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5- Global Impact Factor (Impact Factor for year 2015 is =0.544**) http://globalimpactfactor.com/afro-egyptian-journal-of-infectious-and-endemic-disease/**

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CONTENTS

Effects of High Dose Methotrexate and Delayed Elimination on Myelotoxicity Progression in Children with Acute Lymphoblastic Leukemia

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Key words:
HDMTX, ALL, myelosupression, blood count

Background and study aim: Methotrexate (MTX) as an antineoplastic agent inhibits dihydrofolate reductase. The frequency of high dose methotrexate (HDMTX) associated toxicity is variable. In this study we investigate the frequency of myelosuppression following 5 and 9 days of HDMTX infusion and MTX delayed elimination in subsequent MTX cycles in children with Acute lymphoblastic Leukemia (ALL).

Patients and Methods: This study included 28 children diagnosed with ALL during the period between May2014 to April 2016. Complete blood counts were measured before and after 5 and 9 days of HDMTX infusion and MTX levels at 42h in 28 children with ALL. The HD-MTX dose is 5 gm/m2 during 102 infusion of HD MTX at consolidation phase of ALL therapy. The MTX levels at 42 h in patients with and without toxicity were compared to evaluate the correlation between MTX levels and myelotoxicity.

Results: MTX infusion induced significant reduction in levels of TLC, ANC, RBCs, Hb and significant elevation of PLT count

after 5 days of MTX administration. Additionally, after9 days of MTX infusion, there is significant decrease in TLC, ANC, and RBCs levels. However, no significant difference in the PLT count and Hb level occurred. There is gradual decrease in myelotoxicity after 5 days and increase after 9 days of MTX administration with regard to MTX cycles. There is no statistical difference in MTX level at 42 h between patients with and without myelotoxicity after 5 and 9 days of MTX infusion. MTX delayed elimination observed in MTX cycles 1, 2, 3 and 4 was 42.8% (n=12), 42.8% (n=12), 57.1% (n=16) and 72% (n=13) respectively.

Conclusion: Myelotoxicity was decreased after 5 days of MTX administration and increased after 9 days with regard to MTX cycles. There is no correlation between MTX plasma concentration after 42 h and hematologic toxicity. Therefore, we cannot depend on MTX levels at 42 h to anticipate and predict hematologic toxicity.

INTRODUCTION

Methotrexate (MTX) is an analogue of aminopetrin and the most widely antifolate used in the treatment of certain neoplastic disease, sever psoriasis and adult rheumatoid arthritis **[1]**. Methotrexate (MTX) was first applied as a treatment for malignant diseases in oncology in 1948 **[2]**. It is an important component of the consolidation and maintenance therapy of childhood ALL **[2,3]**. It inhibits dihydrofolate reductase

and was initially developed as an antineoplastic agent **[4]**.

High-dose methotrexate (HDMTX) chemotherapy with leucovorin (LV) rescue is administered to prevent extra-medullary infiltration and it is very important ALL therapy **[5]**. Un-fortunately, MTX therapy may lead to myelosuppression, acute liver toxicity, nephrotoxicity, mucositis, and neuro-toxicity**[4,6-11]**.MTX-associated toxicity is associated with several factors

including dose, the duration of administration, patient risk factors, and genetic factors [12,13]. There are no sufficient data involving the use of HDMTX pharmacokinetic and toxicity information to anticipate hematologic toxicity in children with ALL.

Delayed MTX elimination was defined by either MTX concentration >1 μmol/L at 48 h or >0.1 μmol/L at 72h [14,15]. Kidney and/or liver dysfunction, bone marrow suppression, oral mucosal lesions, secondary infection, and delays in the following course of chemotherapy may be a consequence of MTX delayed elimination [6, 16]. Therefore, adjustments of MTX and leucovorin dose, hydration and alkalization were made to minimize the risk of elimination delay/MTX toxicity [16]. Previous reports referred to the effect of some drugs as proton-pump inhibitors, non-steroidal anti-inflammatory drugs, trimethoprim, sulfamethoxazole, penicillins, ciprofloxacin, anticonvulsants such as phenobarbital on delaying MTX elimination [17-19].

In this study, we proposed to determine first: the hematologic toxicity and MTX delayed elimination frequency and second: evaluate the relationship between hematologic toxicity and MTX level at 42 hour in children taking 5 gm/m^2 MTX infusion during the consolidation phase of ALL therapy.

PATIENTS AND METHODS

Patient Selection :
This study was approved by the Committee of Medical Ethics of Zagazig University (IRB number: 2184). ALL subjects include 28 patients, 16 female (57.1%) and 12 male (42.9%) were recruited from Pediatric Hematology and Oncology Unit Zagazig University during the period between May 2014 and April 2016.

Inclusion criteria :
a- Both sex included
b- Age 2-18 years
c- ALL patients on high dose methotrexate.
d- In consolidation phase

Exclusion criteria :
a- Renal failure
b- Liver failure

Protocol of Study :
According to TOTAL XV protocol, all patients in this study received four HD-MTX doses (5 g/m^2) at 2-week intervals on days 1,15,29 and 43 of consolidation therapy and 6-mercaptopurine

(50 mg/m^2/day) on days 1 to 56 of consolidation therapy. These chemotherapeutics were administered when ANC is ≥300/μL, and platelet count is ≥50x10^9/L. HDMTX will be held if total bilirubin 2 mg/dl and direct bilirubin 1.4 mg/dl. However, 6- mercaptopurine may be held in the presence of ANC 300/ μL, platelet count 50000/ μL or grade 3 or 4 mucositis. Dosage of 6-mercaptopurine subsequent courses may be reduced to 25 mg/m^2/day in patients who have prolonged neutropenia after HDMTX and 6-mercaptopurine treatment. At least two hours before HDMTX, prehydration IV fluid (D$_5$W+ 40 mEq NaHco$_3$/L) will be administered at the rate of 200 ml/m^2/hr provided that urinary pH is ≥6.5. Leucovorin [15mg/m^2 IV or PO for standard/high-risk] will be started at 42 hrs after the start of MTX and repeat every 6 hrs. The dosage of leucovorin will be increased in patients with high plasma MTX concentrations (1.0 μM at 42 hrs) and continued until the MTX concentration is less than 0.10 μM.

Complete Blood Counts and MTX Level Assessments
In the course of 102 infusions of HD-MTX, the MTX plasma level was measured at 42 h after HD MTX infusion. Hemoglobin (Hb), absolute neutrophil count (ANC), platelet (PLT) count, red blood count (RBCs) and TLC (total leukocytes count) were determined before MTX administration and on the 5th and 9th day following MTX infusion using Automated Hematology Analyzer. MTX concentration at 42 h was measured by high performance liquid chromatography (HPLC) assay.

Evaluation of Myelotoxicity
Hematological toxicity or myelotoxicity signs were determined by absolute neutrophil count and hemoglobin according to Common Terminology Criteria for Adverse Events (CTCAE) [20] (Table 1).

Statistical Analysis :
Statistical analyses of data were done by Prism 6, Graph pad, CA, USA. Results were expressed as mean ± standard deviation. Statistical differences were sought using Student's t-test or one way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) and or post hoc test (if more than two sets of data were being compared). Differences were considered significant at a P<0.05.

RESULTS

In this study, there are 102 infusions of HD-MTX delivered to twenty eight newly diagnosed acute lymphoblastic leukemia (ALL) patients aged 2-18 years. There is a statistically significant reduction in TLC (23%, P<0.0001), ANC (22.8%, P<0.0001), RBCs level (8.3%, P<0.0001), Hb level (5.01%, P<0.05) and elevation in PLT count (19.8%, P<0.01) after 5 days of MTX infusion compared to before administration data. Additionally, there is a statistically significant reduction in TLC (18.6%, P<0.0001), ANC (20.3%, P<0.0001), RBCs level (5.5%, P<0.05) after 9 days of MTX infusion. Whereas, there is no statistically significant difference in the PLT count and Hb level after 9 days of MTX infusion (Table 2).

Gradual decrease in myelotoxicity after 5 days of MTX administration was shown. However, there is gradual increase in myelotoxicity after 9 days of MTX administration with regard to MTX cycles (Tables 3-4, Figure 1).

In addition, Table (5,6) show that there is no statistical difference in MTX level at 42 h between patients with or without myelotoxicity after 5 and/or 9 days of MTX infusion. Figure (2) also illustrates that there is a gradual increase in% MTX delayed elimination with regard to MTX cycles.

Table (1): Toxicity criteria according to the Common Terminology Criteria for Adverse Events 2010 guideline

	Grade 1	Grade 2	Grade 3	Grade 4
Hb (g/L)	LLN-10	8-10	<8	Life threatening anemia
ANC(x10^9//L)	LLN-1.5	1.5-1	1-0.5	<0.5

Table (2): TLC, ANC, RBCs, Hb, and Platelets concentrations before and following 5 and 9 days of administration of MTX in ALL patients. All results were expressed as mean ± SD

	Before MTX Infusion	5 days	9 days
TLCx10^3/µL	3.6 ± 1.07	2.76 ±1.03*	2.93 ±1.13*
ANC/µL	1835 ±709.9	1416 ±538.9*	1462±578*
RBCs x10^6/µL	3.6 ±0.5	3.3 ±0.46*	3.4±0.53 a
Hb(g/dL)	10.56 ±1.36	10.03 ± 1.422a	10.37 ±1.28
PLTx10^3/µL	291 ±111.9	348.7 ±138.2b	303.5 ±120.5

Significantly different from before MTX administration at *p< 0.0001, [a]p<0.05and [b]p<0.01

Table (3): Toxicity frequencies after 5 days of MTX administration with regard to MTX cycles in ALL patients

	1st MTX (n=28)	2nd MTX (n=28)	3rd MTX (n=28)	4th MTX (n=18)
ANC<1 x10^9/L	2(7.1%)	4(14.3%)	2(7.1%)	1(5.5%)
Hb< 10g/L & ANC<1 x 10^9/L	9(32.1%)	7(25%)	3(10.7%)	2(11.1%)
Myelotoxicity%	39.2%	39.3%	17.8%	16.6%

Table (4): Toxicity frequencies after 9 days of MTX administration with regard to MTX cycles in ALL patients

	1st MTX (n=28)	2nd MTX (n=28)	3rd MTX (n=28)	4th MTX (n=18)
ANC<1 x10^9/L	1(3.6%)	4(14.3%)	7(25%)	3(16.7%)
Hb< 10 g/L & ANC<1 x 10^9/L	7(25%)	7(25%)	3(10.7%)	3(16.7%)
Myelotoxicity %	28.6%	39.3%	35.7%	33.4%

Table (5): Correlation of MTX levels at 42 hour in patients with and without hematologic toxicity after 5 days of MTX infusion and distribution with reference to the cycles. All values were expressed as mean $\pm$ SD

	Patient with Myelotoxicity	**Patient without Myelotoxicity**	**P value**
Cycle 1(n=28)	1.01 $\pm$ 0.37(**n=11**)	0.96 $\pm$ 0.37(**n=17**)	>0.05
Cycle 2(n=28)	0.99 $\pm$ 0.35(**n=11**)	0.82 $\pm$0.32(**n=17**)	>0.05
Cycle 3(n=28)	0.88 $\pm$ 0.27(**n=5**)	0.93 $\pm$0.34(**n=23**)	>0.05
Cycle 4(n=18)	0.96 $\pm$ 0.33(**n=3**)	1.04 $\pm$0.29(**n=15**)	>0.05

Table (6): Correlation of MTX levels at 42 hour in patients with and without hematologic toxicity after 9 days of MTX infusion and distribution with reference to the cycles. All values were expressed as mean $\pm$ SD

