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Evaluation of Serum Nitric Oxide as a Diagnostic and Prognostic Marker in Patients with Hepatocellular Carcinoma Starting Loco-Regional Therapy

KEYWORDS: Serum nitric oxide; Hepatocellular Carcinoma; loco-regional therapy

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ABSTRACT *Background: Hepatocellular carcinoma (HCC) is a primary malignancy of the liver and occurs predominantly in patients with underlying chronic liver disease and cirrhosis. HCC is now the third leading cause of cancer deaths worldwide, with over 500,000 people affected. The incidence of HCC is highest in Asia and Africa, where the endemic high prevalence of hepatitis B and hepatitis C strongly predisposes to the development of chronic liver disease and subsequent development of HCC.*

Aim of the study: To evaluate the level of serum nitric oxide as a diagnostic and prognostic marker for patients with HCC subjected to Loco-Regional therapy.

Patients and Methods: The study included 30 patients with HCC candidate for loco-regional therapy and 15 cirrhotic non-HCC patients as control subjects. Basal serum nitric oxide level was measured in both groups with assessment of its level after one month for HCC patients subjected to loco-regional therapy.

Results: Serum nitric oxide level was proved to rise in patients with HCC rather than cirrhotic non-HCC patients (120 ± 37.9 vs. 68.7 ± 20µmol/L respectively; p<0.001). Significant reduction of serum nitric oxide level was recorded one month after loco-regional therapy when compared to the basal level (120 ± 37.9 vs. 89 ± 29.7 respectively; p<.001) together with good clinical and radiological response.

Conclusions: The study concluded that serum nitric oxide can be used as a novel diagnostic marker for HCC together with a strong impact on the success of loco-regional therapy giving it a prognostic value.

INTRODUCTION

Liver Cancer rapidly reduces quality of life and typically causes death within 6 months-1year from diagnosis **[1]** Globally, it's the fifth leading cause of cancer and the third leading cause of cancer death. This cancer varies widely in incidence throughout the world with rising incidence in Egypt. The primary risk factors for hepatocellular carcinoma (HCC) include Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), dietary Aflatoxin exposure and chronic alcohol consumption**[2]**

More recently, HCV has begun to eclipse HBV in incidence in many countries throughout North America, Europe and the Middle East**[3]** The rates of HCV in Egypt are among the highest in the world, with a prevalence rate up to 20% **[4]** Recent investigations in Egypt have shown the increasing importance of HCV infection in the etiology of liver cancer estimated to account for 40-50% of cases, and the declining influence of HBV and HBV/HCV infection (25% and 15% respectively) **[5]**

There are several potentially curative or palliative approaches to the treatment of hepatocellular carcinoma**[6]** Loco-Regional therapy is considered among the important lines for treatment of hepatocellular carcinoma including: Radiofrequency ablation that has become the most frequently used form of local ablation therapy. It is the best treatment alternative for patients with early stage hepatocellular carcinoma who are not eligible for surgical resection or transplantation. Several recent randomized trials of adequate quality have shown radiofrequency ablation to be more effective than the once-conventional method of ethanol injection in treating patients with small hepatocellular tumors (2 to 3 cm in diameter), with lower rates of local recurrence and higher rates of overall and disease- free survival **[7]**

Trans Arterial Chemo-Embolization (TACE) has been shown to improve survival among patients with preserved liver function, particularly those with Child–Pugh class A cirrhosis who do not have extrahepatic metastases, vascular invasion, or prominent cancer-related symptoms. A meta-analysis of randomized, controlled trials assessing the use of arterial embolization, chemoembolization, or both as primary palliative treatment for hepatocellular carcinoma showed that these procedures were associated with an improved 2-year survival rate as compared with conservative treatment. TACE is also used as a neo-adjuvant therapy or as a means of down staging a patient's condition before liver transplantation, but whether these approaches provide a survival benefit is unclear **[8]**

