1306.
26. Yang H, Chen GD, Fang F, Liu Z, Lau SH, Zhang JF, et al. Dickkopf-1: as a diagnostic and prognostic serum marker for early hepatocellular carcinoma. *Int. J Biol. Markers* 2013; 28(3): 286-297.
27. Nakamura S, Nouso K, Sakaguchi K, Ito YM, Ohashi Y, Kobayashi Y, et al. Sensitivity and specificity of des-gamma-carboxy prothrombin for diagnosis of patients with hepatocellular carcinomas varies according to tumor size. *The American journal of gastroenterology* 2006; 101(9), pp.2038-2043.
28. Farinati F, Marino D, De Giorgio M, Baldan A, Cantarini M, Cursaro C, et al. Diagnostic and prognostic role of alpha-fetoprotein in hepatocellular carcinoma: both or neither? *Am J Gastroenterol*. 2006; 101: 524-32.
29. Debruyne EN, Delanghe JR. Diagnosing and monitoring hepatocellular carcinoma with alpha-fetoprotein: new aspects and applications. *Clin Chim Acta*. 2008; 395:19-26.
30. Ge T, Shen Q, Wang N, Zhang Y, Ge Z, Chu W, et al. Diagnostic values of alpha fetoprotein, dickkopf - 1, and osteopontin for hepatocellular carcinoma. *Med Oncol*. 2015; 32(3):59.
31. Jang ES, Jeong SH, Kim JW, Choi YS, Leissner P, Brechot C. Diagnostic Performance of Alpha-Fetoprotein, Protein Induced by Vitamin K Absence, Osteopontin, Dickkopf-1 and Its Combinations for Hepatocellular Carcinoma. *PloS one.* 2016; 11(3):e0151069.
32. Forner A, Reig ME, de Lope CR, Bruix J. Current strategy for staging and treatment: the BCLC update and future prospects. *Semin Liver Dis.* 2010; 30:61-74.
33. Livraghi T, Goldberg SN, Lazzaroni S, Meloni F, Solbiati L, Gazelle GS, et al. Small hepatocellular carcinoma: treatment with radio-frequency ablation versus ethanol injection. *Radiology* 1999; 210: 655-61.
34. Huo TI, Huang YH, Wu JC, Lee PC, Chang FY, Lee SD. Survival benefit of cirrhotic patients with hepatocellular carcinoma treated by percutaneous ethanol injection as a salvage therapy. *Scand J Gastroenterol.* 2002; 37(3): 350-5.
35. Izumi R, Shimizu K, Ii TO, Yagi M, Matsui O, Nonomura A, et al. Prognostic factors of hepatocellular carcinoma in patients undergoing hepatic resection. *Gastroenterology* 1994; 106(3): 720-7.
36. Bruix J, Sherman M. Management of Hepatocellular Carcinoma. *Hepatology* 2005; 42(5): 1208-36.
37. Yamabuki T, Takano A, Hayama S, Ishikawa N, Kato T, Miyamoto M, et al. Dikkopf-1 as a novel serologic and prognostic biomarker for lung and esophageal carcinomas. *Cancer Res.* 2007; 67: 2517-25.

Peer reviewer: Maysaa Abdalla, Professor of Tropical Medicine, Faculty of Medicine, Zagazig University, Egypt.

Editor: Tarik Zaher, Professor of Tropical Medicine, Faculty of Medicine, Zagazig University, Egypt

Monocyte Chemotactic Protein-1 Gene Expression in Blood and Ascitic Fluid of Cirrhotic Patients with Spontaneous Bacterial Peritonitis

Maha Z. Omar[1] and Shuzan A Mohammed[2]
[1]*Department of Hepatology, Gastroenterology and Infectious Diseases, Faculty of Medicine, Benha University, Egypt*
[2]*Department of Medical Biochemistry, Faculty of Medicine, Benha University, Egypt*

Corresponding Author
Maha Omar

***Mobile**:+2012232873 12*

E mail:
mahazeinelabedin@yahoo.com

Key words:
SBP, MCP-1, gene expression, cirrhosis

Background and study aim: Cirrhotic patients with ascites show a higher susceptibility to bacterial infections, monocyte chemotactic protein-1 (MCP-1) secretion is up-regulated during chronic hepatitis and correlates with the severity of hepatic inflammation. The aim of this work is to determine the level of expression of MCP-1 gene in blood and ascitic fluid in cirrhotic patients with and without spontaneous bacterial peritonitis (SBP) to evaluate its role in pathogenesis of SBP and its role in diagnosis.

Patients and Methods: This study included 15 healthy subjects served as control group in addition to 35 cirrhotic patients due to HCV infection with ascites; classified into two groups, cirrhosis without SBP (15 patients) and cirrhosis with SBP (20 patients). All groups were subjected to quantitative estimation of MCP-1 gene expression in blood by real time PCR. In SBP and non SBP groups the gene expression were assessed in ascitic fluid also at diagnosis and reassessed in SBP group after treatment.

Results: Blood and ascitic fluid expression of MCP-1 gene were significant higher in SBP group than non SBP group and control group. SBP group showed a significant decrease in level of MCP-1 gene expression in blood and ascitic fluid after resolution of infection by appropriate treatment of SBP.

Conclusion: MCP-1 gene expression in both blood and ascitic fluid may be related to pathophysiology and course of SBP and can be used as a marker for diagnosis.

INTRODUCTION

Liver cirrhosis is the clinical end stage of different entities of chronic liver disease **[1]**. Ascites is the most common complication; about 60% of patients with compensated cirrhosis develop ascites within 10 years of disease onset **[2]**. Patients with cirrhosis and ascites show higher susceptibility to bacterial infections, because of inadequate defense mechanisms **[3,4]**. Spontaneous bacterial peritonitis (SBP) is a common and potentially life-threatening complication in patients with cirrhosis. It is a prototypical infective disease in cirrhotic patients characterized by peritoneal neutrophil infiltration, which also serves as a diagnostic criterion for SBP (e.g. an ascites neutrophil count ≥250 cell/mm^3) **[5]**. Factors influencing the development of SBP in patients with liver cirrhosis are poorly understood **[6]**. SBP can be caused by many reasons due to alterations of the immune system that are very common in patients with end-stage liver disease and associated with an increased risk of infection and death **[7,8]**. Consequently, elevated concentrations of pro-inflammatory cytokines are found in ascitic fluid of these patients **[9, 10].** In addition, hepatitis C virus (HCV) infection is associated with increased hepatic expression of monocyte chemotactic protein-1 [MCP-1also known as chemotatic cytokine ligand 2 (CCL2)] **[11]**. CCL2 is the first discovered human CC chemokine located on chromosome 17 (chr.17, q11.2).Human MCP-1 is composed of 76 amino acids and is 13

kDa in size **[12]**. Chemotactic cytokines are known to be critical mediators of inflammatory cell trafficking into sites of injury and are crucial for the modulation of tissue injury, inflammation and repair **[13]**. MCP-1 is one of the most potent chemokines for monocytes/macrophages and activated lymphocytes during infections **[14]**. In addition, several studies have shown that neutrophil infiltration is affected either directly or indirectly via MCP-1 **[15,16]**. The aim of this study was to investigate the expression of MCP-1 gene in blood and ascitic fluid of patients with decompensated cirrhosis with and without SBP to evaluate its role in pathogenesis of SBP.

PATIENTS AND METHODS

A prospective case-control study was conducted on (35) cirrhotic patients with ascites attending to Hepatology, Gastroenterology and Infectious Diseases Department in addition to (15) health subjects in the period from September 2015 to April 2016, samples of blood from studied groups were analyzed at Molecular Biology Unit, Faculty of Medicine, Benha University.

The studied subjects were classified into three groups; Control group: 15 healthy subjects (11 males and 4 females; mean age was 28.60±4.12 years), Cirrhosis without SBP: 15 cirrhotic patients without SBP (7 males and 8 females; mean age was 61.60±9.75 years) and Cirrhosis with SBP: 20 cirrhotic patients with SBP (13 males and 7 females; mean age was 55.55± 8.94years). SBP was diagnosed by ascitic fluid poly morphonuclear leukocyte (PMN) count ≥ 250 cells/mm^3.

Patients with malignant ascites, tuberculous ascites, evidence for secondary peritonitis, alcoholic liver cirrhosis, HBV infection, antibiotic treatment before paracentesis were excluded from this study. All groups were subjected to full history taking, thorough clinical examination and routine laboratory investigations (complete blood picture, liver profile tests and kidney function tests).