	Patient with Myelotoxicity	**Patient without Myelotoxicity**	**P value**
Cycle 1(n=28)	0.94 $\pm$0.40(**n=8**)	0.96 $\pm$0.38(**n=18**)	>0.05
Cycle 2(n=28)	1.05 $\pm$0.36(**n=11**)	0.79 $\pm$0.29(**n=17**)	>0.05
Cycle 3(n=28)	1.00 $\pm$ 0.32(**n=10**)	0.88 $\pm$0.33(**n=18**)	>0.05
Cycle 4(n=18)	0.85 $\pm$0.28(**n=6**)	1.11 $\pm$0.25(**n=12**)	>0.05
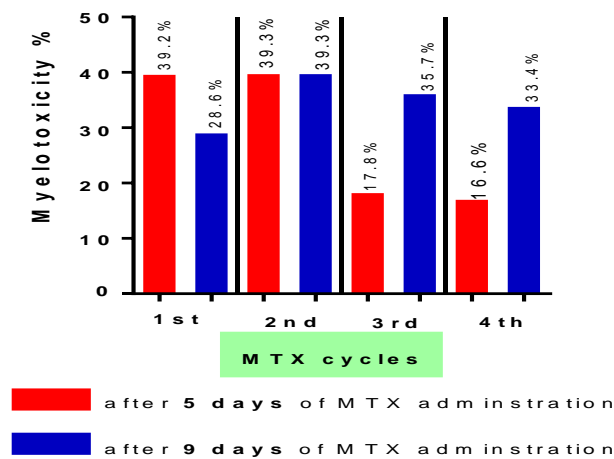
Figure (1): Myelotoxicity percent in different high dose MTX cycles after 5 and 9days of MTX administration in ALL patients
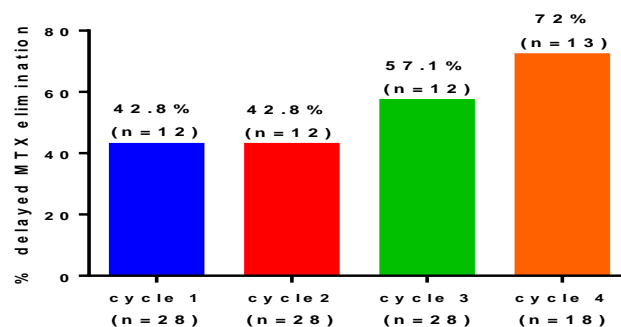
Figure (2): Percentage of delayed MTX elimination with regard to MTX cycles in ALL patients

DISCUSSION

Consolidation phase is considered avaluable and important step in the treatment of children with ALL. The use of high dose methotrexate, sometimes in combination with 6-mercaptopurine (6-MP) has significantly contributed to cure children (70-80%). In this study administration of MTX infusion for 5 days induced significant reduction in TLC, ANC, RBCs, Hb levels and PLT count after 5 days of MTX infusion. Similar data was observed during 9 days administration except the effect on PLT count and Hb level. These results are in agreement with previous studies [21]. This effect on blood counts especially RBCs and TLC is attributed to higher concentration of MTXPG in patients whose blast count decreased within 24 h . Blast cells are the origin of platelets, red blood cells, neutrophils and other types of white blood cells in the myeloid cell line [22]. Therefore, decrease in blast concentration of MTXPGLC (MTX polyglutamate long chain) may be strongly related to the rate of inhibition of de novo purine synthesis in ALL blasts [23].

The significant reduction of Hb after 5 days and the non statistical significant difference of Hb after 9 days may be justified by the results of de Rotte et al. [24]. Methotrexate can affect HbA1c where, methotrexate use and higher concentrations of erythrocyte MTX Glu (erythrocyte methotrexate polyglutamate) are associated with decreased levels of HbA1c. Therefore, concentrations of erythrocyte MTX Glu may be still high after 5 days of MTX infusion. However, after 9 days, concentrations of erythrocyte MTX Glu may be decreased.

Lexicomp [25] has been reported that not all patients should suffer low blood counts in RBCs, WBCs and platelets but this low blood counts is only common in more than 30% of patients supporting the present finding where there is no statistically significant difference in the PLT count and Hb level after 9 days of MTX infusion.

The current study illustrated gradual decrease in hematologic toxicity and myelotoxicity after 5 days of MTX infusion with repeated MTX administrations. These results are consistent with others [26]. Attenuation of hematologic toxicity and myelotoxicity with repeated MTX administrations may be related to HDMTX may be held if total bilirubin 2mg/dl and direct bilirubin 1.4mg/dl [27]. So the myelotoxicity will be decreased. Additionally, dosage of 6- mercaptopurine may be reduced to 25 mg/m^2/day in patients who have

prolonged neutropenia after HDMTX and 6-mercaptopurine treatment [27]and this may be a good reason for the gradual decrease of myelotoxicity [21], especially while the degree of myelosuppression and duration of treatment interruptions following HD-MTX is related to the dose of concurrently administered oral 6MP [21,28-30] and can be avoided by reductions of the dose of 6MP in the weeks before and after HD-MTX [31].

On the other hand, there is gradual increase in hematologic toxicity and myelotoxicity after 9 days of MTX infusion with repeated MTX administrations. These results were compatible with other previous reports [6,32]. Rask et al[6]have been demonstrated that increase in myelosuppression in subsequent cycles of MTX may be related to the accumulation of cytotoxic metabolites of MTX and 6MP. In addition, other studies [21,29] referred to using high dose MTX with increased doses of 6MP may also increase hematologic toxicity.

Interestingly, this study revealed that there is no correlation between MTX plasma concentration after 42 hour and hematologic toxicity. These results were supported by Özdemir et al. [32]. They documented that the MTX levels at 42 h in patients with myelotoxicity were not different from patients without toxicity. Additionally, Csordas et al. [33] did not find any correlation between myelotoxicity and the levels of serum MTX. However, other studies reported that there is a relationship between elevated serum MTX levels and hematologic toxicity [6].

This study explore that there is a gradual increase in MTX delayed elimination with regard to MTX cycles. This may be consistent with the results of Bauters et al. [34]. They reported that high MTX levels (72 h) were frequently observed upon intake of cola beverages. Higher MTX levels were more common after intake of cola during the first and/or second day after the start of HD-MTX infusion. Santucci et al. [35] explained that Cola beverages have a low pH due to their phosphoric acid content and that may explain its effect on MTX elimination.

CONCLUSION

There is gradual decrease in myelotoxicity after 5 days of MTX administration with regard to MTX cycles. However, there is gradual increase in myelotoxicity after 9 days of MTX administration with regard to MTX cycles. There

is no correlation between MTX plasma concentration after 42h and hematologic toxicity. Therefore, we cannot depend on MTX levels at 42 h to anticipate and predict hematologic toxicity. Moreover, there is a gradual increase in MTX delayed elimination with regard to MTX cycles.

Conflicts of interest: None.

Ethical Approval: The study was approved by the Committee of Medical Ethics of Zagazig University (IRB number: 2184) and a written informed consent was taken from each participant that follows the principals in the declaration of Helsinki.

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The Role of Renal Resistive Index in Assessment of Functional Renal Impairment in Patients with Liver Cirrhosis

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Functional renal impairment, Liver cirrhosis, renal resistive index

Background and study aim: Renal dysfunction often develops in patients with liver cirrhosis. Hepatorenal syndrome (HRS) represents the end-stage of reduction in renal perfusion. Duplex Doppler ultrasonography of the kidneys is a non-invasive method to assess blood flow and arterial vascular resistance as a parameter for vasoconstriction. This study aimed to assess the role of renal resistive index as a non-invasive marker for early detection of functional renal impairment in patients with liver cirrhosis.

Patients and Methods: This study was conducted on 20 patients with liver cirrhosis without ascites vs. 60 patients with liver cirrhosis and ascites and 20 healthy subjects as control group. Patients and control were subjected to complete blood picture, liver and kidney functions, serum electrolytes, twenty four hours urinary sodium, abdominal ultrasonography and duplex Doppler evaluation of the renal arteries with calculation of mean renal RI.

Results: Highly significant difference between cirrhotic patients with ascites and cirrhotic patients without ascites and controls regarding mean renal resistive index for both kidneys (P<0.001). At Cutoff point of renal RI 0.71, the sensitivity, specificity and accuracy to predict HRS were 100%, 80% and 82% respectively with AUC= 0.997. A statistical high significant positive correlation between RI and serum creatinine, child class, MELD score and MELD-Na (r=0. 0.818, r= 0. 0.539, r= 0.739 r= 0.807 respectively and P<0.001). A statistical high significant negative correlation between RI and serum sodium, 24 hours urinary sodium, and platelets (r= -0.778, r= 0. -0.688, r= -0.422 respectively and P<0.001).

Conclusion: Renal duplex Doppler ultrasound is useful as a non-invasive method for the evaluation of the renal hemodynamic changes in cirrhotic patients and can predict HRS.

INTRODUCTION

Advanced liver cirrhosis is associated with poor clinical outcome. Therefore, assessment of prognosis is important in the management of these patients [1].

Renal dysfunction often develops in patients with liver cirrhosis. In its most severe form, this kidney dysfunction is termed the hepatorenal syndrome, which is one of many potential causes of acute kidney injury in patients with acute or chronic liver diseases. Affected patients usually have portal hypertension due to cirrhosis, but can also have fulminant hepatic failure from any cause [2].

Hepatorenal syndrome represents the end-stage of reduction in renal perfusion induced by increasingly severe hepatic injury [3]. Despite notable splanchnic arterial vasodilatation and hyperdynamic circulation, patients with cirrhosis show increased renal arterial tone, resulting in poor renal perfusion [4].

Decreased peripheral vascular resistance with activation of compensatory mechanisms [the sympathetic nervous system (SNS), renin-angiotensin-aldosterone system (RAAS) and antidiuretic hormone (ADH)] leads to renal vasoconstriction [5].

In liver cirrhosis, serum creatinine is inaccurate in diagnosis of renal dysfunction as it overestimates renal function due to decreased creatinine production by the liver, protein calorie malnutrition and muscle wasting, therefore, better methods to diagnose this early stage of renal disease are needed [6].

Duplex Doppler ultrasonography of the kidneys is an easy and non-invasive method to assess blood flow and arterial vascular resistance as a parameter for vasoconstriction [7]. The arterial resistive index (RI) is the most widely used parameter to estimate the arteriolar vascular resistance. It is regularly used for screening of transplant rejection or to diagnose renal artery stenosis [8].

A positive correlation has been described between intrarenal RI and plasma renin activity as well as, plasma aldosteron concentration. The activation of the renin-angiotensin-aldosterone system plays an important role in the pathogenesis of hepatorenal syndrome [9]. The aim of the present work was to assess the role of renal resistive index as a non-invasive marker for early detection of functional renal impairment in patients with liver cirrhosis.

PATIENTS AND METHODS

This study was conducted on 80 patients with liver cirrhosis (proved by clinical examination, laboratory and radiological studies) and 20 healthy subjects without evidence of any liver or kidney disease as controls. Patients and controls were selected from the outpatient and/or inpatient Department of Tropical Medicine, Menoufia University hospital in the period between November 2015 to May 2016. Patients with renal impairment due to any cause other than hepatorenal syndrome, diabetes mellitus, hypertension, acute gastrointestinal bleeding and malignant diseases were excluded. Patients were 53 (66.25%) males and 27 (33.75%) females. Their ages ranged from 19 to 80 years with a mean age of 47.54 ± 15.52 as well as, 20 healthy persons of matched age and sex as a control group. This study was approved by the Committee for Ethics of Faculty of Medicine, Menoufia University, Egypt and written informed consent was obtained from each subject before blood was collected.

Patients and controls were classified into the following groups:
Group I: 20 patients with liver cirrhosis without ascites.
Group II: 60 patients with liver cirrhosis and ascites.

Group III: 20 healthy subjects as controls.