Radio-embolization with yttrium-90 microspheres has recently been used as palliative treatment for patients with Child–Pugh class A cirrhosis and intermediate stage hepatocellular carcinoma **[9]**

Nitric oxide, known as the 'Endothelium-Derived Relaxing

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Factor', or 'EDRF', is biosynthesized endogenously from L-Arginine, oxygen and NADPH by various Nitric Oxide Synthase (NOS) enzymes. Reduction of inorganic nitrate may also serve to make nitric oxide. The endothelium (inner lining) of blood vessels uses nitric oxide to signal the surrounding smooth muscles to relax, thus resulting in vasodilation and increasing blood flow. Nitric oxide is highly reactive (having a lifetime of a few seconds), yet diffuses freely across membranes. These attributes make nitric oxide ideal for a transient paracrine (between adjacent cells) and autocrine (within a single cell) signaling molecule **[10]**

Nitric oxide (NO) contributes to vessel homeostasis by inhibiting vascular smooth muscle contraction and growth, platelet aggregation, and leukocyte adhesion to the endothelium. Humans with atherosclerosis, diabetes or hypertension often show impaired NO pathways **[11]**It was found that NO acts through the stimulation of the soluble guanylate cyclase, which is a heterodimeric enzyme with subsequent formation of cyclic GMP. Cyclic GMP activates protein kinase G, which causes phosphorylation of myosin light chain phosphatase, and therefore inactivation of myosin light-chain kinase, and leads ultimately to the dephosphorylation of the myosin light chain, causing smooth muscle relaxation **[12]**

SUBJECTS AND METHODS

The study had been carried out in The Advanced Center for Liver diseases – Zagazig University Hospitals. The study was done on 45 subjects who were classified into 2 groups:

Patients' group consists of 30 patients previously diagnosed for having Hepatocellular Carcinoma and are eligible for Loco-Regional therapy either by Radiofrequency Ablation, Microwave Ablation or Trans arterial Chemo-Embolization and a control group consisting of 15 patients previously diagnosed as Chronic Liver Disease patients at different stages of liver cirrhosis due to HCV infection.

Inclusion criteria:

- Patients previously diagnosed for having Hepatocellular Carcinoma and are eligible for Loco-Regional therapy either by Radiofrequency Ablation, Microwave Ablation or Trans arterial Chemo-Embolization.
- Patients previously diagnosed as Chronic Liver Disease patients at different stages of liver cirrhosis due to HCV infection .

Exclusion criteria:

- Any patients with Hepatocellular Carcinoma who are eligible for surgical resection or conservative treatment according to standard criteria for management of patients with hepatoma.
- Auto-immune hepatitis.
- Alcoholic hepatitis.
- Patients with Chronic liver Disease due to HBV infection.
- Patients with Renal impairment.
- Cardiac patients receiving Nitrate therapy.
- diabetes mellitus, hypertension, obesity, dyslipidemia, metabolic syndrome.

Ethical Clearance: Written informed consent from the patients if possible or from their first degree relatives to participate in the study.

Steps of performance and techniques used:

- Brief History taking.
- Clinical examination.
- Routine Laboratory investigations including Complete Blood Count, Liver Function Tests, Kidney Function Tests, Prothrombin Time, Partial Thromboplastin Time, International Normalization Ratio and Alpha Feto Protein as a marker for Hepatocellular Carcinoma.
- Routine Radiological investigations including Pelvi-Abdominal Ultrasonography and Triphasic Computed Tomography on the Abdomen and the Pelvis to assess the detailed presence of Focal lesions in the liver for accurate diagnosis of Hepatocellular Carcinoma.
- Serum Nitric Oxide will be assessed using ELISA at first to compare between its levels in patients with HCC and patients with Chronic Liver Disease then series of samples to be taken from patients with HCC subjected to loco-regional therapy within before and one month after the procedure aiming to assess the correlation in between Serum Nitric Oxide and tumor regression post Loco-Regional Therapy.