Assessment of MCP-1 gene expression by real time PCR using sybr green:

MCP-1 gene expression was performed in blood for the control group and in both blood and ascitic fluid for all patients at the time of diagnosis and after 5 days of treatment for SBP (cefotaxime administrated 2g IV/8 hours, recommended treatment of SBP) **[17]**.

Total RNA Extraction

Total RNA extraction from 100µl EDTA blood and from 100µlasciticfluidfor each subject was performed using Direct-zol™ RNA MiniPrep from Zymo Research according to the manufacturer instructions, with addition of 300µl Trizolreagent to each sample to be extracted.

Spectrophotometric Quantification of RNA

Total RNA concentration was measured by Nanodrop spectrophotometer 2000 (USA) at A260 and A280. To ensure significance, A260 readings should be greater than 0.15. An absorbance of 1 unit at 260nm corresponds to 44µg of RNA per mL **[18]**. The ratio of the reading at (A260/A280) provides an estimate of the purity of RNA. Pure RNA has an A260/A280 ratio of 1.9 to 2.3.

Two Steps RT-PCR

1^{st} step:The 1^{st} step RT-PCR was for conversion of RNA into complementary DNA (cDNA) in a Veriti™ Thermal Cycler (Applied Biosystems), using Sensi FAST™ cDNA Synthesis Kit (Bioline Reagents Ltd, United Kingdom). PCR mix for cDNA included Total RNA (5µl), 5x TransAmp Buffer (4µl),Reverse Transcriptase (1µl) and up to 20µl nuclease free-water with the thermal profile; 25°C for 10min, 42°C for 15min and 85°C for 5min.

2^{nd} step: RT-PCR for quantitation of MCP-1 gene expression was done using ABI7900HT fast real time PCR, (Applied Biosystem, USA). Single plex reactions were done. This step was performed using Sensi FAST™ Sybr Hi-Rox Kit (Bioline Reagents Ltd, United Kingdom). The primers sequences were human MCP-1; FP: 5´-AACTGAAGCTCGCACTCTCG-3´, RP: 5´-TCAGCACAGATCTCCTTGGC-3´) and human b-actin (FP: 5´-GACTACCTCATGAAGATC-3´, RP: 5´-GATCCACATCTGCTGGAA-3´) **[19]**. A single plex real time PCR reaction was performed with addition of 2x SensiFAST SYBR Hi-ROX Mix (10µl), FP (0.8µl), RP (0.8µl), cDNA (2µl) and up to 20µl nuclease free water. The thermal cycling conditions were 95°C for 5min (holding), cycling (40 cycles: denaturation; 95°C for 15sec, annealing; 56°C for 1min and extension; 72°C for 20sec). Melting curve analysis was performed in each run to confirm specificity of real-time PCR assay (95°C for 15sec, 60°C for 1min and 95°C for 15sec).

Data Analysis

The data, produced as sigmoid-shaped amplification plots (the cycle number is plotted against fluorescence on the linear scale), were analyzed by the RQ manager program 1.2 ABI SDS software (ABI 7900HT). Because the control samples are used as calibrators, their expression levels are set to 1. But because the expression levels were plotted as log10 values (log10 of 1 is 0), the expression level of the control samples appear as 0 in the graph.

Because the relative quantities of the MCP-1 gene are normalized against the relative quantities of the endogenous control β-actin gene, β-actin has no bars in the graph. Fold expression changes are calculated using the equation $2^{-\Delta\Delta CT}$ **[20]**.

Statistical analysis:

The results were analyzed using the SPSS software package version 20 (Chicago, IL, USA) and Microsoft office Excel. Quantitative data are expressed as mean±SD. ANOVA test was used to test the significance of difference between the mean values of more than two groups. Differences between two groups were compared by the studied t-test. The comparison of categorical variables was determined by x^2 test. Correlations between data were performed using Pearson correlation tests as required. Roc curve was used to detect the diagnostic performance of MCP-1 gene expression in both blood and ascitic fluid in diagnosis of SBP. Differences were considered significant at $p<0.05$.

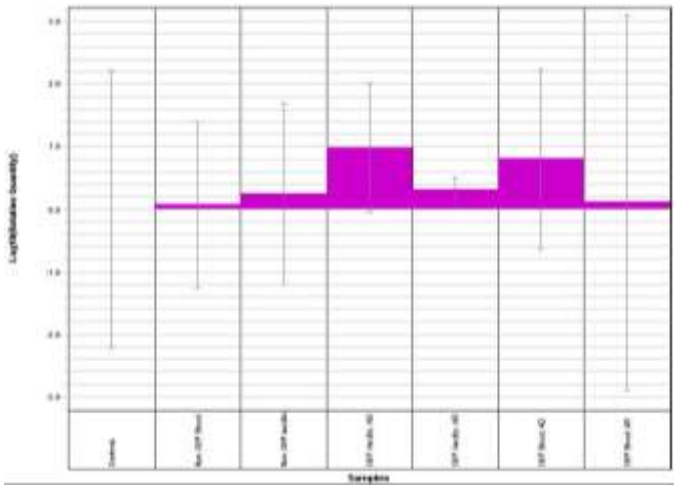

Figure (1): Gene expression plot of MCP in the studied groups

Relative quantitation of MCP-1 mRNA gene expression for all samples (AD: at diagnosis, AR: after resolution), represented as Log10. The expression level of the control samples appear 0 in the graph because the log10 for 1 is 0. The relative quantities for MCP-1 are normalized against relative quantities of GAPDH (endogenous control).

RESULTS

In the current study, the majority of studied patients in SBP group were males (65%), there was very highly statistically significant difference between the studied groups as regard age which was higher in group II (without SBP) than SBP and control groups (p=0.000), also there was very highly statistically significant difference as regard DM (p=0.000). Regarding clinical data there was highly statistically significant difference between SBP group and non SBP group regarding jaundice (p=0.000), which more common in SBP (80% vs 53.3% respectively), and both Child-Pugh and MELD score (p=0.036, 0.034 respectively) (Table 1).

There was highly statistically significant difference between studied groups regarding CBC, liver functions tests, serum creatinine. Between SBP and non-SBP group there was highly statistically significant difference regarding SAAG (p=0.024), mean level of MCP-1 gene expression in blood was higher in SBP group than non-SBP group and control group with very highly statistically significant difference (p=0.000), also MCP-1 gene expression in ascitic fluid was higher in SBP than non SBP group (p=0.021) (Table 2).

Within SBP group the level of expression of MCP-1 gene in both blood and ascitic fluid was decreased after treatment of SBP (in blood; 4.93

±0.320 vs 4.31±0.0472 before and after treatment, respectively and in ascitic fluid; 5.11 ± 0.323 vs 4.50 ± 0.0438 before and after treatment, respectively) with highly statistically significant difference (p=0.001 for both) (Figure 2).

Regarding correlation studies we found that there was significant positive correlation between MCP-1 gene expression in blood and the expression in ascitic fluid in both non-SBP and SBP (r=0.739, p=0.002 and r=0.985, p=0.000 respectively), also there was significant positive correlation between MCP-1 gene expression in blood and Child-Pugh score in non SBP group. While there was significant positive correlation between MCP-1 gene expression in ascitic fluid and platelet count in non-SBP group (r=0.56, p=0.049), with gene expression in blood in both SBP and non SBP (r=0.985, p=0.000 and r=0.739, p=0.002 respectively) also there was significant positive correlation with Child-Pugh in SBP group (r= 0.842, p= 0.000) (Table 3).

Ascitic expression of MCP-1 gene at cutoff 4.51 had higher sensitivity, specificity, PPV and NPV than its blood expression (90 %, 80%, 85.7%, 85.7%vs85 %, 76.7%, 70.83%, 88.5% respectively) with AUC was 0.913 and 0.892 (p<0.001) (Table 4, Figure 3).