All patients and controls were subjected to the following:
- Proper and detailed history taking.
- Complete clinical examinations (general and local abdominal examinations).
- Laboratory investigations: Complete blood picture, random blood sugar, liver function tests includes [serum bilirubin, serum albumin, Alanin transaminase (ALT), Aspartate transaminase (AST), prothrombin time & concentration and INR], renal function tests [blood urea and serum creatinine], serum electrolytes [serum sodium concentration and serum potassium concentration], urine analysis, twenty four hours urinary sodium and serological tests for viral markers [HBsAg by and HCVAb by ELISA].
- Child-Pugh classification was calculated for all studied patients to assess the severity of liver disease, depending on patients' clinical and laboratory data (ascites, hepatic encephalopathy, serum albumin, serum bilirubin and international normalized ratio INR [10]. MELD score and MELD-Na also were calculated for all studied patients according to the following formulae: MELD = 9.57 loge [Creatinine (mg/dL)] + 3.78 loge [Bilirubin (mg/dL)] +11.2 loge [International Normalized Ratio] + 6.43 [11]. MELD-Na = MELD - Na - [0.025 X MELD X (140 – Na)] + 140 [12].
- Radiological evaluation; Abdominal ultra-sonography was done to evaluate liver, spleen, portal vein, the amount of ascites and both kidneys. According to the amount of ascites and the criteria for hepatorenal syndrome which include; liver cirrhosis with ascites, serum creatinine >1.5 mg/dL, no improvement of serum creatinine (a decrease in serum <1.5 mg/dL) after 2 days off diuretics and volume expansion with albumin (1g/kg body weight up to a maximum of 100 g/d), absence of shock, no current or recent treatment with nephrotoxic drugs, absence of signs of parenchymal renal disease, as suggested by proteinuria (>500 mg/d) or haematuria (>50 red blood cells per high-power field) and/or abnormal renal ultrasound [13], group II (cirrhotic patients with ascites) was subdivided into:
G IIa: 27 patients with mild to moderate ascites.
G II b: 22 patients with massive ascites.
G IIc: 11 patients with hepatorenal syndrome.

- The renal Doppler US technique; patient fast for 8 hours prior to the Doppler ultrasonographic examination of the native kidney. The transducer was positioned so as to visualize the lateral or posterolateral aspect of the kidney. In this position, Doppler examination could performed with the lowest appropriate angle (0-60), establishing an appropriate approach toward vascular structures in the periphery of the hilum and permitting visualization of the kidney without obstruction by gases present in the segments of the intestine and causing artifact. The patient was placed in the decubitus or semi-decubitus position with the kidney to be examined on top, thus permitting visualization of the kidney and including an image of the abdominal aorta. The lateral tip of the transducer was angled slightly toward the caudal aspect, permitting appropriate imaging of the course of the main artery or vein [14].
- Doppler analysis; patients and controls underwent abdominal ultrasonography using US equipment with color Doppler capability using convex linear (2.8-5 MHz) transducer (General electric LOGIQ P6 device). The renal resistive index was automatically calculated by the US equipment. Intra-renal resistance was measured on inter-lobar arteries three times in different regions of each kidney (upper, middle and lower poles) and then the mean value was calculated. Subsequently, a mean RI was calculated for each subject (mean of both kidneys) [15].

Statistical Analysis

Data was statistically analyzed using SPSS (statistical package for social science) (IBM, New York, USA) program version 22 for windows and for all the analysis a p value < 0.05 was considered statistically significant.

RESULTS

Demographics of the studied groups

There was no statistical significant difference between studied groups as regards age and sex distribution (p value >0.05). Cirrhotic patients without ascites (group I) were 14 males (70%) & 6 females (30%) with their mean age 45.5±15.4. Cirrhotic patients with ascites (group II) were 39 males (65%) & 21 females (35%) with their mean age 51.6±15.5. Control group (group III) were 12 males (60%) and 8 females (40%) with their mean age 50.4±16.3.

There was a statistical significant difference between the cirrhotic patients without ascites and cirrhotic patients with ascites regarding history of hematemsis and/or melena, liver and splenic size (p value <0.05) as well as, there was a statistical high significant difference between the same groups regarding jaundice, lower limb edema and history of hepatic encephalopathy (p value <0.001).

There was a statistical high significant difference between the studied groups regarding hemoglobin concentration and platelets count (p value <0.001) as well as there was a statistical significant difference between the studied groups regarding WBCs count (p value <0.05). There was a statistical high significant decrease in the mean values of hemoglobin concentration (10.10 gm/dl), WBCs (3.96 x103 /cm3) and platelet count (70.8 x10^3 /cm^3) in cirrhotic patients with ascites in comparison with cirrhotic patients without ascites (12.34 gm/dl, 6.04 x103/cm3 and 159.2 x10^3 /cm^3 respectively) and control group (13.0 gm/dl, 6.47 x10^3/cm^3 and 225.9 x10^3/cm^3 respectively).

There was a statistical high significant difference between the studied groups regarding total bilirubin, serum albumin, prothrombin concentration, AST and ALT (p value <0.001). There was a statistical high significant increase in the mean values of serum bilirubin (4.25 mg/dl) as well as, a statistical high significant decrease in serum albumin (2.44 gm/dl) and prothrombin concentration (48.27%) in cirrhotic patients with ascites in comparison with cirrhotic patients without ascites (1.04 mg/dl, 3.98 gm/dl and85.5% respectively) and control group (0.69 mg/dl, 4.13 gm/dl and 98.8% respectively)

There was a statistical high significant difference between cirrhotic patients without ascites and cirrhotic patients with ascites regarding Child classification (p value <0.001). Most patients in GI were Child A (18 patients, 90%) on the other hand, the majority of patients in GII were Child C (49 patients, 81.7%).

Regarding abdominal ultrasound findings among studied groups, there was a statistical high significant difference between the studied groups regarding size of liver and spleen, portal vein dilatation and the amount of ascites (p value <0.001). There was no statistical significant difference between the cirrhotic patients without ascites and cirrhotic patients with ascites regarding the etiology of liver cirrhosis (p value = 0.69). Chronic HCV was the commonest etiology of cirrhosis in both groups.

There was a statistical high significant difference between the studied groups regarding kidney function tests and serum Na (p value < 0.001). There was a statistical high significant increase in blood urea and serum creatinine as well as, a statistical high significant decrease in serum Na in patients with massive ascites and patients with hepatorenal syndrome in comparison with other groups. Table (1)

Table (1): Kidney function tests and serum Na+ among studied groups (No=100)

Studied variable	GI (N=20)	GII (N=60)			GIII (N=20)	K	P value
		GIIa (N=27)	GIIb (N=22)	GIIc (N=11)			
	$\overline{X}$ ±SD	$\overline{X}$ ±SD	$\overline{X}$ ±SD	$\overline{X}$ ±SD	$\overline{X}$ ±SD		
Blood urea Range	28.9±4.58 21 - 38	27.0±3.80 21 - 35	39.5±12.6 23 – 89	78.6±18.3 38 - 105	26.8±4.20 21 - 34	47.1	0.001
S. creatinine Range	0.82±0.30 0.30 – 1.30	0.73±0.27 0.30 – 1.20	1.40±0.70 0.40 – 4.20	3.02±0.65 2.30 – 4.50	0.73±0.83 0.30 – 1.20	46.1	0.001
Serum Na	139 ±3.22	136.5 ±5.57	129.1 ±5.57	116.7 ±6.37	139.1 ±3.22	52.1	0.001

There was a statistical high significant difference between the studied groups regarding MELD and MELD-Na scores (p value <0.001). There was high significant increase in MELD and MELD-NA scores in patients with massive ascites and patients with hepatorenal syndrome in comparison with other groups as well as when compared with each other, however there was no statistical significant difference between cirrhotic patients without ascites (GI) and cirrhotic patients with mild to moderate ascites (GIIa). Table (2)

There was a statistical high significant difference between the studied groups regarding 24 hour urine output and 24 hour urinary Na (p value < 0.001). There was a significant decrease in 24 hour urinary Na in cirrhotic patients with massive ascites and patients with hepatorenal syndrome in comparison with other groups, however there was no statistical significant difference between cirrhotic patients with mild to moderate ascites (GIIa) and cirrhotic patients with massive ascites (GIIb). Table (2)

Table (2): MELD and MELD-Na score among patients in GI and GII (NO=80)

Studied variables	GI (N=20)	GII (N=60)			F	P value	Post hoc test
		GIIa (N=27)	GIIb (N=22)	GIIc (N=11)			
	$\overline{X}$ ±SD	$\overline{X}$ ±SD	$\overline{X}$ ±SD	$\overline{X}$ ±SD			
MELD score	9.79±0.73	10.1±0.98	11.2±1.21	13.3±0.99	36.7	0.001	P1:0.323 P2:<0.001** P3:0.001** P4:<0.001** P5:<0.001** P6:<0.001**
MELD-Na score	10.6±2.51	12.7±4.30	18.9±4.59	28.8±4.41	59.1	0.001	P1:0.079 P2:<0.001** P3:0.001** P4:<0.001** P5:<0.001** P6:<0.001**
24 hr urinary Na	79.0±33.6	55.2±24.9	46.7±20.89	9.30±6.53	35.6	0.001	P1:0.007 P2:<0.001** P3:0.001** P4:0.061 P5:<0.001** P6:<0.001**

** highly significant

P1:between GI and GIIa , P2:between GI and GIIb , P3:between GI and GIIc , P4:between GIIa and GIIb , P5:between GIIa and GIIc , P6:between GIIb and GIIc

There was a statistical high significant difference between the studied groups regarding renal resistive index (p value <0.001). In this study, the mean renal resistive index was significantly higher in all cirrhotic patients groups (GI and GII a,b,c) than in control group. There was a statistical high significant increase in resistive index in patients with massive ascites and patients with hepatorenal syndrome in comparison with other groups. On the other hand, there was no statistical significant difference between cirrhotic patients without ascites and cirrhotic patients with mild to moderate ascites (Table 3).

Table (3): Renal resistive index among studied groups (No=100)

Renal duplex	GI (N=20)	GII (N=60)			GIII (N=20)	F	P value	Post hoc test
		GIIa (N=27)	GIIb (N=22)	GIIc (N=11)				
	$\overline{X}$ ±SD	$\overline{X}$ ±SD	$\overline{X}$ ±SD	$\overline{X}$ ±SD				
Right	0.62±0.07	0.64±0.03	0.71±0.03	0.83±0.04	0.59±0.01	79.5	0.001	P1:<0.001** P2<:0.001** P3:<0.001** P4<:0.001** P5:>0.05 P6:<0.001** P7:<0.001** P8<:0.001** P9:<0.001** P10:<0.001**
Left	0.63±0.02	0.65±0.02	0.73±0.05	0.84±0.02	0.61±0.01	281.7	0.001	P1:<0.05* P2:<0.05* P3:0.001** P4:<0.001** P5:>0.05 P6:<0.001** P7:<0.001** P8:<0.001** P9:<0.001** P10:<0.001**
Mean resistive index for both kidneys	0.62±0.01	0.64±0.03	0.71±0.04	0.83±0.03	0.59±0.01	151.3	0.001	P1:<0.001** P2:<0.001** P3:<0.001** P4:<0.001** P5:>0.05 P6:<0.001** P7:<0.001** P8:<0.001** P9:<0.001** P10:0.001**

*Significant ** highly significant   F: ANOVA test
P1:between GIII and GI , P2:between GIII and GIIa , P3:between GIII and GIIb , P4:between GIII and GIIc , P5:between GI and GIIa , P6:between GI and GIIb , P7:between GI and GIIc , P8:between GIIa and GIIb , P9:between GIIa and GIIc , P10:between GIIb and GIIc

There was a statistical high significant positive correlation between renal resistive index and child score (r = 0.539), age (r = 0.226), total bilirubin (r = 0.678), blood urea (r = 0.815), serum creatinine (r = 0.818), MELD score (r = 0.739) and MELD-NA score (r = 0.807) and there was a statistical high significant negative correlation between RI and serum albumin (r = -0.621), prothrombin concentration (r = -0.535), platelets count (r = -0.422), serum sodium (r = -0.778), 24 hours urinary sodium (r = -0.688). Table (4). Figure. (1a&b). Figure. (2a&b).