Measurement of Serum Nitric Oxide:

Total Nitric oxide samples were obtained using ***R&D Systems'® Total Nitric oxide kit.***

R&D Systems' Total Nitric Oxide kit has two assay options.

Serum samples were obtained using a serum separator tube (SST).

Centrifugation was done for 15 minutes at 1000 x grand samples were stored at ≤ 20°c.

Statistical Methods:

Data collected throughout history, thorough clinical examination, laboratory investigations, radiological investigations and outcome measures were coded, entered and analyzed using Microsoft Excel software.

Data were then imported into Statistical Package for the Social Science **(SPSS version 20.0)** software for analysis

- Mean ± standard deviation with median and range when appropriate described quantitative data. Numbers with percentages described qualitative data. Chi-Square Test was used to compare proportions whilst ANOVA, LSD and independent t test were used to compare different parameters.
- Correlations were estimated using Pearson's Correlation.
- ***P*** value is considered significant at ≤0.05 level, highly significant at ≤0.001 and not significant at >0.05 level.

RESULTS

Table (1) : Laboratory Data of different studied groups (Liver Function Tests)

Variables	Patient Group (HCC) N = 30	Control Group (Cirrhosis) N = 15	***Kruskal Wallis Test***	***Significance***
Total Bilirubin	mg/dl	mg/dl		
Mean ± SD	1.17 ± 0.28	2.4 ± 1.1		
Median	1.1	2.3	5.1	<0.001
Range	0.7 – 1.9	1.2 – 5.4		(HS)

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Serum Albumin	gm/dl	gm/dl		
Mean ± SD	3.46 ± 0.31	2.89 ± 0.47	3.2	0.002
Median	3.45	2.9		(HS)
Range	2.9 - 4	1.8 – 3.7		
ALT	U/L	U/L		
Mean ± SD	39.7 ± 5.8	47 ± 8.87		0.001
Median	40.5	48	3 .5	(HS)
Range	30 - 54	31 - 60		
AST	U/L	U/L		
Mean ± SD	48.6 ± 7.6	62 ± 11		<0.001
Median	49	63	4.1	(HS)
Range	35 - 63	40 - 88		

Table (2) : Laboratory Data of different studied groups (Platelet Count , INR and Serum Creatinine)

Variables	*Patient Group (HCC)* ***N = 30***	*Control Group (Cirrhosis)* ***N = 15***	*Kruskal Wallis Test*	*Significance*
Platelet Count	/cmm	/cmm		
Mean ± SD	155400 ± 16443	99466 ± 24456		
Median	155000	101000	6.02	<0.001
Range	112000 - 186000	51000 - 143000		(HS)
INR				
Mean ± SD	1.16 ± 0.1	1.47 ± 0.27	4.1	<0.001
Median	1.2	1.4		(HS)
Range	1 – 1.5	1.1 - 2		
Serum Creat.	mg/dl	mg/dl		
Mean ± SD	0.91 ± 0.25	0.92 ± 0.27		0.609
Median	0.9	0.9	6.5	(NS)
Range	0.5 – 1.4	0.5 – 1.4		

Table (3) : Laboratory Data of levels of AFP and Serum Nitric Oxide in different studied groups

Variables	*Patient Group (HCC)* ***N = 30***	*Control Group (Cirrhosis)* ***N = 15***	*Kruskal Wallis Test*	*Significance*
AFP	ng/L	ng/L		
Mean ± SD	59 ± 34	5.79 ± 2.8	5	<0.001
Median	57	6.5		(HS)
Range	8.8 - 154	1.5 – 10.9		
Serum Nitric Oxide	µmol/L	µmol/L		
Mean ± SD	120 ± 37.9	68.7 ± 20	4.8	<0.001
Median	116	66		(HS)
Range	33 - 206	32 – 95.8		

Table (4) :Clinical and Radiological Data of Patients' Group (HCC)