Table (1): Baseline demographic and clinical characteristics of studied groups

Variables	Controls	Non-SBP Cirrhosis	SBP Cirrhosis	Test	p
	n.=15	n.=15	n.=20		
Sex (♂/♀) (n., %)	11(73.3%)/ 4(26.7%)	7(46.7%)/ 8(53.3%)	13(65%)/ 7(35%)	2.391[#]	0.300
Age (years) (mean±SD)	28.60±4.12	61.60±9.75[a]	55.55±8.94[a,b]	72.171[‡]	0.000
Diabetes mellitus	0 (0%)	11 (73.3%)	8 (40%)	17.176[#]	0.000
Hypertension	0 (0%)	1 (6.7%)	1 (5%)	0.955[#]	0.62
Jaundice	0 (0%)	8 (53.3%)	16 (80%)	22.22[#]	0.000
Gasterointestinal bleeding	0 (0%)	6 (40%)	9 (45%)	0.088[#]	0.767
Hepatic encephalopathy	0 (0%)	0 (0%)	5 (25%)	8.333[#]	0.016
Child-Pugh score(B/C)	-	6(40%)/ 9(60%)	2(10%)/18(90%)	4.375[#]	0.036
MELD score (mean±SD)	-	15.378±3.58	24.324±6.362	4.907[^]	0.034

[#]: X^2 test, [^]: t test, [‡]: Anova test
[a]: significant against controls, [b]: significant against Non-SBP, significant p values are in bald

Table (2): Baseline laboratory characteristics of studied groups

Variables	Controls	Non-SBP Cirrhosis	SBP Cirrhosis	Test	p
	n.=15	n.=15	n.=20		
Hemoglobin (g/dl)	13.65±1.185	9.373±2.04[a]	8.715±2.350[a]	29.724[‡:]	0.000
Platelets×10^3 (cell/mm^3)	297.933±	97.668±33.375[a]	118.85±37.82[a]	116.89[‡]	0.000
Total leukocyte count×10^3 (cell/mm^3)	7.302±1.729	6.598±1.96	12.72±6.72[a,b]	9.927[‡]	0.000
Aspartate Aminotransferase (IU/L)	26.26±5.93	45.053±20.38	83.25±79.66[a]	3.72[‡]	0.032
Alanine Aminotransferase (IU/L)	24.60±7.423	45.73±17.09	70.80±61.60[a]	5.68[‡]	0.006
Albumin (g/dl)	4.113±0.42	2.9400±0.354[a]	2.615±0.424[a,b]	62.28[‡]	0.000
Total Bilirubin (mg/dl)	0.622±0.307	2.72±1.77	7.57±5.25[a,b]	18.59[‡]	0.000
Prothrombin time (sec)	12.97±0.652	15.62±1.94[a]	17.61±3.404[a,b]	15.51[‡]	0.000
Creatinine (mg/dl)	0.855±0.207	1.426±0.44	2.18±1.611[a,b]	6.86[‡]	0.002
SAAG(g/dl)	-	2.25±0.554	1.85±0.45	5.57[^]	0.024
MCP-1 gene expression in blood (log10 RU)	4.199±0.019	4.27±0.0399	4.93±0.320[a,b]	69.70[‡:]	0.000
MCP-1 gene expression in Ascitic fluid (log10 RU)	-	4.44±0.094	5.11±0.323[b]	60.17[^]	0.021

[#]: X^2 test, [^]: t test, [‡:] anova test

[a]: significant against controls, [b]: significant against Non-SBP, significant p values are in bald

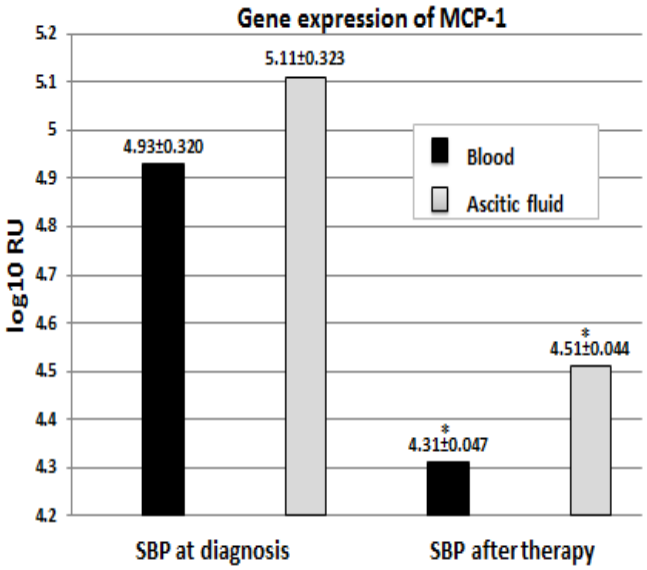

Figure (2): MCP-1 gene expression in blood and ascitic fluid in SBP before and after treatment

Table (3): Correlation between MCP-1 gene expression in blood and ascitic fluidat diagnosis and some studied parameters in both SBP and non-SBP groups

Variables	MCP-1 gene expression in blood				MCP-1 gene expression in ascitic fluid			
	Non-SBP		SBP		Non-SBP		SBP	
	r	p	r	p	r	p	r	p
Hemoglobin(mg/dl)	-0.307	0.189	-0.155	0.581	-0.078	0.783	-0.284	0.225
Platelets×10^3 (cell/mm^3)	0.0155	0.515	0.375	0.168	0.516	0.049	0.179	0.450
Total leukocyte count×10^3 (cell/mm^3)	0.175	0.461	0.403	0.136	0.423	0.116	0.219	0.354
Aspartate Aminotransferase (IU/L)	-0.038	0.875	-0.449	0.093	-0.163	0.567	-0.059	0.804
Alanine Aminotransferase (IU/L)	-0.035	0.882	-0.282	0.309	-0.091	0.747	-0.050	0.835
Albumin (g/dl)	-0.148	0.532	0.414	0.125	0.113	0.688	-0.216	0.361
Total Bilirubin (mg/dl)	-0.082	0.354	-0.410	0.129	-0.406	0.134	0.243	0.301
Prothrombin time (sec)	-0.082	0.731	-0.036	0.899	0.049	0.863	-0.012	0.958
Creatinine (mg/dl)	0.263	0.261	0.306	0.267	0.244	0.382	0.253	0.281
SAAG (g/dl)	-0.341	0.141	0.351	0.199	0.147	0.535	-0.346	0.135
MCP-1 gene expression in Ascitic fluid (log10 RU)	0.739	0.002	0.985	0.000	-	-	-	-
MCP-1 gene expression in blood (log10 RU)	-	-	-	-	0.739	0.002	0.985	0.000
Child–Pugh score	0.791	0.000	-0.479	0.073	-0.415	0.124	0.842	0.000
MELD score	0.410	0.072	-0.194	0.488	-0.147	0.602	0.442	0.051

Table (4): Diagnostic performance of MCP-1 gene expression in blood and ascitic fluid for diagnosis of SBP

Variables	Cutoff	Sensitivity	Specificity	PPV	NPV	AUC	Accuracy	95% CI	p
MCP-1 gene expression in blood (log10 RU)	4.28	85 %	76.7%	70.8%	88.5%	0.892	80%	0.77-1.00	<0.001
MCP-1 gene expression in Ascitic fluid (log10 RU)	4.51	90 %	80%	85.7%	85.7%	0.913	85.7%	0.80-1.00	<0.001

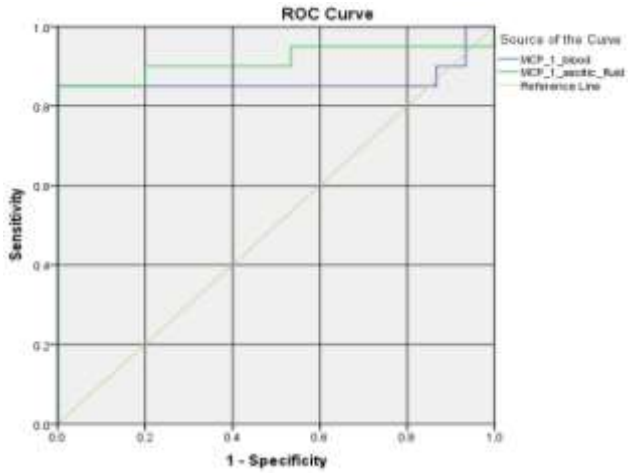

Figure (3): Roc curve for performance of MCP-1 gene expression in blood and ascitic fluid for diagnosis of SBP**.**