Table (4): Correlation between renal resistive index and different variables among studied patients

Studied variables	Resistive index	
	r	**P value**
CHILD classification	0.539	0.001**
Age	0.226	0.043*
Serum albumin	-0.621	0.001**
Prothrombin concentration	-0.535	0.001**
Bilirubin	0.678	0.001**
Platelets count	-0.422	0.001**
Serum Na	-0.778	0.001**
24hr Urinary Na	-0.688	0.001**
Blood urea	0.815	0.001**
Serum creatinine	0.818	0.001**
MELD score	0.739	0.001**
MELD-Na	0.807	0.001**
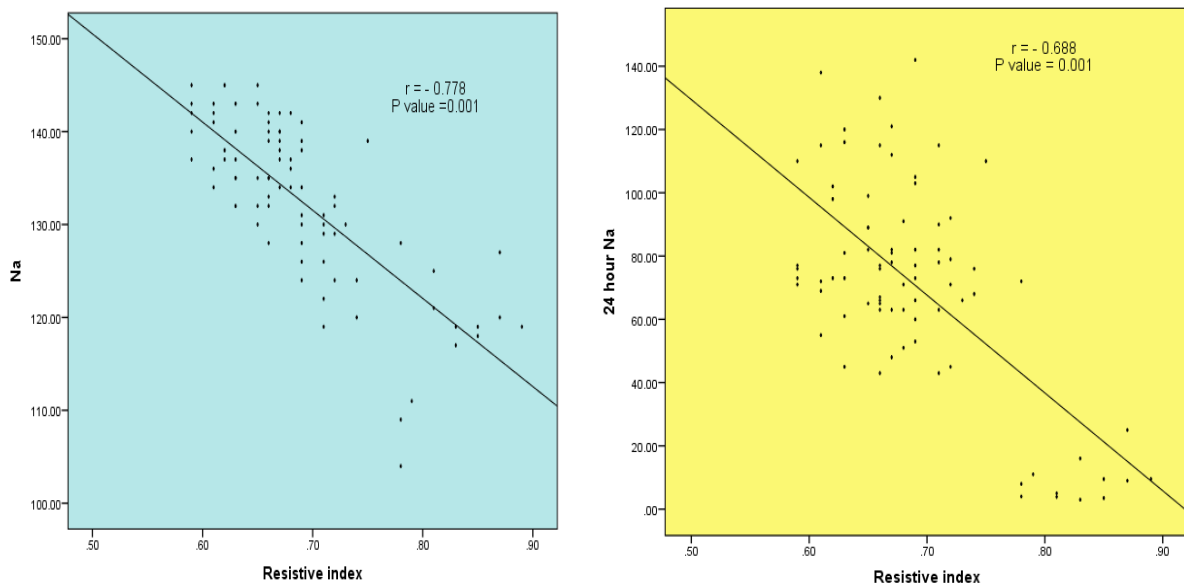
Fig. (1a&b): Correlation between renal resistive index and Serum Na+ &24hr Urinary Na among patients in GI and GII
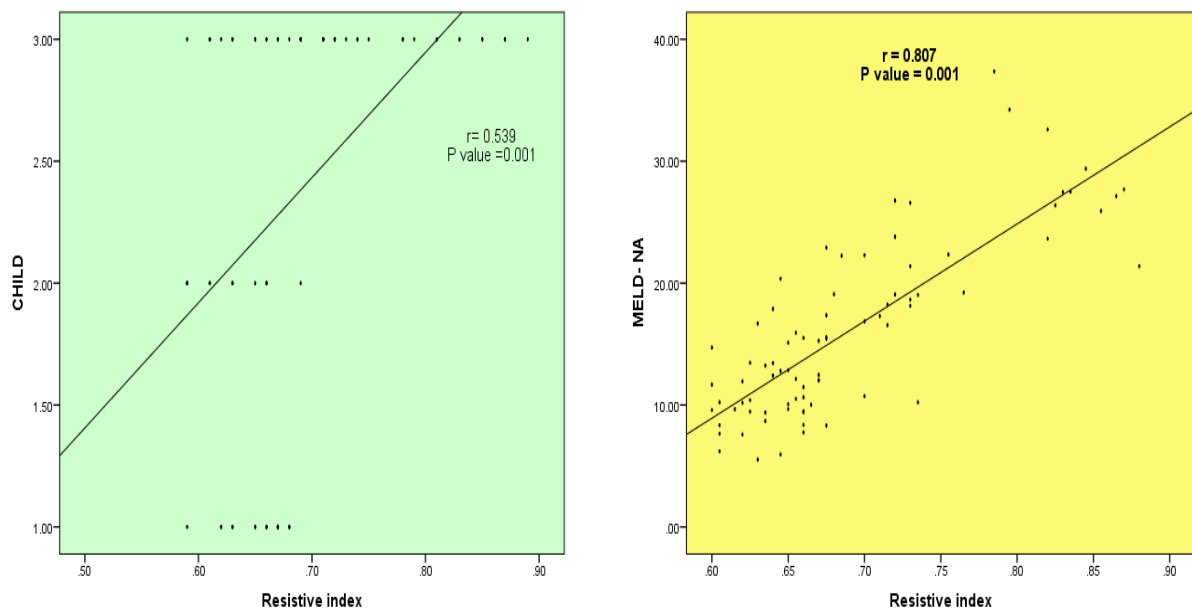
(Fig. 2 a&b): Correlation between renal resistive index and CHILD& MELD-Na score among patients in GI and GII

At Cutoff point of renal RI 0.71, the sensitivity of the test to predict hepatorenal syndrome was 100%, the specificity was 80%, PPV was 44%, NPV was 100%, accuracy was 82% and AUC = 0.997. Figure (3).

Cutoff point	Sensitivity %	Specificity %	Positive predictive value%	Negative predictive value%	Diagnostic accuracy%
0.71	100	80	44	100	82
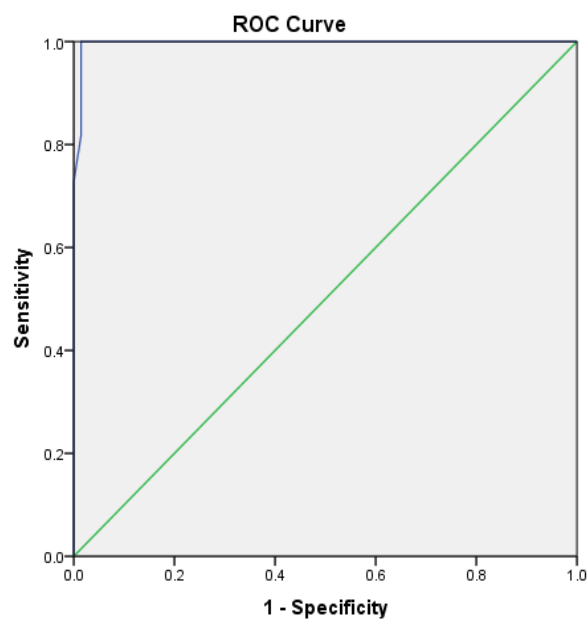
(Fig. 3): *Renal resistive index ROC curve*

DISCUSSION

Cirrhosis is an increasing cause of morbidity and mortality in more developed countries. It is the 14th most common cause of death in adults worldwide but the fourth in central Europe; it results in 1.03 million deaths per year worldwide [16]. Renal dysfunction frequently complicates the clinical course of advanced liver disease and is invariably associated with poor clinical outcomes. So, optimal management of renal dysfunction in cirrhosis is extremely important. Renal dysfunction in chronic liver disease is characterized by impaired natriuresis, decreased free water clearance, and decreased glomerular filtration rate (GFR). Hyponatremia, ascites and hepatorenal syndrome (HRS) represent the clinical consequences of disturbances in renal functions [17]. Despite notable splanchnic arterial vasodilatation and hyperdynamic circulation, patients with cirrhosis show increased renal arterial tone, resulting in poor renal perfusion. Decreased peripheral vascular resistance with activation of compensatory mechanisms [the sympathetic nervous system (SNS), renin-angiotensin-aldosterone system (RAAS) and antidiuretic hormone (ADH)] leads to renal vasoconstriction [5].The intra-renal resistive index (RI) is the most frequently used parameter to assess intra-renal resistance and is calculated based on intra-renal duplex Doppler ultrasound measurements [18].

In this study the statistical analysis revealed no significant difference between the studied groups as regards age and sex distribution, this ensures that the demographic data has no effect on the results of the study indicating no bias in it.

Regarding liver function tests, there was a statistical high significant difference between the studied groups regarding total bilirubin, serum albumin, prothrombin concentration, AST and ALT. These results were in agreement with those reported by Goyal et al. [19] who stated that, there were significant differences in prothrombin concentration, AST, ALT, serum albumin and serum bilirubin between cirrhotic patients without ascites and cirrhotic patients with ascites. Also, Fouad et al. [20] reported that, prothrombin concentration and serum albumin were significantly higher while, serum bilirubin was significantly lower in patients with compensated liver cirrhosis than patients with decompensated cirrhosis and patients with hepatorenal syndrome.

In this study, there was no statistical significant difference between the cirrhotic patients without ascites and cirrhotic patients with ascites regarding the etiology of liver cirrhosis. Chronic HCV was the commonest etiology of cirrhosis in both groups (95%) in GI and (91.7%) in GII. This result agreed with the study done by Amer et al. [21] who reported that, the prevalence of HCV infection in Egypt is the highest reported worldwide of 14.7% and about 85% of those infected with HCV will develop chronic hepatitis of varying severity, nearly 20% of patients develop cirrhosis in 10-20 years.

Regarding the blood urea and serum creatinine, this study detected that, there was a statistical high significant difference between the studied groups (p value <0.001). There was a high significant increase in blood urea and serum creatinine in patients with massive ascites and patients with hepatorenal syndrome in comparison with other groups while, there was no statistical significant difference between cirrhotic patients without ascites and both control and cirrhotic patients with mild to moderate ascites. In agreement with this result, the study done by Nix et al. [22]who determined that, serum creatinine and blood urea levels in patients with the hepatorenal syndrome was significantly higher than that of other different groups (p<0.05) but there was no significant changes in creatinine levels between cirrhotic patients without ascites and control group. While, creatinine levels in cirrhotic patients with ascites was higher than that in cirrhotic patients without ascites.

In the present study, there was a statistical high significant difference between studied groups as regards serum sodium levels and there was highly significant decrease in serum sodium in patients with massive ascites and patients with hepatorenal syndrome in comparison with other groups. These results were in agreement with Sikarwar et al. [23] who found that, there was decrease in serum sodium in patients with decompensated cirrhosis in comparison with compensated cirrhosis mostly due to dilutional hyponatremia. Also, Gines and Guevara [24] reported that, low serum sodium levels are very common finding in patients with hepatorenal syndrome.

Regarding 24 hours urinary sodium of patients in GI and GII, there was a statistical high significant difference between the two groups. There was a significant decrease in 24 hours urinary Na in cirrhotic patients with massive ascites and patients with hepatorenal syndrome in comparison with

other groups. Also, there was a statistical significant difference between cirrhotic patients without ascites and cirrhotic patients with mild to moderate ascites. This was in agreement with Kenawi et al. [25] who reported that, the urine sodium excretion in patients with chronic liver disease decreases with progression of disease and also with Fouad et al. [20] who reported that, urinary sodium excretion decrease in patients with hepatorenal syndrome. Sikarwar et al. [23] detected that, the mean urinary sodium concentration was significantly higher in cirrhotic patients without ascites than in cirrhotic patients with ascites and it was also higher in cirrhotic patients with ascites than in patients with hepatorenal syndrome.

In this study, the mean renal resistive index was significantly higher in all cirrhotic patients groups (GI and GIIa,b,c) than in control group. This result was in agreement with Cazzaniga et al. [26], Ustundag et al. [27] and Fouad et al. [20] who showed that, intra-renal RI was significantly higher in patients with cirrhosis than in healthy subjects. Also Masahiko et al. [28] demonstrated that, resistive index was significantly higher in cirrhotic patients compared to controls and compared to patients with chronic hepatitis.

Abuelo [29] reported that, the increase in renal vascular RI in cirrhotic patients with ascites can be explained by a physiological homeostatic response to vascular under filling occurring in ascitic patients. When the vascular under filling is moderate, the renal vasoactive substances are effectively counterbalanced by increased renal synthesis of prostaglandins so that, renal blood flow and GFR remain normal. In contrast, when the vascular under filling is severe, intense stimulation of endogenous vasoconstrictor systems occurs, producing renal vasoconstriction and impairment of renal blood flow and GFR. Colle et al. [30] reported that, intra-renal blood flow is preserved in cirrhotic patients by intra-renal mechanisms until the ascites becomes refractory. When this regulation fails renal ischemia causes tubular necrosis, azotemia and oliguric renal failure.

In the current study, there was a statistical high significant increase in mean renal resistive index of both kidneys in patients with massive ascites and patients with hepatorenal syndrome in comparison with other groups. On the other hand, there was no statistical significant difference between cirrhotic patients without ascites and cirrhotic patients with mild to moderate ascites.