Characters	No.	***Percentage %***
CTP Score		
A	28	93.33
B	2	6.66
C	0	0
CT classic findings		
YES	29	96.66
NO	1	3.33
Ascites		
YES (minimal)	5	16.66
NO	25	83.33
Jaundice		
YES (mild)	7	23.33
NO	23	76.66
Splenomegaly		
YES	4	13.33
NO	26	86.66
Procedure Done		
RFA	16	53.33
TACE	14	46.66

Table (5) : Association between Serum Nitric Oxide and Size of Lesion as documented by Triphasic CT

Variables	Pearson's Coefficient	Significance
Serum Nitric Oxide	**r**	**p**
Size of Lesion	0.22	0.2 (NS)

Table (6) : Association between Alfa Feto Protein and Size of Lesion as documented by Triphasic CT

Variables	Pearson's Coefficient	Significance
AFP	**r**	**p**
Size of Lesion	0.099	0.6 (NS)

Table (7) : Comparison between CTP Score in Patients' Group (HCC) before and after being subjected to locoregional therapy

CTP score	Before LRT	After LRT	Wilcoxon-Sign Rank Test
A	28	24	***p***
B	2	5	0.024
C	0	1	(HS)

Table (8) : Association between levels of Serum Nitric Oxide before and one month after being subjected to locoregional therapy

Serum Nitric Oxide	Mean ± SD	Wilcoxon – Sign Rank Test	***Significance***
Before Locoregional Therapy	120 ± 37.9	***r = 0.92***	***p =<0.001***(HS)
After Locoregional Therapy	89 ± 29.7		

Table (9) : Association between levels of Alfa Feto Protein before and one month after being subjected to locoregional therapy

Alfa Feto Protein	Mean ± SD	Wilcoxon – Sign Rank Test	***Significance***

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Before Locoregional Therapy	59±34		
After Locoregional Therapy	50±35	***r = 0.86***	***p = < 0.001(HS)***

Table (10) : Association between levels of Serum Nitric Oxide after being subjected to locoregional therapy in relation to successful and failed patients

Serum Nitric Oxide after Locoregional Therapy			
Failed (6)	***Successful (24)***	Mann – Whitney Test	
Mean ± SD	133 ± 16.6	78.4 ± 20	***p = <0.001*** (HS)

Table (11) : Outcome of Locoregional therapy on Patients' Group (HCC)

Outcome	No.	Percentage %
Response		
Good	24	80
Poor	6	20
Recurrence		
Present	4	13.33
Absent	24	80
Failure and Death	2	6.66%

Table (12) : Validity of Serum Nitric Oxide in diagnosis of Hepatocellular Carcinoma

Subjected Groups	HCC N = 30	Cirrhotics N = 15	***Total***
Above 110.65 µmol / L	16	1	17
Below 110.65 µmol/ L	14	14	28
Total	30	15	45

N.B **Cut-off value for serum Nitric Oxide = 110.65 µmol/L**

* Sensitivity = (16/30) x100 = 53.3%
* Specificity = (14/15) x100 = 93.3%
* Positive Predictive Value = (16/17) x 100 = 94%
* Negative Predictive Value = (14/28) x 100 = 50%

Table (13) : Validity of Serum Nitric Oxide in prognosis of Hepatocellular Carcinoma

Subjected Groups	Failed N = 6	Successful N = 24	***Total***
Above 110.65 µmol / L	6	1	7
Below 110.65 µmol/ L	0	23	23
Total	6	24	30

N.B **Cut-off value for serum Nitric Oxide = 110.65 µmol/L**

* Sensitivity = (6/6) x100 = 100%
* Specificity = (23/24) x100 = 95.8%
* Positive Predictive Value = (6/7) x 100 = 85.7%

* Negative Predictive Value = (23/23) x 100 = 100%

Table (14) : Validity of Alfa Feto Protein in diagnosis of Hepatocellular Carcinoma

Subjected Groups	HCC N = 30	Cirrhotics N = 15	***Total***
Above 200 ng/ ml	0	0	0
Below 200 ng/ ml	30	15	45
Total	30	15	45