DISCUSSION

SBP is the most frequent infection in patients with liver cirrhosis. In these patients, SBP bacterial protein is recognized, and proinflammatory cytokines are released to blood and ascites **[21]**. In the current study, we found that SBP was common in males than females (65% vs 35% respectively) with mean age (55.55±8.94 years) which lower than non SBP and higher than control groups (p=0.000), this was coincided with **Syed et al., [22]** who found that SBP occurs more with higher ages due to more chance of infection in those patients also these results were in agreement with the study of Kim et al. **[23]** and Salama et al. **[6]** which showed that the majority of studied SBP patients were males (70% for first study and 72% for second study) with mean age was (53.3± 8.8, 51.24±9.3 years) respectively**.**On the same hand, these results were in line with those obtained by Kasztelan-Szczerbinska et al. **[24]** and Mostafa et al. **[25]** who found that SBP was more frequently among individuals of the masculine sex in percentages ranging from 72.8% to 83.7% and the mean age observed between the individuals with SBP ranging from 52.8 to 58.4 years. Regarding the clinical data, the present work found that, jaundice was more evident in SBP than non-SBP (80% vs 53.3% respectively) with highly significant difference between both groups (p=0.000). This was in accordance with that elicited by Thiele et al. **[26],** who found that jaundice was the most common complication of SBP (73.3%) but with insignificant difference between SBP and non SBP (p=0.336). In the present work we noted that the majority of SBP cases were Child-Pugh class C (90%) with statistically significant difference between SBP and non-SBP (p=0.036). This result matches with that reported by Cirera et al. **[27]**, Syed et al. **[22]** and Paul et al. **[28]** who elicited that 70%, 85%,80% respectively of SBP patients had Child class C. There was a significant increase of MELD score in SBP (p=0.034) with mean value (24.324±6.362). This coincided with Thiele et al. **[26],** who found higher MELD score in SBP than non-SBPwith mean (22.2±7.6 vs 17.9 ± 6.7). Also, Kraja et al. **[29]** and Gayatri et al. **[30]** observed that individuals with moderate to high MELD score present a substantially greater risk for SBP development.SBP patients in this study had lower mean SAAG value (1.85±0.45 g/dl) as compared to non-SBP patients (2.25± 0.554 g/dl). Similar results were reported by Thiele et al. **[26]** as mean value of SAAG in SBP was 1.3 g/dl and in non-SBP was 1.7 g/dl. This can be explained by Tarn and Lapworth **[31]** who stated that SBP is advanced liver disease is associated with low serum albumin concentration and so on lower SAAG than cirrhotic patients without SBP, and reinforced by Albillos et al. **[32]** who reported that SAAG should be the test of choice with the addition of an ascitic fluid PMN count to diagnose/exclude bacterial peritonitis. In contrast to these findings Nouman et al. **[33]** observed a lower mean SAAG value (1.2 g/dl) in non-SBP patients as compared to SBP patients (1.5 g/dl), this difference may be related to different numbers of studied patients.MCP-1 is one of the key chemokines that participate in the recruitment of inflammatory cells and is highly expressed under inflammatory conditions **[34]**. MCP-1 acts as a chemotactic factor for monocytes and macrophages, thus, these cells migrate to the ascetic fluid. These monocytes and macrophages release TNF-α and other cytokines, which in turn induces the expression of adhesion molecules on endothelial cells, therapy mediating a systemic reaction to the infection **[23]**. There was significant increase, reported in the present work, in the mean of level of MCP-1 gene expression in both blood and ascitic fluid in SBP than non-SBP which was in agreement with Gabele et al**. [13]**. There was significant decrease in MCP-1 expression in both blood and ascitic fluid after SBP treatment. This finding was in agreement with Kim et al. **[23]** who reported a change in various cytokines levels after treatment of SBP as decrease in MCP-1 and interleukin-10 levels on follow up after treatment. Our results could suggest that this chemokine (MCP-1) may play a pathophysiological role during the course SBP**.** Also this study found that MCP-1 gene expression in both blood and ascitic fluid can be used to diagnose SBP but ascitic expression had higher sensitivity, specificity than blood expression (90%, 80% vs 85%, 76.7% respectively) and we not found any literatures discus this point.

CONCLUSION

MCP-1 gene expression in both blood and ascitic fluid may be related to development and course of SBP, and can be used as a marker for diagnosis of SBP.

Funding: None.
Conflicts of interest: None.
Ethical approval: The protocol of this study was approved by ethical committee of Benha

University and written informed consent was taken from all patients for participation in this work.

REFERENCES

1. Damico G, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. *J Hepatol* 2006; 44:2017-231.
2. Gines P, Auintero E, Arroyo V, Teres J, Bruguera M, Rimola A, et al. Compensated cirrhosis: Natural history and prognostic factors. *Hepatology* 1987; 7:122-128.
3. Pluta A, Gutkowski K, Hartleb M. Coagulopathy in liver diseases. *Adv Med Sci*. 2010;55:16–21.
4. Vincent JL, Gustot T. Sepsis and cirrhosis: many similarities. *ActaGastroenterol Belg.* 2010; 73: 472–478.
5. Sheer TA, Runyon BA. Spontaneous bacterial peritonitis. *Dig Dis.* 2005;23:39–46
6. Salama MK, Sabry D, Al-Ghussein MA, Ahmed R, AbdAllah S, Taha FM et al. Molecular detection of monocyte chemotactic protein-1 polymorphism in spontaneous bacterial peritonitis patients. *World J Gastroenterol.* 2014; 20(33): 11793–11799.
7. Arvaniti V, D'Amico G, Fede G, Manousou P, Tsochatzis E, Pleguezuelo M, Burroughs AK. Infections in patients with cirrhosis increase mortality four-fold and should be used in determining prognosis. *Gastroenterology*. 2010; 139:1246–1256, 1256.e1-5.
8. Gustot T, Durand F, Lebrec D, Vincent JL, Moreau R. Severe sepsis in cirrhosis. *Hepatology*. 2009; 50:2022–2033.
9. Andus T, Gross V, Holstege A, Ott M, Weber M, David M, Gallati H, Gerok W, Schölmerich J. High concentrations of soluble tumor necrosis factor receptors in ascites. Hepatology. 1992; 16: 749–755.
10. Andus T, Gross V, Holstege A, Schölmerich J. High interleukin-6 concentrations in hepatic ascites. *Dig Dis Sci.* 1994; 39: 219–220.
11. Narumi S, Tominaga Y, Tamaru M, Shimai S, Okumura H, Nishioji K, Itoh Y, Okanoue T. Expression of IFN-inducible protein-10 in chronic hepatitis. *J Immunol*. 1997;158:5536–5544.
12. Van Coillie E, Van Damme J, Opdenakker G. The MCP/eotaxin subfamily of CC chemokines. *Cytokine Growth Factor Rev*. 1999; 10(1):61-86.
13. Gabele E, Muhlbauer H, Paulo H, Johann M, Meltzer C, Leidl F et al. Analysis of monocyte chemotactic protein-1 gene polymorphism in patients with spontaneous bacterial peritonitis. *World J Gastroenterol* 2009; 15(44): 5558-5562.
14. Luster AD. Chemokines--chemotactic cytokines that mediate inflammation. *N Engl J Med*. 1998; 338: 436–445.
15. Li P, Garcia GE, Xia Y, Wu W, Gersch C, Park PW, Truong L, Wilson CB, Johnson R, Feng L. Blocking of monocyte chemoattractant protein-1 during tubulointerstitial nephritis resulted in delayed neutrophil clearance. *Am J Pathol*. 2005; 167: 637–649.
16. Maus U, Huwe J, Maus R, Seeger W, Lohmeyer J. Alveolar JE/MCP-1 and endotoxin synergize to provoke lung cytokine upregulation, sequential neutrophil and monocyte influx, and vascular leakage in mice. *Am J RespirCrit Care Med*. 2001; 164: 406–411.
17. European Association for the study of liver (EASL): Gines P, Angeli P, et al. : Clinical practice guidelines on the management of ascites, spontaneous bacterial peritonitis, hepatorenal syndrome in cirrhosis. *J. Hepatol*. 2010; 53:397-417.
18. Wilfinger WW, Mackey K, Chomczynski P. Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. *Biotechniques*. 1997; 22(3):474-6, 478-81.
19. Gong JH, Shin D, Han SY, Kim JL, Kang YH.Kaempferol suppresses eosionphil infiltration and airway inflammation in airway epithelial cells and in mice with allergic asthma. *J Nutr.* 2012; 142(1):47-56.
20. Livak, K. , Schmittgen, T. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 2001; 25: 402-408.
21. Wiest R, Krag A, Gerbes A. Spontaneous bacterial peritonitis: recent guidelines and beyond. *Gut*. 2012; 61:297-310.
22. Syed VA, Ansari JA,Karki P, Regmi M , Khanal B. Spontaneous bacterial peritonitis (SBP) in cirrhotic ascites: A prospective study in atertiary care hospital, *Nepal. Kathmandu University Medical Journal*. 2007; 51:48-59.
23. Kim JH, Lee JS, Lee SH, Bae W K, Kim N, Kim K , Moon Y. The association between the serum sodium level and the severity of complications in liver cirrhosis. *Korean J Intern Med*. 2009; 24(2):106-12.
24. Kasztelan-Szczerbinska B, Slomka M, Celinski K, SerwackiM,Szczerbinski M , Cichoz-Lach H. Prevalence of spontaneous bacterial peritonitis in asymptomatic inpatients with decompensated liver cirrhosis – a pilot study. *Adv Med Sci*. 2011; 56(1):13-7.
25. Mostafa MS, El-Seidi EA, Kassem AM, Shemis MA, Saber M , Michael MN. Detection of ascitic fluid infections in patients with liver cirrhosis and ascites. *Arab J Gastroenterol*. 2011; 12(1):20-4.
26. Thiele G, Silva O, Fayad L, Lazzarotto C, Ferreira M, Marconcini M, et al.,. Clinical and laboratorial features of spontaneous bacterial peritonitis in southern Brazil. *Sao Paulo Med. J*. 2014; 132(4):205-210.