These results were in agreement with Maroto et al. [31] who demonstrated that, RI was significantly higher in decompensated cirrhotic patients with ascites than in compensated cirrhotic patients and that the RI of compensated cirrhotic patients is higher than in the controls. They reported that, these results were highly sensitive and specific for the diagnosis of HRS. Also, in another study, Bardi et al. [31] reported that, patients with HRS had significantly higher values of RI than those without HRS. The relative risk of developing HRS in patients with an RI = 0.70 was high. RI is a useful indicator in patients with cirrhosis and ascites for the diagnosis and prognosis of HRS.

In this study, there was a statistical high significant difference between the cirrhotic patients without ascites and cirrhotic patients with ascites regarding Child classification. Most patients in GI were child A, on the other hand, the majority of patients in GII were Child C. There was a statistical high significant positive correlation between renal resistive index and child classification. This result was in agreement with the study done by Yan and Zhang [33] and Moustafa et al. [34] who detected that, there was positive correlation between RI and Child-Pugh classification. Moreover, Child C patients had the highest RI followed by Child B patients and lastly Child A patients.

In this study, there was a statistical high significant positive correlation between renal resistive index and age and total bilirubin and there was a statistical high significant negative correlation between renal resistive index and serum albumin, prothrombin concentration and platelets count. This result agreed with Abdel-Bary et al. [35] who detected that, RI had a significant positive correlation with age and total bilirubin (r =0.593, P<0.001) and there was a significant negative correlation between renal RI and prothromin concentration and serum albumin (r =_0.407, P<0.001) (35).This result disagreed with Moustafa et al. [34] who reported that, there was no significant correlation between renal RI and serum albumin.

Regarding the correlation between renal resistive index and serum sodium there was a statistical high significant negative correlation between them (p value <0.001). In agreement with this result Abdel-Bary et al. [35] who reported that, there was negative correlation between RI and serum sodium (r= -0.341, P value <0.001).

This study showed a highly significant positive correlation between renal resistive index and blood urea & serum creatinine. This result agreed with Sikarwar et al. [23] who reported that, there was positive correlation between the values of RI and blood Urea and serum creatinine. It was observed that, increased renal RI value in cirrhotic patients was associated with corresponding increase in blood urea level and serum creatinine. This is in contrast to Pompili et al. [36] who reported that, there was no significant correlation between renal RI and serum creatinine.

In this study, there was a statistical high significant positive correlation between renal resistive index and MELD (r= 0.739, p value < 0.001) and MELD-Na scores (r= 0.807, p value <0.001). Theses results agreed with Abdel-Bary et al. [35] who detected that, there was a significant positive correlation between RI and MELD (r= 0.859, P<0.001) and MELD Na (r= 0.769, P<0.001) (35). Patients with high MELD score had higher RI. These results also were in agreement with those of Umbro et al. [37] and Moustafa et al. [34]

In this study, Resistive index ROC curve analysis showed that, RI had AUROC = 0.997 and if the Cutoff point of renal RI was 0.71, the sensitivity of the test was 100%, the specificity was 80%, PPV was 44%, NPV was 100% and accuracy was 82%. The results of this study were close to that reported by Abdel-Bary et al. [35] who stated that, RI ROC curve analysis showed that RI had AUROC = 0.903 (95% CI: 0.835–0.949 and P<0.001). At a cutoff value of RI >0.73, renal resistive index had sensitivity of 100% and specificity of 66.36% (35).

CONCLUSION

From the present study, we conclude that, renal duplex Doppler ultrasound is useful as a non-invasive method for the evaluation of the renal hemodynamic changes in cirrhotic patients with good correlation to the severity of liver disease. The RI may help identify a subgroup of high-risk patients with a poor prognosis that require special therapeutic care.

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Serum Zinc Levels in Egyptian Patients with HCV Induced Chronic Liver Diseases: Evaluation and Clinical Significance

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Key words: Zinc; Hepatitis C; Chronic liver diseases

Background and study aim: HCV infection is a major health problem worldwide. In Egypt the estimated prevalence is about 22%. As Zinc (Zn) is the second most prevalent trace element in the body, we aimed to evaluate serum Zn levels in patients with HCV induced chronic liver diseases, study the relationship between these levels and clinical profiles, histopathological criteria and HCC characters of the studied cases. **Patients and Methods:** Sixty nine patients aged from (18 to 67) years were included in addition to 23 age- and sex-matched healthy subjects serving as a control, all were stratified into, G 1: 23 patients biopsy proven CH. G 2: 23 cirrhosis patients. G 3 :23 HCC patients proved by abdominal ultrasonography, triphasic spiral C.T Scan and AFP. Group 4 : 23 healthy persons as controls. All underwent routine investigations and serum Zn levels were analyzed on atomic absorption spectrophotometer, meanwhile cirrhotic subjects were assessed for severity of disease by Child-Pugh classification.

Results: Serum zinc was significantly lower in chronic hepatitis than control on one hand and HCC group on the other hand (p<0.001) and they were significantly decreased in Child class C patients than Child class A (p= 0.023). Significant positive correlation was found between serum Zn and age in cirrhotic group moreover, there was no significant correlation between serum Zn and any of laboratory parameters in the studied groups and fibrosis stages of chronic hepatitis group. Negative correlation was detected between serum Zn and tumor multiplicity and BCLC in HCC group. **Conclusion:** We can conclude that serum zinc decreased significantly in chronic HCV patients and these levels decreased by increasing severity of liver disease according to Child classification. It is recommended to evaluate the role of zinc supplementation in treating clinical manifestation of liver cirrhosis and liver cell failure due to HCV.

INTRODUCTION

Hepatitis C virus (HCV) is a major cause of acute and chronic liver disease. HCV infection frequently leads to chronic hepatitis aggravated by hepatic fibrosis and steatosis subsequently leads to increase the risk of hepatic cirrhosis and hepatocellular carcinoma that are responsible for high morbidity and mortality rate [1]. In Egypt, HCV infection is a major health problem. According to World Health Organization (WHO), the estimated prevalence is about 22% [2], because of the very high prevalence rate of HCV in the general Egyptian population, it accounts for most chronic liver diseases and HCC cases in Egypt [3].

Trace metals are integral part of metalloenzymes and participate in biological functions, such as oxygen transport, free radical scavenging, structural organization of macromolecules, and hormonal activity [3]. Zinc (Zn) is the second most prevalent trace element in the body. It is integrally involved in the normal life cycle and it has many important regulatory, catalytic, and defensive functions [4]. Acute and chronic viral

hepatitis are associated with reduced serum zinc levels, without any difference among groups classified according to the etiology or the clinical course of liver disease and the linkage between zinc homeostasis and viral infection could be identified with the assumption that viruses produce severe oxidative stress in the hepatocytes, leading to apoptosis or hepatocytic necrosis and causing a decrease in protein synthesis [6]. The low serum zinc level is common in patients with liver cirrhosis (due to decreased intake, decreased absorption, decreased bioavailability, and increased losses (because of malabsorption) [5], HCC and in HCC tumor tissue [7]. The decrease in intracellular zinc levels in HCC could be explained by a down regulation of ZIP14 gene expression and the near absence of the protein within hepatoma cells in core biopsy samples. ZIP14 localizes to the cell membrane of normal hepatocytes and is a functional transmembrane transporter involved in the uptake of zinc into the cell [8]. In this study, we evaluated serum zinc levels in patients with HCV induced chronic hepatitis, Liver cirrhosis, and HCC and studied the relationship between these levels and clinical profiles, histopathological criteria and HCC characters of the studied cases.

SUBJECTS AND METHODS

This cross-sectional study was conducted on 69 adult patients with HCV-related chronic liver diseases who were referred to Hepatology, Gastroenterology and Infectious diseases department, Benha University Hospital during the period from August 2013 to April 2014. Twenty three healthy, age and sex matched subjects were selected as controls. An informed medical consents was taken for their participation in the study. The protocol of the study was approved by the human ethical committee of Benha University Hospital. Patients with diseases affecting serum zinc levels such as renal impairment and chronic heart failure, alcohol abuse, diabetes mellitus, previous antiviral treatment, concurrent infections or malignancy other than HCC, chronic HBV infection or concomitant chronic HBV and HCV infections, zinc therapy or using hormonal drugs was excluded in addition to patients subjected to surgery or chemotherapy.

The Studied subjects were stratified into : Group 1 (chronic hepatitis group) that included 23 patients who were diagnosed by liver biopsy. Group2 (cirrhotic group) that included 23 patients in whom cirrhosis was diagnosed by clinical, laboratory data and ultrasonographic examination. Group 3 (HCC group) that included 23 patients with HCV related HCC who were diagnosed by abdominal ultrasonography, triphasic C.T and AFP. Group 4 (control group) that included 23 healthy persons as a control group with no clinical, laboratory or ultrasonographic evidence of illness.

All subjects underwent CBC, liver profile (ALT, AST, and bilirubin using Beckman Synchron CX7 Delta Clinical System, prothrombin time, and INR using stago analyzer), Serum creatinine, Viral markers including : HCV-Ab by Enzyme linked immunosorbent assay (ELISA) (IU/ml) in addition to HBsAg by ELISA (IU/ml), and AFP. Abdominal ultrasonography, abdominal triphasic spiral C.T Scan was done for HCC group. The severity of liver cirrhosis was assessed by Child–Pugh score and Barcelona Clinic Liver Cancer (BCLC) staging system was done for HCC group.

Sample preparation: Blood samples were collected under aseptic conditions, then samples protected in evacuated tubes without adding any anticoagulant agent. The samples were left standing for one hour; sera were separated at 3000 rpm centrifugation for 10 min and preserved at - 20°C till further analysis.

Method of serum Zn assay:
The sera were diluted 1: 5 with double deionised water. Zn levels were detected by atomic absorption spectrophotometer instrument (Variant Australia) using internal standard (Merck Germany), then the results were multiplied by 5. Normal range of serum Zn is (0.8 – 1.5 µg/ml).

Statistical analysis:
Sample size calculation:
Sample size was 92 subjects (69 cases and 23 controls). The α level was 0.05 as it represents the type I error probability for a two sided test. The sample size was calculated at 95% confidence level (CL) and power of the study 80%. Z value was 1.96 at 95% CL.

The collected data were tabulated and analyzed using SPSS version 16 software (Spss Inc, Chicago, ILL Company). Statistical analysis was done according to Knapp and Miller [9].

Categorical data were presented as number and percentages while quantitative data were expressed as mean and standard deviation, median range. Chi square test (X^2), Fisher's, ANOVA (F test), student "t", Man Whitney U test, Krauskal Wallis

test and Spearman's correlation coefficient (rho) were used as tests of significance.

RESULTS

Patients of HCC group were significantly older than those of chronic hepatitis and cirrhotic groups (P<0.001). There was no statistically significant difference between the studied groups regarding gender. Serum zinc levels were significantly lower in chronic hepatitis group than control group and HCC group (P<0.001). Patients with Child class (C) had lower serum Zn levels than those with Child class (A) with significant difference between them (P= 0.023). Out of 23

HCC patients, 1 patient (4.3%) had stage 0 of BCLC, 12 patients (52.3%) had stage A, 5 patients (21.7%) had stage B, 1 patient (4.3%) had stage C, 4 patients (17.4%) had stage D.

Significant positive correlation was found between serum Zn levels and age in cirrhotic group but there was no significant correlation between serum Zn levels and any of laboratory parameters of the studied groups on one hand and fibrosis stages of chronic hepatitis group on the other hand. Negative non significant correlation was detected between serum Zn levels and tumor multiplicity and BCLC in HCC group.