N.B **Cut-off value for serum Alfa Feto Protein = 200ng/ ml**

* Sensitivity = (0/30) x100 = 0%

* Specificity = (15/15) x100 = 100%

* Positive Predictive Value = (0/0) x 100 = 0%

* Negative Predictive Value = (15/45) x 100 = 33.3%

Table (15) : Validity of Alfa Feto Protein in prognosis of Hepatocellular Carcinoma

Subjected Groups	Failed N = 6	Successful N = 24	***Total***
Above 200 ng/ ml	0	0	0
Below 200 ng/ ml	6	24	30
Total	6	24	30

N.B **Cut-off value for serum Alfa Feto Protein = 200 ng/ ml**

* Sensitivity = (0/6) x100 = 0%
* Specificity = (24/24) x100 = 100%
* Positive Predictive Value = (0/0) x 100 = 0%
* Negative Predictive Value = (24/30) x 100 = 80%

DISCUSSION

Hepatocellular carcinoma (HCC) is the fifth most common cancer in men and the seventh most common in women**[13]**The incidence of this particular cancer varies throughout the world, with rising incidence in Egypt **[14]**

In Egypt, HCC was considered as a common malignant tumor, accounting for about 4.7% of chronic liver disease patients with most HCC patients being presented at a late stage in approximately 85% of cases **[15]**Risk factors that lead to the multistep development of HCC are well known and it is established that approximately 80% of HCC cases developing individuals suffering from chronic hepatitis B or C viral infection HBV or HCV, cirrhosis, and also those with a high exposure to aflatoxin-B1 as well as those with a high intake of alcohol **[16], [17] , [18].**

NO, a small potent lipophilic gas with divergent biological activities, seems to play an important role in modulating tissue injury and carcinogenesis. Three distinct forms of nitric oxide synthase (NOS) catalyze the formation of NO. Endothelial NOS and neuronal NOS are constitutively expressed in different tissues, whereas inducible nitric oxide synthase (iNOS) is related to a high-output pathway for NO production which contributes to tumor cell angiogen-

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esis as well as the invasion and metastases of HCC**[19] , [20].**

The formation of new functional blood vessels occurs in several phases including endothelial cell budding which is facilitated by vasodilatation, loosening of inter-endothelial contacts and leakage from pre-existing vessels. These phenomena allow extravasation of plasma proteins that together with the extracellular matrix components facilitate the laying down of a provisional scaffold for migrating endothelial cells **[21] , [22].**

HCV infection causes elevated iNOS transcription which might be responsible for carcinogenesis in the cirrhotic liver and these effects depend on NO concentrations **[23].**

The present study included (30) patients diagnosed as HCC on top of liver cirrhosis presented to the Advanced Center for Liver Diseases (ACLD) – Zagazig University Hospitals that were highly selected for subjection to locoregional therapy together with (15) cirrhotic non-HCC patients who were randomly chosen as a control group.

Tables (1) & (2) showed that there was high significant increase in the mean values of total bilirubin , ALT , AST and INR among the cirrhotic non-HCC patients when compared to HCC patients with high significant decrease in the mean values of serum albumin and platelet count in between both subjected groups.That was attributed to high selectivity of HCC patients before being subjected to locoregional therapy while those cirrhotic non-HCC patients showed much deterioration concerning liver functions as they were randomly chosen with no certain criteria for selecting them.

Clinical and radiological data of highly selected HCC supposed to be subjected to locoregional therapy included (28) patients with CTP score (A) (93.33%) with only (2) patients with CTP score (B) (6.66%) with classic triphasic CT findings present in (29) patients (96.66%). Minimal ascites was found in only (5) patients (16.66%) with mild jaundice documented in only (7) patients (23.33%) and mild splenomegaly in (4) patients (13.33%).RFA was done in (16) patients (53.33%) while (14) patients (46.66%) were subjected to TACE as documented in ***table (4).***