27. Cirera J, Bauer T M,Navasa M, Vila J, Grande L, Taura P, et al.Bacterial translocation of enteric organisms in patients with cirrhosis. *J. Hepatol*. 2001; 34: 32-37.
28. Paul K, Kaur J , Kazal HL. To Study the Incidence, Predictive Factors and Clinical Outcome of Spontaneous Bacterial Peritonitis in Patients of Cirrhosis with Ascites. *Journal of Clinical and Diagnostic Research.* 2015; 9(7): 9-12.
29. Kraja B, Sina M, Mone I, Pupuleku F, Babameto A, Prifti S, Burazeri G. Predictive value of the model of end-stage liver disease in cirrhotic patients with and without spontaneous bacterial peritonitis. *Gastroenterol Res Pract.* 2012; 1-5.
30. Gayatri AA, Suryadharma IG, Purwadi N , WibawaID. The relationship between a model of end stage liver disease score (MELD score) and the occurrence of spontaneous bacterial peritonitis in liver cirrhotic patients. *Acta Med Indones*. 2007; 39(2):75-8
31. Tarn A , Lapworth R. Biochemical analysis of ascitic (peritoneal) fluid: what should we measure? *Ann. Clin. Biochem*. 2010; 47:397-407.
32. Albillos A, Cuervas-Mons V, Millan I, Canton T, Montes J, Barrios C, et al. Ascitic fluid polymorphonuclear cell count and serum to ascites albumin gradient in the diagnosis of bacterial peritonitis.*Gastroenterology*. 1990; 98(1): 134-40.
33. Nouman S, Hussain A, Hussain M , Ahmed M. Frequency of spontaneousbacterial peritonitis in chronic liver disease. *Annals*. 2010; 16:112-5.
34. Graupera I, Solà E, Fabrellas N, Moreira R, Solé C et al. Urine Monocyte Chemoattractant protein-1 is an independent predictive factor of hospital readmission and survival in cirrhosis. *PLoS One*. 2016; 11(6):e0157371. doi: 10.1371/journal.pone. 0157371. eCollection 2016.

Peer reviewer: Amany Ibrahim, Professor of Internal Medicine, Faculty of Medicine, Zagazig University, Egypt.

Editor: Tarik Zaher, Professor of Tropical Medicine, Faculty of Medicine, Zagazig University, Egypt

.

Prednisolone can Prevent Post-Herpetic Neuralgia in Post-Kidney Transplant Recipient

Mohammad Ahmed Saraya[1], Mariam Abdulrhman Al-Fadhli[2] and Jafar Abdulrida Qasem[3]

[1]*Department of Tropical Medicine, Faculty of Medicine, Zagazig University, Zagazig, Egypt.*
[2]*Department of Medicine, Infectious Disease Hospital, Ministry of Health, Kuwait.*
[3]*Department of Applied Medical Sciences, College of Health Sciences, Public Authority for Applied Education and Training, (PAAET).*

Corresponding Author
Mariam Al-Fadhli

Mobile:
+965 99660936

E mail:
medicine209@gmail.com

Key words:
Kuwait, Prednisolone, herpes zoster, post herpetic neuralgia

Background and study aim: Post-herpetic neuralgia (PHN) is a neuropathic painful condition that is one of the most common complications of an acute herpes zoster infection. To date, there is no consensus on the definition of PHN. Early studies used a wide range of definitions, including any pain that follows disappearance of the rash of herpes zoster, whereas other studies used the definition of pain present for more than 1 or 2 months after rash onset.This study aimed to assess the effect of oral prednisolone with antiviral therapy on prevention of post-herpetic neuralgia in post-kidney transplant recipient.

Patients and methods: 40 patients were divided into two groups, the first one (group A) involved 20 patients who had renal transplant within the past five years and have received oral prednisolone (10 mg/day) and the second one (group B) involved also 20 patients but they are immune-competent without co-morbidity. Follow up was done at one, three and six months to assess the pain.

Results: At time of admission and discharge, there was significant difference between group A and group B as regard zoster pain. The same significance was observed between two groups during follow up after one and three months and not observed after six months.

Conclusion: Oral corticosteroid can provide modest benefits in reducing the pain of herpes zoster and the incidence of post-herpetic neuralgia in post-kidney transplant recipient.

INTRODUCTION

Shingles, or herpes zoster, may occur at any stage in a person's life. Herpes zoster is the clinical manifestation of the reactivation of a lifelong latent infection with *Varicella zoster* virus, usually contracted after an episode of chickenpox in early life. *Varicella zoster* virus tends to be reactivated only once in a lifetime, with the incidence of second attacks being <5% **[1].** Herpes zoster occurs more commonly in later life (as T cell immunity to the virus wanes) and in patients who have T cell immune-suppression. The objectives of treating herpes zoster (HZ) are to control acute pain, accelerate rash healing, minimize complications and reduce the risk of post-herpetic neuralgia (PHN) and other late appearing sequels. An additional objective, particularly important for immune-suppressed patients, is to reduce the risk of cutaneous and visceral dissemination of the varicella zoster virus (VZV) **[2]**.

Post-herpetic neuralgia is a neuropathic painful condition that is one of the most common complications of an acute herpes zoster infection. There are three forms of pain defined as follows. First, pain at presentation is acute pain and the extent of its resolution can be quantified over the first 30 days. Second, and the most debilitating form of pain, is PHN. Several definitions of PHN have been used over the past 30 years, and all have different implications for clinical studies. PHN is defined by the US Food and Drug Administration (FDA) as pain that has not resolved 30 days after disease onset. An alternative definition, including any pain that follows disappearance of the rash of herpes zoster **[3]**. The third form of pain is that

of zoster-associated pain (ZAP), whereby pain is viewed as a continuous from the time of acute zoster until its complete resolution, if it occurs. To date, there is no consensus on the definition of PHN. Early studies used a wide range of definitions, including any pain that follows disappearance of the rash of herpes zoster, whereas other studies used the definition of pain present for more than 1 or 2 months after rash onset **[3]**. However, recent models of pain resolution and statistical analysis suggest that the most appropriate definition of PHN is pain that persists 90 days or more after the onset of HZ rash **[4]**.

Corticosteroids have a potent anti-inflammatory action which might minimize nerve damage and thereby relieve or prevent the pain of people suffering from this condition. It is theorized that treatment with steroids before the PHN develops may reduce the risk of PHN developing **[5]**.

We aimed in this study to assess the effect of oral prednisolone with antiviral therapy on Prevention of post-herpetic neuralgia in post-kidney transplant recipient.

PATIENTS AND METHODS

We undertook 40 patients were diagnosed as herpes zoster at Infectious Disease Hospital (IDH) which is the only tertiary infectious hospital in Kuwait. Diagnosis of herpes zoster was based on the presence of the characteristic skin lesions. The skin lesions begin as a maculopapular rash that follows a dermatomal distribution, commonly referred to as a "belt-like pattern". The maculopapular rash evolves into vesicles with an erythematous base.

Patients were excluded if they had a known congenital or acquired immunodeficiency, kidney failure, liver damage, peptic ulcer, generalized or skin infections.