Table (1): Demographic criteria of the studied groups

	Group				Total
	Chronic hepatitis group (N=23)	Cirrhotic group (N=23)	HCC group (N=23)	Control group (N=23)	
Gender Male No (%) Female No (%)	16(69.6%) 7(30.4%)	15(65.2%) 8(34.8%)	20(87.0%) 3(13.0%)	12(52.3%) 11(47.7%)	63(68.5%) 29(31.5%)
Total	23(100.0%)	23(100.0%)	23(100.0%)	23(100.0%)	92(100.0%)
P value	0.004				
X^2=6.59				**P=0.09**	

Group	Age (years)		ANOVA (F test)	P
	Mean	**± SD**		
Chronic hepatitis group	†38.65	9.79	15.95	<0.001*
Cirrhotic group	‡$^\diamond$49.0	8.22		
HCC group	‡†$^\diamond$56.4	5.25		
Control group	46.43	10.92		

Table (2): Laboratory criteria of the studied groups

Variable	Chronic hepatitis group (N=23)		Cirrhotic group (N=23)		HCC group (N=23)		Control group (N=23)		P	F test	Krauskal Wallis test	ANOVA
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD				
Haemoglobin (g/dl)	13.87	1.316	‡12.72	1.303	‡12.03	1.319	13.00	1.421	<0.001*	7.48		
WBCs (x10³)	6.31	2.041	5.93	1.295	5.28	1.640	7.58	2.031	0.26	6.84		
PLT (x10³)	204.7	48.076	†‡113.7	51.038	†‡124.2	58.141	246.5	63.880	<0.001*	30.5		
ALT (U/L)	†62.20	60.278	50.91	46.497	51.86	24.074	31.60	12.276	0.01*		11.5	
AST (U/L)	58.30	56.840	48.43	35.279	†66.08	39.485	34.52	12.474	0.001*		15.8	
Total bilirubin (mg/dl)	0.62	0.380	†1.04	0.651	†‡1.94	0.870	0.52	0.244	<0.001*		42.1	
Serum albumin (gm/dl)	†4.43	0.43	†3.83	0.47	†‡Δ3.00	0.53	4.03	0.38	<0.001*		F test= 39.5	
α-feto protein (ng/ml)	◊4.52	3.14201	◊44.44	61.63213	1422.86	2124.48964	◊7.30	3.84282	<0.001*		52.2	
Serum creatinine (mg/dl	0.937	0.202	1.013	0.225	1.009	0.258	1.011	0.250	0.64			0.56
PC (%)	89.8	8.61	†‡76.8	10.36	†‡Δ65.2	13.45	92.1	5.47	<0.001*			36.6
INR	1.11	0.113	†‡1.26	0.204	†‡Δ1.49	0.306	1.07	0.059	0.001*			5.78
PT (second)	12.75	1.423	†13.22	1.388	†‡Δ14.71	2.00	11.84	0.427	<0.001*			16.2

Table (3): Mean and standard deviation of serum zinc levels among the studied groups

Group	Serum Zn (µg/ml)		Krauskal Wallis test	P
	Mean	± SD		
Chronic hepatitis group	†◊0.56	0.246	25.25	<0.001*
Cirrhotic group	0.87	0.236		
HCC group	0.97	0.482		
Control group	1.15	0.40		

Table (4): Number and percent of studied cases within each group according to serum Zn levels

| Group	Low Zn levels cases	Normal Zn levels cases	High Zn levels case
Chronic hepatitis group	19 (82.7%)	3 (13%)	1 (4.3%)
Cirrhotic group	15 (65.2%)	4 (17.4%)	4 (17.4%)
HCC group	7 (30.4%)	7 (30.4%)	9 (39.2%)

Table (5): Spearman's correlation coefficient between mean serum Zn levels and age and some laboratory parameters of the studied groups

With	Serum Zn (µg/ml)							
	Chronic hepatitis group		Cirrhotic group		HCC group		Control group	
	Rho	P	Rho	P	Rho	P	Rho	P
Age (years)	0.328	0.12	0.499	0.015*	0.317	0.14	0.630	0.001*
Haemoglobin(g/dl)	-0.233	0.28	-0.134	0.54	0.059	0.79	-0.133	0.54
WBCs (x10^3)(C/mm3)	0.393	0.06	0.117	0.59	0.192	0.38	0.343	0.11
Platelet (x10^3)(C/mm3)	0.277	0.2	0.0	1.0	0.326	0.12	0.08	0.72
ALT(U/L)	-0.156	0.47	-0.039	0.85	-0.159	0.46	-0.171	0.43
AST(U/L)	-0.001	0.99	-0.038	0.86	0.022	0.92	0.233	0.28
T. bilirubin(mg/dl)	0.064	0.77	0.205	0.34	0.223	0.31	0.124	0.57
Albumin(mg/dl)	0.155	0.48	0.038	0.86	0.180	0.41	0.043	0.84
PT(second)	0.007	0.97	0.204	0.35	0.149	0.49	-0.259	0.23
PC(%)	-0.095	0.66	-0.141	0.52	-0.164	0.45	0.259	0.23
INR	0.06	0.78	0.197	0.36	0.132	0.54	-0.266	0.21
S. Creatinine(mg/dl)	0.226	0.3	0.082	0.71	0.186	0.39	0.356	0.096
AFP(ng/ml)	0.071	0.74	0.235	0.28	0.291	0.17	-0.286	0.18
F stage	0.08	0.72	-----	-----	-----	-----	-----	-----

Table (6): Among Correlation between serum Zn levels and some studied variables HCC group

With	Serum Zn (µg/ml)	
	Rho	P
Tumor size	0.358	0.093
Tumor multiplicity	-0.043	0.84
BCLC staging	-0.307	0.15
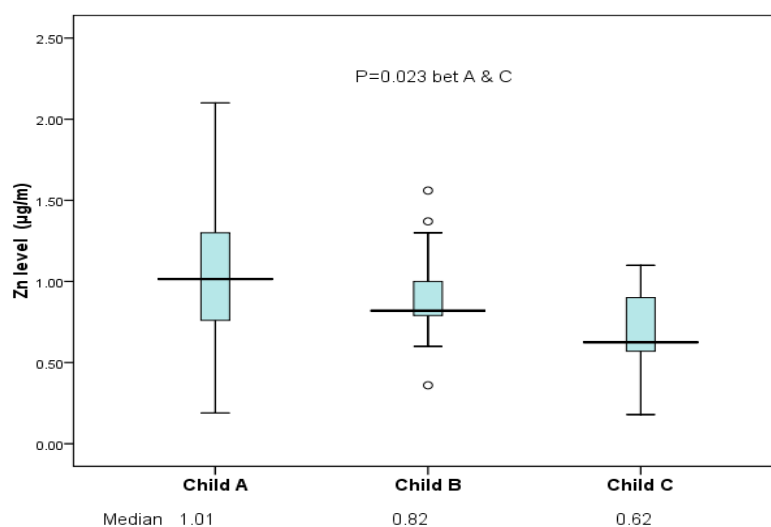
Figure (1): Serum Zn levels according to Modified Child-Turcotte- Pugh scoring system
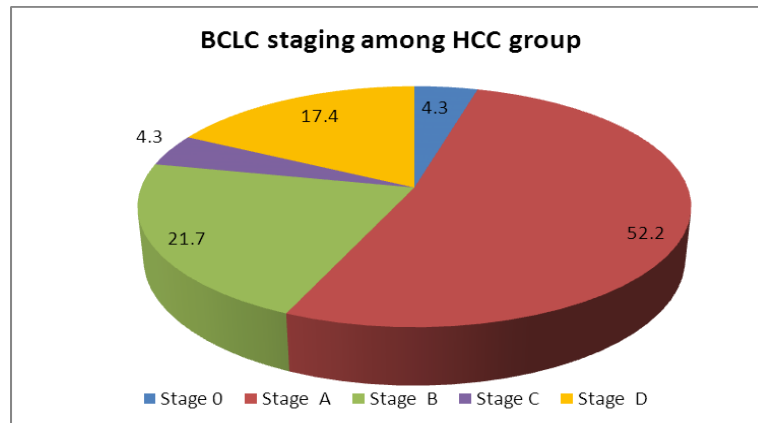
Figure (2): BCLC staging among HCC group

DISCUSSION

Hepatitis C virus infection is one of the main causes of chronic liver disease worldwide in addition the long-term impact of its infection is highly variable, from minimal changes to extensive fibrosis and cirrhosis with or without hepatocellular carcinoma [10]. In the present study, patients of HCC group were significantly older than those of chronic hepatitis and cirrhotic groups (p<0.001). This result was similar to that found by Wang et al. [11] and El-Zayadi et al. [12]; where the latter reported that HCC in Egypt is significantly more prevalent among older age groups than younger age groups and suggested that HCV infection in old patients induces a rapid progression to HCC independent of HCV genotype. Old age is a risk factor for HCC, especially in areas where HCV infection is endemic as Egypt [13]. Regarding sex distribution among patients of the current work, there was a High percentage of males in all groups. In agreement with our results, Johnson et al. [14] reported that males had higher rate of cirrhosis than females. Male to female ratio differs among countries as greater ratios were noticed in the high incidence regions such as Africa, China, Taiwan and Japan [15]. This finding may be attributed to more exposure to risk factors like HCV among male patients. However, sex hormones and other x-linked genetic factors may also be important role [12]. The current study reported that there was significant decrease in the mean serum Zn levels in chronic hepatitis group compared to control and HCC group (p<0.001). This finding agreed with Nakayama et al. [16] and Ko et al. [17] who reported that serum zinc levels was significantly

lower in chronic hepatitis patients than in healthy controls. However, Saghir et al. [18] stated that there was no any statistically significant difference between chronic hepatitis C patients and controls according to serum zinc and copper concentrations. The difference may be attributed to different sample sizes (as the previous study included 71 cases for each group). However Moriyama et al. [19] elicited that the median zinc concentration in the HCC with liver cirrhosis group (68 patients) was significantly lower than those in the chronic hepatitis group, a result that was against that of the current study. The results of the current study may be explained by the fact that high level of IL6 that occurs in response to acute inflammation and infection causes induction of ZIp14, which is Zn transporter, resulting in uptake of zinc into liver and serum hypozincemia associated with inflammation and infection [20,21]. The current work revealed that in chronic hepatitis, cirrhotic and HCC groups : 19 cases (82.7%) ,15cases (65.2%) and 7 cases (30.4%) had low serum Zn levels while 3 cases (13%) , 4 cases (17.4%) and 7 cases (30.4%) had normal serum Zn levels and lastly 1 case (4.3%), 4 cases (17.4%) and 9 cases (39.1%) had high serum Zn levels respectively. Port et al. [22] assessed serum zinc levels in 22 HCV-related HCC patients and they found that 7 (31.8%), 14 (63.6%) and 1(4.5%) of patients were below upper limit of normal, normal and above upper limit of normal, respectively. Also Atia et al. [23] revealed that among the total of 50 cirrhotic patients 36 (72%) had low serum zinc levels while remaining 14 (28%) patients had normal serum zinc levels. The results obtained in this work showed that the mean of serum zinc levels was significantly lower in patients with

Child class C than those with Child class A (P= 0.023), a result that came in agreement with Moriyama et al. **[19]** and Vijaylaxmi et al. **[24]** who found that the median zinc concentration in the Child-Pugh C group was significantly lower than those in the Child A (P<0.0001) and Child B (P=0.0033) classes and that serum zinc levels were significantly decreased with advancement of liver disease as compared to early stage of liver cirrhosis with significant negative correlation with

Child-Pugh Score. Significant positive correlation between serum Zn levels and age in cirrhotic group was observed in the current work, meanwhile there was no Significant correlation between serum Zn levels and any of the other laboratory parameters of the studied groups and fibrosis stages of chronic hepatitis group. These results came on line with El Bassuoni et al. **[7]** and Anber et al. **[25]** who found no significant correlations when serum Zn levels were correlated with ALT, AST, total bilirubin, INR, α-feto protein, serum albumin, haemoglobin % and WBCs count. On the other hand Moriyama et al. **[19]** reported highly significant positive correlation between serum Zn levels and haemoglobin %, platelets count, WBCs count and serum albumin in chronic hepatitis and liver cirrhosis groups and a highly significant negative correlation between serum Zn levels and α-feto protein, total bilirubin and AST level. Also the present study showed that there was no significant correlation between serum Zn levels and BCLC staging (P=0.15), tumor size (P= 0.093) and multiplicity (P= 0.84) of HCC group. The same results were reported by Moriyama et al. **[19]** who stated that the size and numbers of cancerous nodules were not correlated with the serum concentration of zinc or with BCLC staging system.