In the present study, we found a high significant increase in the serum concentration of AFP in HCC patients as compared to cirrhotic non-HCC patients as shown in ***table (3)***. This was in agreement with **[24]** who stated that the distinction between HCC and cirrhosis has become challenging because regenerative nodules may mimic tumors in cirrhotic livers and also because of elevated levels of AFP in cirrhotic patients. Similar results were obtained throughout many studies including **[25] , [26] , [27].**

The present study has shown that AFP median value was significantly higher in the studied HCC patient group before being subjected to locoregional therapy (59 ng/ml) than the matched control group (5.79 ng/ml) where $p< 0.001$.These results agreed with **[28]**whostated that AFP appeared in many parechymatous liver diseases such as acute viral hepatitis and chronic hepatitis but higher levels were found most frequently in HCC.

Table (9) apparently showed significant reduction in median values of AFP in those patients with HCC within one month after the procedure done during regular follow up (50 ng/ml) as compared to values before doing the procedure (59 ng/ml) where $p< 0.001$ together with an awesome clinical and radiological response.

Tables (14) and (15) discussed the validity of AFP in diagnosis of HCC and possibility of predicting the prognosis of those HCC patients after being subjected to locoregional-therapy. The cut-off value of AFP was estimated to be (200 ng/ml) according to the results obtained from the present study.

Interestingly, (zero) patients were above the estimated cut-off value while (15) cirrhotic non-HCC patients were below this cut-off value giving it a sensitivity of (0%), a specificity of (100%) with a positive and a negative predictive values of (0% and 33.3%) respectively. Thus, it may give an idea about the uselessness of AFP as a marker for diagnosis of HCC anymore.These results were consistent with **[29]**who suggested that AFP is unsatisfactory for the purpose of detection of hepatocellular carcinoma due to its poor sensitivity and specificity, thus, he suggests the need for novel biomarkers for the detection of early HCC.

Other studies have shown nearly similar results with specificity close to (100%) but with a sensitivity below (45%) **[30].**

Concerning the impact of AFP on the success of the procedure , the results gave a sensitivity , a specificity , a positive and a negative predictive values of (0% , 100% , 0% & 80%) respectively giving a brilliant impact for successful procedure of locrogeional therapy.

In the present study, there was a significant increase in the median values of serum nitric oxide in the HCC patients' group before the procedure done (120 µmol/L) as compared to the cirrhotic non-HCC control group (68.7 µmol/L) where $p < 0.001$ as in ***table (3)***. Those results were in agreement with **[31]** who stated that the relationship between chronic inflammation and tumor genesis has been long suspected. It is well known that malignant tissues are infiltrated by leukocytes, which locally secrete cytokines, chemokines, matrix-degrading enzymes, growth factors, free radicals, and oxidants. This creates a microenvironment that may enhance cell proliferation, survival, migration, as well as angiogenesis, thereby promoting tumor development.

[32] as well confirmed that a particularly important role of increased NO generation in this microenvironment is now well recognized as an essential step initiating neoplastic transformation.

[33]stated that not only immune cells infiltrating the tumor, but the tumor cells themselves, are able to produce large amounts of NO due to induced expression of iNOS, which may prevail in rapidly growing tumors.

[34]confirmed that there was an evidence for a role of NO overproduction as a mechanism initiating and promoting tumor genesis, also **[35]** reported that the double edged sword of NO in tumor biology clearly depends on cell type, NO concentration, oxidiative stress and tumor milieu.

Interestingly, ***table (8)*** compared the association between mean levels of serum NO before and one month after being subjected to locoregional therapy during the routine follow up. It showed significant reduction in those patients during the short term follow up one month after the procedure done (89 µmol/L) when compared to those levels before being subjected to the procedure (120 µmol/L)

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where $p < 0.001$. This result was consistent with **[36]**who stated that overproduction of NO might represent an essential link between inflammation and carcinogenesis with playing a crucial role in tumor genesis when they observed significant reduction in levels of NO two weeks following ablation by radiofrequency.