The patients were divided into two groups, the first one (group A) involved 20 patients who had renal transplant within the past five years and have received oral prednisolone (10mg/day) and the second one (group B) involved also 20 patients but they are immune-competent without co-morbidity. All patients were subjected to history taking and thorough clinical examination. We measured liver function test (LFT), kidney profile (KP), complete blood count (CBC), blood glucose, C-reactive protein (CRP) and levels of the pro-inflammatory cytokine interleukin-6 (IL-6) (Biomedix medical group, Synlab, German) on the day of presentation and on the day of discharge from the hospital. Follow up was done at one, three and six months to assess the pain.

All patients in both groups received acyclovir intravenously in proper dose according to body weight for 7 to 10 day **[6]**.

Statistical analysis :

The data was analyzed using the statistical package for social sciences (SPSS) version 8.0 software. The significance of differences between mean values of the study variables was evaluated by using *t*-test. The significance of differences between proportions was performed using the Chi-square test. The P value less than 0.05 is considered significant.

RESULTS

From September, 2013, to November 2014, we enrolled 40 patients in this study and were divided into two groups, group A (post renal transplant patients) and group B (patients without co-morbidity); each of them involved 20 patients. 26 (65%) of 40 patients in both groups A and B were males and 14 (35%) were females.

The mean length of hospital stay in group A was 9.5 days (±0.76) compared with 7.15 days (±0.98) in group B. Length of hospital stay differed significantly between two groups (p-value = 0.02). There was significant increase in the duration of rash in group A as compared with group B (Table 2).

At time of admission, there were significant differences between group A and group B as regard C-reactive protein, interleukin-6 concentrations and zoster pain (Table 1). At time of discharge, the same significant in C-reactive protein, interleukin-6 concentrations and zoster pain were observed between two groups (Table 2).

Follow up after one month, there were two patients had PHN in group A and nine patients in group B and there were significant difference between both groups (Table 3). Follow up after three months, there were no patients had PHN in group A and there were six patients had PHN in group B and there were significant difference between both groups (Table 3). Follow up after six months, no significant difference regarding PHN were observed between groups.

Table (1) : Comparison between studied groups at time of admission

	On admission		
	Group A N (20 patients)	**Group B N (20 patients)**	**P-value**
Age	49.51±6.7	50.40±7.1	0.51
Sex			
Male n (%)	13 (65%)	13 (65%)	1.0
Female n (%)	7 (35%)	7 (35%)	1.0
ALT	41.5±48.3	39.2±47.3	0.71
AST	51.0±36.8	48.1±31.7	0.65
CRP	22.05±5.04	45.05±5.05	0.000
IL6	8.57±4.09	19.79±3.07	0.000
Plt	162.95±31.31	163.45±33.16	0.94
WBCs	5.9±1.15	6.23±1.25	0.24
S. creatinine	117.5±14.12	101.5±13.23	0.21
Herpes Zoster site:			
Thoracic	13 (65%)	10 (50%)	0.12
Lumber	6 (30%)	7 (35%)	0.73
Sacral	1 (5%)	3 (15%)	0.32
Pain at presentation:			
No pain	10 (50%)	0 (0%)	0.000
Mild	7 (35%)	4 (20%)	0.01
Moderate	3 (15%)	11 (55%)	0.001
Severe	0 (0%)	5 (25%)	0.001

Table (2) : Comparison between studied groups at time of discharge

	On discharge		
	Group A N (20 patients)	**Group B N (20 patients)**	**P-value**
ALT	36.2±46.2	34.5±45.3	0.74
AST	49.0±34.6	45.1±36.4	0.71
CRP	12.06±3.3	18.04±3.1	0.001
IL6	6.31±2.02	11.29±2.07	0.01
Plt	180.73±22.4	172.75±25.6	0.61
WBCs	6.3±1.2	6.9±1.3	0.89
S. creatinine	119.4±12.1	101.5±14.3	0.05
Duration of rash	13.6±0.42	8.5±0.54	0.01
Length of hospital stay	9.5±0.76	7.15±0.98	0.02
Pain at discharge:			
No pain	13 (65%)	6 (30%)	0.001
Mild	6 (30%)	3 (15%)	0.001
Moderate	1 (5%)	8 (40%)	0.000
Severe	0 (0%)	3 (15%)	0.001

Table (3) : Comparison between studied groups at 1, 3, 6 months regarding the pain

	Follow up		
	Group A N (20 patients)	**Group B N (20 patients)**	**P-value**
Pain at one month:			
No pain	18 (90%)	11 (55%)	0.001
Mild	2 (10%)	6 (30%)	0.01
Moderate	0 (0%)	3 (15%)	0.01
Severe	0 (0%)	0 (0%)	1.0
Pain at three months:			
No pain	20 (100%)	14 (70%)	0.001
Mild	0 (0%)	5 (25%)	0.001
Moderate	0 (0%)	1 (5%)	0.97
Severe	0 (0%)	0 (0%)	1.0
Pain at six months:			
No pain	20 (100%)	19 (95%)	0.96
Mild	0 (0%)	1 (5%)	0.97
Moderate	0 (0%)	0 (0%)	1.0
Severe	0 (0%)	0 (0%)	1.0

DISCUSSION

Post-Herpetic Neuralgia (PHN) is observed in 9–45% of all cases of herpes zoster (HZ) and the incidence has been reported to be as high as 50–60% among elderly or immune-suppressed patients **[7]**. PHN is simply defined as a form of nerve pain (neuropathy, neuritis) that persists in the area of the rash once the HZ lesions have healed **[3].**

There is general agreement for the need of early therapy that prevent virus multiplication thereby reducing damage to the dorsal horn, dorsal root ganglion, and peripheral sensory receptors in the skin. The early treatment of herpes zoster can not only treat the acute infection but can also prevent the onset of PHN by limiting viral induced nerve damage **[7].**

The difficulties in verifying the validity of the use of acyclovir and steroids in treatment of HZ are due to lack of standardization of concepts. So, we chosen group A to be the patient group because they have already given corticosteroid. The likelihood of patients suffering from HZ after RTX to develop post-herpetic neuralgia or a disseminated VZV disease is up to nine times increased compared with the general population **[8]**. Considering that cellular immunity in patients with end-stage renal disease (ESRD) on dialysis is impaired **[9]** and that cellular immunity in these patients will be massively suppressed at the time of transplantation by current induction therapies **[10]**.

The results of this study demonstrate that the administration of intravenous acyclovir and low dose of corticosteroid in group A can prevent the development of post-herpetic neuralgia (PHN) to a significant greater extent than intravenous acyclovir only in group B at time of discharge, after one month and after three month and this difference is not observed after six months (Table 2 & 3). As regard of acute zoster pain, the difference between the studied groups was observed as early as at time of presentation.

Many authors agree that the use of steroid is effective for both the acute phase pain and for the prevention of PHN, and attribute its efficacy to the anti-inflammatory effects and to lysosomal protection which could reduce neuronal damage **[7].** Other authors, however, doubt the efficacy of steroid and suggest that steroids increase the risk of herpes dissemination **[5]**. In this study, the dermatomal distributions in patients of group A who are immune-compromised and with low dose of corticosteroid do not support this later viewpoint that the use of steroid is ineffective and contribute to systemic dissemination.

Despite, we compare between immune-compromised patients in group A and immune-competent patient in group B, the risk of development of PHN in group B is significantly higher than in group A.

The oral administered of corticosteroids in conjunction with acyclovir has been shown to

reduce the pain associated with herpes zoster. The likely mechanism involves decreasing the degree of neuritis caused by active infection and, possibly, decreasing residual damage to affected nerves **[11]**.

Some studies designed to evaluate the effectiveness of prednisolone therapy in preventing post-herpetic neuralgia have shown decreased pain at three and 12 months **[5]** .Other studies have demonstrated no benefit **[11]**.

In summary, this study demonstrated that the patients in group A, who treated with acyclovir and low dose of corticosteroid, are not at higher risk for developing PHN than the immune-competent patients in group B, who treated only with acyclovir.

CONCLUSION

An orally administered low dose of corticosteroid can provide modest benefits in reducing the pain of herpes zoster and the incidence of post-herpetic neuralgia in post-kidney transplant recipient.

Funding: None.
Conflicts of interest: None.
Ethical approval: Approved.