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Intestinal Parasitic Infections and Iron Deficiency Anaemia among School Children in El Khalige Village, Dakhalia, Egypt

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Key words:
anaemia, intestinal parasite, school children, Dakhalia, Egypt

Background and study aim: Intestinal parasitic infection and iron deficiency anaemia are still nowadays, an important public health problem worldwide, mainly in developing countries. The present study aimed to study the relationships between intestinal parasitic infections and iron deficiency anaemia in school children 6-12yrs.

Patients and Methods: A cross-sectional study was performed among (102) school children at El Kalige Village-Dakahlia. Children having signs or symptoms related to other causes of microcytic hypochromic anemia such as thalassemia, diabetes mellitus, cancers, receiving chemotherapy or radiotherapy were excluded from the study. Examination of blood and stool were done for all children.

Results: The prevalence of parasitic infections was 37% of total 102 school children. Parasitic infection was subdivided into 3 major group; helminthes, protozoa and mixed infection. Overall, helminthes infection was more prevalent 22% compared with both protozoa infection 8% and mixed infection 8%. *Ancylostoma duodenale* (9.8%), *Hymenolepis nana* and *Giardia lamblia* (7.8%), *Strongyloides stercoralis* (5.9%) *Entamoeba histolytica* and *Cryptosporidium* (3.9%) each and lastly, *Enterobius vermicularis*, *T. trichura* and *Schistosoma mansoni* with 2 cases each (1.9%). The parasitic infection was higher in females (52.6%) more than males (47.4%) insignificantly. Anemia was mild in infected group with Hb (Mean ± SD) 11.12±1.35 g/dl. 51% of selected children were anaemic. The prevalence of anemia was slightly highly non-significant prevalent among infected children (52.6%) compared with non infected (50%). Iron deficiency anemia (IDA) represents 88.5% of anemic cases in all children, 90% of anemic cases in the infected children and 87.5% of anemic cases in non infected children.

Conclusion: This study showed a high prevalence of parasitic infections among the children in the rural areas of Egypt and IDA is associated with intestinal parasitic infection.

INTRODUCTION

Parasitic infection is considered a major public health problem in children all over the world. In developing countries, low social and financial status of the individual leads to the increase in the prevalence of intestinal parasitic infections [1]. Epidemiological studies in several countries, has shown around 3.5 billion people affected globally; 300 million of them are ill, 50% of them being school age children [2]. In Egypt, the intestinal parasites affect 56% of the school children [3].

Due to anaemia, growth retardation and some developmental and mental problems, parasitic infections are considered a dangerous health problem [4].

In children (0.5-5.0 yrs), hemoglobin level < 11 g/dl and for children (12-15 yrs) hemoglobin level < 12 g/dl is defined as anaemia [5].

Anemia is a nutritional disorder worldwide especially in children. In various less developed countries in both Asia and Africa, 40% of children are anemic. In the early periods of life of children, the Deaths reached 726,000 caused by iron deficiency, with the highest percentage in Africa and Southeast Asia [6].

Disturbance in the behavior of the children can be occurred due to chronic anemia, due to its hazard effect upon neurological development in infants and also reduced scholastic performance in children of school age. Rest-less legs syndrome is common in those with iron deficiency anemia, with several symptoms include swelling of the arms or legs, heartburn, vague bruises, vomiting, sweating, pallor and blood in stool [7].

The main treatment of anemia is by treatment of the underlying disease which in most cases leads to cure or at least improvement of anemia. So, World Health Organization depends upon regular deworming of school age children in its control strategy. Regular treatment reduces the intensity of infection and gives a protection to those already infected [8]. Therefore, this study was undertaken to investigate the prevalence of intestinal parasitic infections in school going children in El Khalige village despite the various precautional measures and also, the relationship between intestinal parasitic infections and iron deficiency anemias. The results will be taken by the school authority to help them to adopt deworming measures.

PATIENTS AND METHODS

Study type:
This study was a cross sectional prevalence study. The study was conducted from January to December 2016. The practical work was done at the Department of Tropical Medicine and the Medical Parasitology Department of the Faculty of Medicine, Zagazig University, Zagazig, Egypt.

Study area
The current study was performed at El Kalige Village lies 10 kilometers away from EL Mansoura city, the village supplied with a clean water supply and central sanitary sewage systems.

Sample Size:
The calculated sample size of the study was102 children, using the following formula [9].

$$n = \frac{Z^2 + P^* (1-P)}{d^2}$$

Where
Z = 1.96 for 95% confidence level.
p = expected prevalence of satisfaction (0.50).
d = precision (Margin of error) = 0.05

Inclusion criteria:
All children aged 6-12years attending EL-Khalige primary school were included in this study.

Exclusion criteria:
Children suspected to have signs or symptoms related to other causes of microcytic hypochromic anemia such as thalassemia, or chronic debilitating diseases as malignancy, chronic renal diseases, diabetes mellitus, immune diseases were avoided .

A full history through a special sheet was obtained by the investigator after interviewing the mothers of the selected children and receiving a questionnaire. The parents of the children completed consent forms, but because many of them refused to give information about their income, we could not interpret the socioeconomic standards and could not estimate the amount of taken iron in diet.

All selected children were subjected to the following, Full history taking including: Age, Gender, Residence, education attainment, socio-economic status (father or mother occupation, household income), medical and drug history, complaint (diarrhea, abdominal pain, anorexia, nausea, vomiting and anal itching) pallor, jaundice, clubbing, organomegally and ascities.

Collection of stool samples:
The stool was examined macroscopically for the presence of blood and mucus or adult worm of helminths.

The stool was examined microscopically also for ova and cysts of intestinal parasites by direct wet smear which was done within twelve hours of the collection of the sample. Direct examination was performed by wet mount technique using saline, iodine and lacto phenol. In case of negative samples, concentration technique by formalin acetone sedimentation method was done [10]. Baermann's technique was used for demonstration of *Strongyloides* larva [11]. In addition modified acid fast staining was done for demonstrating coccidian parasites like *Cryptosporidium, Isospora*.

Blood Collection and Determination of Iron Status:
Test performance for Hemoglobin concentrations was done using the Cyanmethemglobin method to form the stable hemoglobin derivative

cyanmethemoglobin. The Potassium Ferricyanide converts the Hemoglobin to Methemoglobin by the action of Potasium Cyanide and was standed for 3 minutes. For conversion of Hemoglobin to Cyanmethemoglobin, before the absorbance is measured against a reagents blank at a wave length of 540 mm using a Spectrophotometer **[12].**

Data were analyzed with SPSS version 21. The normality of data was first tested with one-sample Kolmogorov - Smirnov test. Qualitative data were described using number and percent. Association between categorical variables was tested by Chi-square test. Continuous variables were presented as mean ± SD (standard deviation). The two groups were compared with Student t-test while more than two groups compared by ANOVA test. The significance's threshold is fixed at 5% level (p-value). P-values < 0.05 were considered significant and > 0.05 was insignificant. But < 0.001 was highly significant.

Table 1: Comparison between infected and non-infected group regarding to gender, age and anemia for 102 school children aged 6-12ys

Items	Infected group (n=38) (37%)		Non-infected (n=64) (63%)		Test of sig. p-value
	No	%	No	%	
Sex					
Male	18	47.4	26	40.6	X^2=.442 p=.506
Female	20	52.6	38	59.4	
Age/years					
Mean ± SD	8.94±2.19		9.18±1.82		t=.596 p=.553
Min-Max	6.00-12.00		6.00-12.00		
Anemia					
Anemic	20	52.6	32	50.0	X^2=.066 p=.797
Non anemic	18	47.4	32	50.0	

*p value is significant when p ≤ 0.05

Table 2: Comparison between all selected children, infected and non infected children regarding to Iron deficiency anemia (IDA) and anemia

Items	Total selectedchildren (102)		Infected child (38)		Non infected children(64)		Test of sig. p-value
	NO	%	NO	%	NO	%	
IDA	46	45	18	47.3	28	43.8	X^2= .077 p=.962
Anemia	52	51	20	52.6	32	50	

*p value is significant when p ≤ 0.05

Table 3: Subtypes of parasitic infection and Comparison between them regarding to gender, age and anemia

Items	Helminthes group (n=22) (22%)		Protozoal group (n=8) (8%)		Mixed group (n=8) (8%)		Test of sig. p-value
	No	%	No	%	No	%	
Sex							
Male	10	45.4	6	75.0	6	75.0	P=.483
Female	12	54.5	2	25.0	2	25.0	P=.002
Age/years							
Mean ± SD	8.68±1.75		8.37±1.50		9.12±1.72		p=.691
Min-Max	6.00-12.00		6.00-10.00		6.00-11.00		
Anemia							
Anemic	12	54.5	4	50.0	4	50.0	P=.041
Non anemic	10	45.4	4	50.0	4	50.0	

*p value is significant when p ≤ 0.05
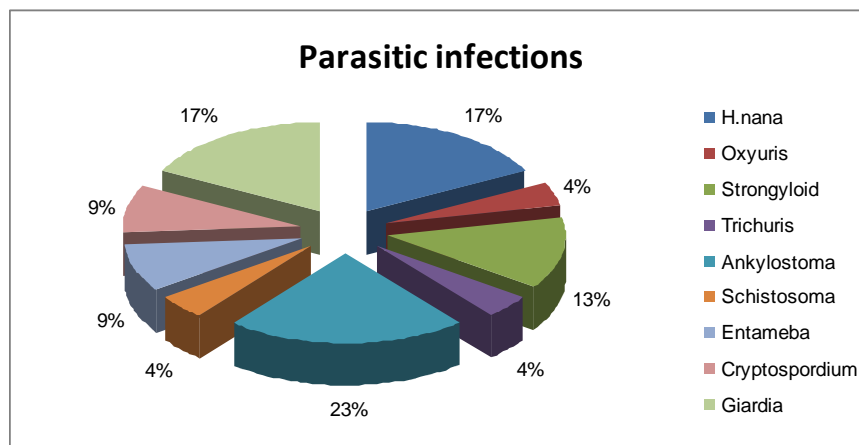
Fig. 1: Subtypes of parasitic infections and their prevalence among total infected children (46 cases).
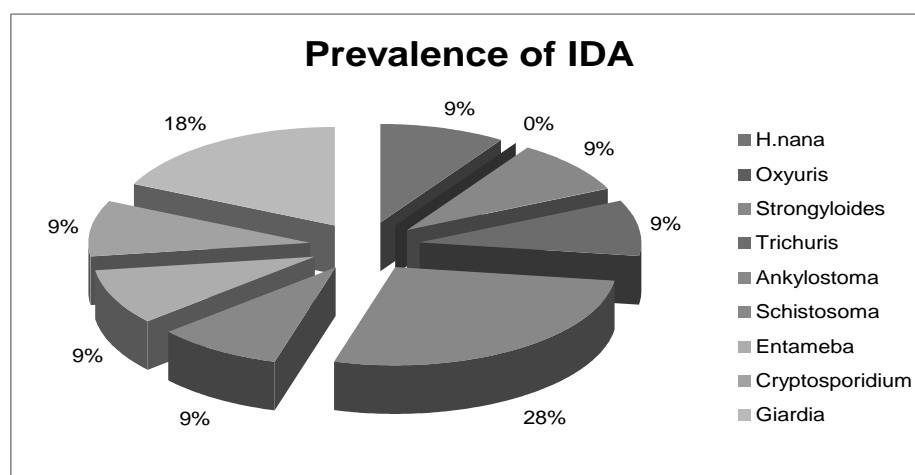
Fig (2): Prevalence of IDA in different subtypes of intestinal parasite

DISCUSSION

In developing countries, intestinal parasitic infections are one of the most prevalent infections and also, have highly spread among school aged children [13].

Intestinal parasitic infestations are considered as a serious public health problem as it causes iron deficiency anaemia, growth retardation in children, and other physical and mental health problems [14].

Bad effect of intestinal parasitic infestations on child iron status was observed by Hesham et al. [15] who linked intestinal parasitic infestations with significant reduced mean haemoglobin levels in children.

Prevention of iron deficiency anemia can improve performance in school, avoid behavioral alterations, and assure better growth. Longitudinal studies reported that children who are anemic in infancy continue to have poor cognition, long-lasting adverse effects on hearing and visual function, poor school achievement and more behavioral problems [16].