As a matter of consideration, (24) patients among the HCC patients' group proved success with good clinical and radiological response to the procedure done on the routine follow up after one month while (4) patients didn't show that response and was supposed to try another treatment strategies, while (2) patients developed deteriorated general condition and developed Hepatic encephalopathy in which (1) patient died later on. This was documented briefly in ***table (11)***.

Table (10) discussed in brief the impact of mean values of serum NO on the success of the procedure done in which it showed significant reduction in levels of serum NO in those patients who developed successful procedure (78.4 µmol/L) compared to those whom the procedure didn't work out with (133 µmol/L) where $p < 0.001$. This was in agreement with **[37] , [38] , [39]** who suggested that tumor-promoting influence of NO had been identified by stimulating tumor angiogenesis, inducing angiogenic and lymphangiogenic factor expression, most significantly vascular endothelial growth factor VEGF, and by stimulating blood vessel maturation via the recruitment of perivascular cells pericytes as documented by **[40] , [41].**

This also was consistent with **[42] , [43]**who stated that NO had been associated with enhanced migration and invasion of tumor cells through mechanisms depending on guanylcyclase and MAPK signaling.

[32]stated that these influences of NO depended on the duration and level of NO exposure, the type of iNOS-expressing cells and cellular sensitivity to nitric oxide cytotoxic activity.

Tables (12) and (13) discussed the validity of serum NO in diagnosing HCC and possibility of predicting the prognosis of those patients after being subjected to locoregional therapy. The estimated cut-off value of serum NO obtained from the results of the present study was (110.65 µmol/L). The diagnostic performance of NO showed a moderate sensitivity of (53.3%) with a strong specificity of (93.3%) with positive and negative predictive values of (94% & 50%) respectively giving it a possibility of being a marker for diagnosing HCC. However, due to small sample size, these data need to be validated with a larger sample size.

The results of the present study showed a high sensitivity for failure of the procedure (100%) with a high specificity for success of the procedure done (95.8%) together with positive and predictive values of (85.7% & 100%) respectively. Thus, NO has a strong impact on the success or failure of procedure of locoregional therapy during the regular follow up term. These results were in agreement with **[33]** who stated that hepatocytes produced NO in response to several inflammatory stimuli. Tumor cells themselves were able to produce large amounts of NO due to induced expression of iNOS, which might prevail in rapidly growing tumors.

Also, **[44]** supposed that there was a possibility that NO production by hepatic tissue is accelerated in patients with HCC.

[45]confirmed that increased NO generation was well-recognized as an essential step initiating neoplastic transformation. Added to this, **[23]** stated that NO played an important role in HCC development and its progression.

A possible explanation for increased serum NO levels in HCC is that NO is reactively induced by the hepatic tissue surrounding HCC by three independent mechanisms: (1) tumor cells directly stimulate macrophages and Kupffer cells to produce NO, (2) HCC produces a variety of cytokines that may stimulate hepatocytes to produce NO, and (3) a marked deterioration of liver function in HCC patients may be associated with increased portosystemic shunting and further development of hyperdynamic circulation, leading to an increase in NO production **[46].**

The present study showed that there was no significant correlation in between serum NO, AFP and size of lesion as documented by triphasic CT which was the gold standard in this study to diagnose HCC where $p = 0.2$ for NO and $p = 0.6$ as for AFP in ***tables (5) & (6)*** respectively. These results agreed with those of **[47]** who reported that the overproduction of NO in malignant tissues by iNOS inhibited the immune defense mechanism and increased tumor blood, correlating with carcinogenesis in cirrhotic liver but does not play a role in tumor progression in HCC.

CONCLUSION:

The study concluded that serum nitric oxide can be used as a novel diagnostic marker for HCC together with a strong impact on the success of loco-regional therapy giving it a prognostic value with uselessness of AFP as a marker in diagnosing HCC as it gave (0%) sensitivity to HCC diagnosis, however, it can exclude the presence of HCC when below the cut-off value of 200 ng/ml as it gave a specificity of (100%), together with a positive and a negative predictive values of (0% and 33.3%) respectively.

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