REFERENCES

1. Cunningham AL,Dworkin RH. The management of post-herpetic neuralgia. *BMJ;* 2000, 321(7264): 778–779.
2. Gnann JW Jr, Whitley RJ. Clinical practice. Herpes zoster. *N Engl J Med;* 2002, 347: 340–6.
3. Whitleya RJ, Volpib A, McKendrickc M, van Wijckd A, Oaklandere AL. Management of herpes zoster and post-herpetic neuralgia now and in the future. *Journal of Clinical Virology;* 2010, (48) S1, S20–S28.
4. Arani RB, Soong S-J, Weiss HL, Wood MJ, Fiddian PA, Gnann JW, et al. Phase specific analysis of herpes zoster associated pain data: a new statistical approach. *Stat Med*; 2001, 20: 2429–39.
5. Han Y, Zhang J, Chen N, He L, Zhou M, Zhu C. Corticosteroids for preventing post-herpetic neuralgia. *Cochrane Neuromuscular Disease Group;* 2013, Published Online: 28 MAR; CD005582.
6. dos Santos MC ,Alecrim.MGC. Varicella Pneumonia in an Adult. *N Engl J Med;* 2010, 362:1227.
7. Pasqualucci A, Pasqualucci V, Galla F, De Angelis V, Marzocchi V, Colussi R, et al . Prevention of post-herpetic neuralgia: acyclovir and prednisolone versus epidural local anesthetic and methylprednisolone. *Acta Anaesthesiol Scand*; 2000, 44: 910–918.
8. Arness T, Pedersen R, Dierkhising R, Kremers W, Patel R . Varicella zoster virus-associated disease in adult kidney transplant recipients. Incidence and risk-factor analysis. *Transpl Infect Dis* 2008, 10: 260-268.
9. Kato S, Chmielewski M, Honda H, Pecoits-Filho R, Matsuo S, Yuzawa Y et al. Aspects of immune dysfunction in end-stage renal disease. *Clin J Am Soc Nephrol* 2008, 3:1526-1533.
10. Litjens NH, Huisman M, van den DM, Betjes MG.Impaired immune responses and antigen-specific memory CD4 + T cells in hemodialysis patients. *J Am Soc Nephrol*; 2008, 19:1483-1490.
11. Stankus SJ, Dlugopolski M,Packer D. Management of Herpes Zoster (Shingles) and Postherpetic Neuralgia. *Am Fam Physician*. 2000 Apr 15, 61(8):2437-2444.

Peer reviewer:Nahla Elgammal, Professor of Tropical Medicine, Faculty of Medicine, Zagazig University, Egypt.

Editor: Tarik Zaher, Professor of Tropical Medicine, Faculty of Medicine, Zagazig University, Egypt

Case Report 1:Guillain Barré Syndrome Post Primary Varicella Infection

Mariam Al-Fadhli[1], Saroj Bala Grover[1], Mohamed Ahmed Saraya[2]
[1]*Department of Medicine, Infectious Diseases Hospital, Kuwait*
[2] *Department of Tropical Medicine, Faculty of Medicine, Zagazig University, Zagazig, Egypt*

Corresponding Author
Mariam Al-Fadhli

Mobile:
+965 99660936

E mail:
medicine209@gmail.com

Key words:
Guillain-Barré syndrome, Varicella zoster, IV immunoglobulin

ABSTRACT

Guillain-Barré syndrome (GBS) is a critical condition that usually arises as a late complication of certain infections. Varicella zoster is (VZ) an extraneous antecedent infection that can cause GBS. We report a rare case of GBS following primary VZV infection in an adult.

INTRODUCTION

Guillain-Barré syndrome is an acute demyelinating polyneuropathy, which is autoimmune in nature. Most of the cases follow an antecedent respiratory or gastrointestinal illness one to three weeks after the onset of symptoms. Usual antecedent infections include *Camylobacter jejuni*, Cytomegalovirus and Ebstein-Barr virus. Other causes include HIV, *Mycoplasma* pneumonia, Lyme disease and lymphoma. Varicella zoster is one of the uncommon causes of GBS and when it does occur it is usually following reactivation of VZ. We are reporting a case of GBS following primary VZ infection.

CASE REPORT

A 28 years old Indian male patient presented to us with giddiness, progressive weakness of his extremities of 4 days duration. It began with difficulty in walking, getting up from the floor and then progressed to difficulty in lifting his hands within the next 2 days. Patient was diagnosed to have chicken pox ten days prior to the onset of these symptoms. On examination, he was afebrile, normotensive and had no respiratory distress. General physical examination revealed dry rash over his trunk and limbs suggestive of recent chicken pox infection. Neurological examination showed reduced muscle tone in all limbs with weakness of all muscle groups. Reflexes were absent and sensory system examination was unremarkable. He also had bilateral abducens paresis and bilateral facial palsy. There was no involvement of other cranial nerves. The cerebellar signs were absent with mute plantar response. At presentation there was no neck or respiratory muscle weakness. There were no fundoscopic changes.
Laboratory investigations showed the following values; a westerngreen erythrocyte sedimentation rate of 95 mm/hr, CRP 12 mg/dl, Hb 11.9 g/dl; Hct 36.5%, WBC 8.5 x 109, 30% segmented neutrophils, 62% lymphocytes, 12% band forms, 4.3% monocytes, platelet 225 x 109, AST 39 IU/L, ALT 32 IU/L, GGT 15 IU/L, LDH 286 IU/L. Blood sugar was normal. Anti-varicella virus IgM antibody was positive. A brain CT was normal and a cerebrospinal fluid tap showed 28 red cells, 4 white cells, protein 200 mg/l, glucose 65mg/dl, and chloride 119.5meq/l. No virus or bacteria were isolated. A nerve conduction velocity test showed increase latency and decreased amplitude.

Patient was started on intravenous steroid. On day four of admission patient developed respiratory distress with an episode of tachycardia and hypertension. He was intubated and connected to ventilator. Blood pressure was controlled by propranolol. Patient was given intravenous immunoglobulins daily for five days. He showed signs

of improvement. Three weeks later the patient was extubated and transferred to ward. One week later he had improved from his neurological condition and discharged from hospital. In the end he recovered completely with no neurological deficits.

DISSCUSSION

This patient had all clinical features of GBS such as weakness, paresthesias, diminished and absent deep tendon reflexes. The finding of nerve conduction study and CSF examination also supported the diagnosis of GBS **[1-3]**.

Primary VZ infection can cause neurological complications such as myelitis, aspectic meningitis, vasculitis, optic neuritis, the most common being encephalitis 1:1000 **[4]**. GBS is the least common 1:15000 **[5]**. GBS following varicella zoster typically has a latent period of 2 weeks to 2 months. Shorter latent periods, as in this case, are associated with more severe complication **[6,7]**.

It is thought that steroid can reduce the severity of GBS, but controlled clinical trials have demonstrated that this treatment not only is not effective but it can have detrimental effect on the disease. The immunoglobulin therapy in GBS can lessen the immune attack on the nervous system. Several hypotheses don't know why or how this works **[8]**.

The most critical part of the treatment for GBS consists of keeping the patient's body functioning during recovery of the nervous system. This can sometimes require placing the patient on mechanical ventilatory assistance,a heart monitor and blood pressure. That's why Guillain-Barré syndrome patients are usually treated in hospitals, often in an intensive care ward **[9,10]**.

CONCLUSION

GBS can present as neurological complication of primary HZV. It should be treated by intravenous immunoglobulin or plasmaphoresis together with supportive care which will lead to good recovery in the majority of cases.

REFERENCES

1. Steiner I, Kennedy PG, Pachner AR. The neurotropic herpes viruses: herpes simplex and varicella-zoster. *Lancet Neurol.* 2007 Nov; 6(11):1015–28.
2. Hughes RAC, Hadden RDM, Gregson NA, Smith KJ. Pathogenesis of Guillain–Barré syndrome. *J Neuroimmunol.* 1999 Dec; 100(1–2):74–97.
3. Puchhammer-Stöckl E, Popow-Kraupp T, Heinz FX, Mandl CW, Kunz C. Detection of varicella-zoster virus DNA by polymerase chain reaction in the cerebrospinal fluid of patients suffering from neurological complications associated with chicken pox or herpes zoster. *J Clin Microbiol.* 1991 Jul 1; 29(7):1513–6.
4. Legeune B, Alix D, Le Fur JM, Chastel C. Syndrome de Guillain Barré et Varicella. *Arch Fr Pediatr* 1980; 38:139.
5. Sanders EACM, Peters ACB, Gratana GM, Huges RAC. Guillain Barré Syndrome after Varicella Zoster infection. Report of 2 cases, *J. Neurol* 1987; 235:437-439.
6. Cresswell F, Eadie J, Longley N, Macallan D. Severe Guillain–Barré syndrome following primary infection with varicella zoster virus in an adult. *Int J Infect Dis.* 2010 Feb; 14(2):e161–3.
7. McCrary ML, Severson J, Tyring SK. Varicella zoster virus. *J Am Acad Dermatol.* 1999 Jul; 41(1):1–16.
8. Lehmann HC, Hartung H-P. Varicella-zoster virus: another trigger of Guillain-Barré syndrome? *Clin Infect Dis Off Publ Infect Dis Soc Am.* 2010 Sep 1; 51(5):531–3.
9. Pavone P, Maccarrone F, Sorge A, Piccolo G, Greco F, Caruso P, et al. Guillain-Barré syndrome after varicella zoster virus infections. A case report. *Minerva Pediatr.* 2002 Jun; 54(3):259–62.
10. Roccatagliata L, Uccelli A, Murialdo A. Guillain-Barré syndrome after reactivation of varicella-zoster virus. *N Engl J Med.* 2001 Jan 4; 344(1): 65–6.

Case Report 2 :Rabies Through Organ Transplant

Mariam Al Fadhli[1], Saroj Bella Grover[1], Mohamed Ahmed Saraya[2]
[1]*Department of the Medicine, Infectious Diseases Hospital, Kuwait.*
[2]*Department of Tropical Medicine, Faculty of Medicine, Zagazig University, Zagazig, Egypt.*

Corresponding Author
Mariam Al-Fadhli

Mobile:
+965 99660936

E mail:
medicine209@gmail.com

Key words:
Rabies, Transmission, recipient, infectious

ABSTRACT

The rabies virus causes fatal encephalitis and can be transmitted through tissue or organ transplantation. In March 2015, a kidney recipient with no reported exposures to potentially rabid animals died from rabies after transplantation.

INTRODUCTION

Rabies is an endemic fatal Zoonotic disease commonly transmitted to humans through contact (bites and scratches) with infected animals. Human rabies is rare in Kuwait. Most cases of rabies that have occurred in Kuwait are imported cases, having history of exposure to a rabid animal in their country. Rabies transmission via solid organ transplantation though reported in different parts of the world is rare. We report a case of rabies in a kidney transplant recipient with no reported exposure to a potentially rabid animal. This patient developed fatal encephalitis and died. This is the first case of rabies in an organ recipient in Kuwait.

CASE DESCRIPTION

A 5 year old Kuwaiti child presented with history of high grade fever and status epileptics. She was a known case of renal failure, since birth. 3 months prior to admission the child had been transplanted kidney from an Indian male who had died due to encephalitis, the cause for which was not established.

Anticonvulsants drugs were started, in addition to cefotaxime and acyclovir after doing CT scan brain, and lumbar puncture which were normal. She was put on mechanical ventilation, as had generalized convulsions and tremors. She was febrile, had tachycardia, was well hydrated, BP 120/70 mmHg. The examination of the heart, chest and abdomen were unremarkable. Complete blood counts, renal function tests, liver function tests, biochemistry, blood culture, were normal. PCR for CMV was high but other virology study such as HHV6, HHV7, HHV8, EBV, Enterovirus, Coxackie were IgM negative. She later received injection meropenum, gangcylovir, intravenous immunoglobulin and injection ambisone. Later the child died due to respiratory distress.

DISCUSSION

Our case has received organ (kidney) from a donor who had recently died of encephalititis, the cause for which had not been established. Also from the same donor, recipients who has received kidney, heart, liver, developed signs and symptoms similar to the one that the donor had presented with and died. Suspicion of Rabies was raised and the source was suspected to be the donor.

Rabies is not endemic in Kuwait. Most cases of Rabies are imported cases with the history of the patient being exposed to a rabid animal in their own country. Within the last 10 years we had only 3 cases, with the last one, being 2 years back.

Hence, in our case though the patient had presented with features of encephalitis, diagnosis of Rabies was suspected retrospectively when cluster of cases with similar signs and symptoms occurred raising the suspicion of Rabies in the other organ recipients. An investigation was initiated. The father of the donor was contacted in India and he indicated that the donor was bitten by a street dog in his home town, prior to his return to Kuwait and the dog had died. Furthermore the biopsy for rabies taken from the recipient of the organ had confirmed the diagnosis. The two corneal recipients from the same donor were not infected after their grafts were removed and were given rabies post exposure prophylaxis, and they are being followed closely.

Rabies is a preventable viral disease of mammals, commonly transmitted to humans through contact (bites and scratches) with infected animals. One important mode of no bite transmission is person to person via organ transplantation particularly in corneal transplantation **[1-7] s**uch transmission has occurred in eight recipients in five countries like Thailand (two cases), India (two cases), Iran (two cases) U.S.A (one case) and France (one case) **[8]**.

Human rabies due to organ transplantation has been reported, though quite rare. In 2004, CDC confirmed the first reported cases **[9]**. Although rabies transmission had occurred previously through cornea transplants, this was the first report of rabies transmission via solid organ transplantation. The organ donor had undergone routine eligibility screening, including laboratory testing. One of the organ recipient died during transplant surgery and the other three recipients died later of rabies. It was discovered later (after the death of the donor) from the friends of the donor that the later had recently been bitten by a bat. A similar case had happened in Maryland. The man died from Rabies after receiving a kidney from an infected donor **[9]**. The recipient had no reported animal exposure. CDC laboratories tested tissue samples from the donor and the recipient who had died to confirm diagnosis of rabies through organ transplantation. Both had the same type of virus – a raccoon type **[9]**.

A similar rabies transmission through organ transplantation occurred in Germany **[9]**. Six recipients received organs or tissues from a donor with rabies. Two recipients receiving donor corneas were not infected after their grafts were removed. Recipients who received lung, kidney and combined Kidneys and pancreas died. The liver recipient had been previously vaccinated against rabies and survived.

Usually if Rabies is not clinically suspected laboratory testing for Rabies is not routinely performed, as it is difficult for doctors to confirm results in the short time, the organs have to be kept viable for the recipients. This happened in our case.

Given the mortality with transplant associated encephalitis, it becomes imperative to suspect rabies as a cause for infectious encephalitis among organ donors, especially if the donor is from endemic area.

CONCLUSION

Rabies can be difficult to diagnose especially in countries where it is a rare disease. There is a lack of awareness of rabies being a cause of obscure behavioral and neurologic manifestation especially if a history of bite does not exist. In that event the disease may easily be mistaken for other neurological or psychiatric condition.

We suggest that any organ procurement organization should add a question for exposure to any animal in the screening questioner, even though it may be a rare disease in their countries. What also needs to be looked into more closely that organs from donors especially those from endemic areas of Rabies presenting with infectious encephalitis should only be used in extreme circumstances, especially when screening for rabies prior to donation cannot be done due to lack of facilities for screening.

REFERENCES

1. Chang HG, Eidson M, Noonan – Toly C, et al. Public Health impact of reemergence of rabies, New York. *Emerg Infect Dis* 2002; 8:909-13.
2. Rupprecht CE, Briggs D, Brown C, et al. Evidence for a 4 – dose vaccine schedule for human rabies post exposure prophylaxis in previously non-vaccinated individuals. *Vaccine* 2009; 27:7141-8
3. Dutta JK. Rabies transmission by oral and other no -bite routes. *J Indian Med Assoc*. 1998; 96:359
4. Schoenstadt A. Health information brought to life, rabies transmission. Available at: http://rabies. emedtv.com/rabies/rabies - transmission. html.

5. CDC – Human rabies preventation - United States 2008. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2008; 57 (RR-3): 1-28

6. Javadi MA, Fayaz A, Mirdehghan SA, Ainollahi B. Transmission of Rabies by Corneal graft. *Cornea* 1996; 15(4) 431-3.

7. Johnson N, Lipscombe DW, Stott R, et al. Investigation of a human case of Rabies in the United Kingdom. *J Clin Virol* 2002; 25: 351-6

8. Susan S, Ahmad F, Pooneh R, at el. Six fatal cases of classical rabies virus without biting incidents, Iran 1990 – 2010. *J Clin Virol* 2012; 54:251-254

9. CDC – News (March 15, 2013): Q&A – Human Rabies due to organ transplantation 2013 http://www.cdc.gov/rabies/resources/news/2013-03-15.html.