This cross-sectional survey was carried out at El Kalige village, Dakahlia governorate to demonstrate the relationship between intestinal parasitic infections and iron deficiency anemia in a sample of children in this rural area in Dakahlia. Data showed an interaction between parasitic infections, haemoglobin level suggesting that parasites affect serum iron level of surveyed children.

This study was performed on 102 children (44 males, 58 females) aged 6- 12 years. The results reported that the prevalence of parasitic infections at this rural area was 37% (47.4% for males, 52.6% for females) (Table 1). This was in accordance with Sah et al. [17] who recorded that the prevalence of intestinal parasitic infection was 31.5% in a study performed on 147 school children attending the primary school in Nebal. The prevalence of parasitic infection was reported by Amare et al. [18] was lower than our results (22.7%) in their study on school children in Gondar, Ethiopia.

The study performed by Hegazy et al. [19] on 500 preschool children aged 2-6years in Damanhur city, revealed that overall prevalence of parasitic infections was 51.8%. Also Worku et al. [20] reported parasitic prevalence 55.6%, both results were greater than our study (37%) (Table1) .

The explanation for this result is due to improved sanitation which is the only definitive intervention to eliminate parasitic infection [21]. Sanitation in this village includes sewage systems since 1995 which helps the prevention of contamination of soil and water and prevent transmission of parasitic diseases. It is also due to periodic anti helminthic therapy. Anti helmintic treatment reduced morbidity by decreasing the worm burden. Regular chemotherapy in high-risk groups can reduce the levels of infection and also, result in immediate improvement in the growth and the health of the children [22]. Anti-helminthic therapy is given regularly at the start of each school year by Primary Health Care Unit in this village. Health education also participates in the reduction of the prevalence of parasitic infections. Achievement of reduction of infection and reinfection can be done by encouraging healthy behaviors [23].

Our results reported higher parasitic infection in females (52.6%) more than males (47.4%) but with insignificant difference (Table 1), this was in agreement with Rayan et al. [8] who found a higher parasitic infection in females (59%) more than in males (41%) in a study up on 195 rural school children. On contrast, Pradhan et al. [13] showed different result as the higher parasitic infection was in males (28.2%) compared with females (20.2%). This is explained by the presence of other environmental or behavioral factors other than the gender in parasitosis. Generally, the high risk of infection in male is due to the increased mobility of the male, while in female is due to more soil contact during growing vegetables and eating raw vegetable during preparing food [17].

This study reported that, the prevalence of anemia in all selected children was 51% (table 2).This was in accordance with Al-Mekhlafi et al. [24] who reported that the prevalence of anemia was 48.5% in a study was done on 241 school children (7-12) years in Pos Betau, Malaysia. Our result was higher than the result of a survey conducted by Ngui et al. [25] who reported overall prevalence of anemia 26.2% and Oliveira et al. [26] also found lower results than our result (21.6%). On contrast, Nabakwe et al. [27] found that anaemia was extremely high (92%) in Kenya, which could be attributed to the

increase in the incidence of malaria in the area of the study.

Our study reported a high prevalence of anemia in infected children(52.6%) compared with non-infected (50%)(table 1,2).This was in agreement with a study conducted by Hesham et al. [15] to assess the relationship between intestinal parasites and nutritional status in Thailand among 343 children, who showed that, the incidence of anemia was high among the infected children (59%) than non-infected children (42%). Hegazy et al. [19] also reported that prevalence of anemia was higher in infected children (48.6%) compared with non-infected (28.8%). It affects 20% to 50% of the world's population and it spreads among young children [28].

This study recorded that overall, 51% of children were anemic (Hb<11.5 g/dl). The prevalence of IDA was 45%, which accounted for 88.5% of the anemic cases (Table 2). Globally, these results were in agreement with the data showed by WHO that, in developing countries, the prevalence of IDA is up to 48% of school-age children [29]. This was in accordance with Aini et al. [30] who reported that overall, 41.5% of children were anemic and 36.5% had IDA, which accounted for 88% of anemia in these children. On the other hand, lower result was conducted by Ngui et al. [31] on a total 550 school children aged 7 - 12 years, 26.2% were anemic and 16.9% with IDA .

Our study reported that IDA was slightly highly non-significant among infected children with 47.3% compared with both non infected (43.8%) and all selected children (45%) (p=0.962) (Table 2). This is explained by the fact that blood loss (mostly occult bleeding), reduced appetite, impaired digestion, and malabsorption may be the reasons of poor iron status and iron deficiency anemia that are frequently observed in children suffering from intestinal parasitic infestations [32].

In our study parasitic infection is sub classified into 3 major group, helminthes, protozoa and mixed infection. Helminthic group (22), Protazoa group (8) and 8 cases mixed protozoa and helminthes. Overall helminthes infection were more prevalent 22% (22/102) compared with both protozoa infection 8% (8/102) and mixed infection 8% (8/102) (Table 3). This coincided with Oliveira et al. [26] who reported that the prevalence of infection by helminths was higher than that of protozoa (24.1%, 13.4%) in the studied children. While mixed coinfection by both helminthes and protozoa occurred in 6.7%. Different result was conducted by Pradhan et al. [13] showing that the protozoal infection was higher than of the helminthes and mixed infection (17.5%, 4.6%, 1.5%, respectively). Sah et al. [17] also found that, the infection by helminthes (13%) and of protozoa (18.5%) from the total population of the study.

Anemia was highly non-significantly prevalent in helminthes infection (54.5%) compared with both protozoan and mixed infection (50% each) (table3). This coincide with Jardim-Botelho et al. [33] who reported increase prevalence of anaemia associated with helminth infection compared with both protazoal and mixed infection. Also Alemu et al. [34] showed that helminth parasites was the obvious cause of high anemia prevalence compared to individuals with protozoa and mixed infections (P<0.0001).

Among the helminthes, the highest incidence was Ancylostoma duodenale (33.3%), followed by Hymenolepis nana (26.6%) (Fig. 1). This coincides with Jardim-Botelho et al. [33] who reported also a high incidence of Ancylostoma duodenale (69.8%) with the majority of individuals harbouring helminthes infections. This is in contrast with Gyawali et al. [35] and Khanal et al. [36] on studies conducted on school children of Nepal. These studies have found Ascaris lumbricoides and Trichuris trichiura as the commonest intestinal helminthes in school children of Nepal.

Among the intestinal protozoa, Giardia lamblia was the first one, followed by Entamoeba histolytica and cryptosporidium (fig 1). This was in accordance with Pradhan et al. [13] who found that Giardia lamblia was the most common (58.6%) among protazoal infection. On contrast, Rayan et al. [8] reported different result as Entamoeba histolytica had the highest prevalence (25.3%), then Giardia lamblia (17.9%).

In our study, Ancylostoma duodenale was the most common parasites associated with IDA in anemic infected children 28% (6/22),followed by Giardia lamblia 18% (4/22) then Hymenolepis nana, Strongyloides stercoralis, Trichuris trichiura, Schistosoma mansoni, cryptosporidium 9% (2/22) each (fig 2). This coincides with Aini et al. [30] who showed that the depletion of iron stores was mainly caused by Ancylostoma duodenale infection. It was reported that anaemia was more common among intestinal parasitic

infected especially, giardiasis. This study was performed to show the association between haemoglobin, serum iron, serum ferritin concentrations in Orang Asli children living in endemic areas of intestinal parasitic infections in Malaysia.

Jonker et al. **[37]** also found that *A. duodenale* was (32.1%) from the severe cases of anemia and (23.5%) from the non-severely ones. The explanation for this result may be that the severity of disease is associated with increase of the intensity of the hookworm infection. Due to the blood loss from the bowel mucosa of the infected host caused by the attachment and feeding of the adult hookworms upon it leading to iron deficiency and anemia. Blood loss caused by *A duodenale* is 2 to 10 times more than by *N.americanus*.

All studies which were performed upon the prevalence of hookworm infection, observed that hookworm infection was a strong predictor of IDA and anaemia in school children. In addition, anaemia became worst in heavy hookworm infection. Furthermore, it has been reported that children from high prevalence of *Ancylostoma duodenale* areas had significantly worse IDA than children from low prevalence areas **[38]**.

Many results reported that Giardia infection was associated with lower iron levels. The prevalence of IDA reached 74.4 % among pre-school children infected with giardiasis and coming from a rural area of Egypt **[39]**. *Giardia lamblia* infection had an adverse effect on the growth and the hemoglobin level of the children as a result of damage to the intestinal mucosa and malabsorption **[40]**.

CONCLUSION

The results showed that, the prevalence of parasitic infections is still high among the rural children of Egypt. Iron deficiency anemia is highly prevalent in school-children especially, in children who have parasitic infestations. This study emphasizes the importance of health education, good sanitation and personal hygiene, good cooking of food, safe water supply. And also, Screening for intestinal parasitic infections and appropriate treatment even they were asymptomatic, could be an important part of the program for anemia control in less developed countries.

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Evaluation of N-Terminal Pro Brain Natriuretic Peptide as a biomarker for clinical severity of heart failure in pediatric population

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Background and study aim: Brain Natriuretic Peptide (BNP) and N-Terminal pro-Brain Natriuretic Peptide (NT-pro BNP) are frequently used in the diagnosis of congestive heart failure (CHF), especially for distinguishing between patients with dyspnea of cardiac and pulmonary origin. The present work aimed at evaluating N-Terminal pro-Brain Natriuretic Peptide (NT-proBNP) as a biomarker for diagnosis of congestive heart failure (CHF) in pediatrics and as well as its severity and hence, helping early diagnosis of CHF in absence of rapid echocardiographic examination.

Subjects and Methods: The patients group (group I) consisted of 45 children (24 males and 21 females) aging between 45 days and 12 years. All having the inclusion criterion of CHF. They were subclassified into: group I-1 including 15 patients having dilated cardiomyopathy (DCM), group I-2 including 15 patients having congenital heart disease (CHD) and group I-3 including 15 patients that have developed CHF due to non-cardiac causes. The control group was formed of 15 healthy children (group II) matched in age and gender with the patients groups. All children were subjected to full history taking, physical examination, classification of clinical severity of CHF cases according to Modified Ross Score, imaging and laboratory investigations including serum level of NT-proBNP.

Results: The study revealed that serum NT-proBNP showed a highly statistically significant increase in CHF cases three groups (I-1, I-2 and I-3) in comparison to the control group (group II) (P<0.001). NT-proBNP level showed highly statistically significant positive correlation with CHF class of clinical severity (P<0.001). Regarding echocardiographic parameters NT-proBNP showed a highly significant positive correlation with left ventricular end diastolic dimensions (LVEDD) and left ventricular end systolic dimensions (LVESD), and a highly significant negative correlation with left ventricular (LV) ejection fraction (EF), fractional shortening (FS) and mitral valve E/A ratio. At cutoff level of 1500 pg/ml, the sensitivity of NT-proBNP as a diagnostic biomarker in children with CHF was 98% and the specificity was 100%.

Conclusion: NT-proBNP is significantly statistically correlated with clinical severity of CHF and echocardiographic parameters of CHF cases of different causes .We highly recommend a long-term study on the value of the level of NT-proBNP as a prognostic risk parameter.

INTRODUCTION

CHF is a clinical syndrome where the heart is unable to provide the output required to meet the metabolic demands of the body; however, the causes and mechanisms of CHF are significantly different between adults and children **[1]**. There is no single diagnostic test for CHF because it is largely a clinical diagnosis based on a

careful history and physical examination **[2].** In early stages of CHF, various compensatory mechanisms are evoked to maintain normal metabolic function **[1]**. The clinical syndrome of CHF is a final common pathway of most forms of cardiovascular disease **[3]**. Several studies have reported the prevalence of CHF to vary between 3% and 9% **[4-6]**. Thus, it is a pediatric emergency that must be anticipated and excluded in every acutely ill child **[6]**. CHF has multiple causes: predominant among these in developed countries are the primary cardiomyopathies, which account for 60% of children requiring a cardiac transplant, and the congenital heart diseases **[7]**. In addition, certain systemic processes such as inflammatory diseases, metabolic disorders, endocrine derangements, and kidney disease result in an unknown number of cases **[8]**.

B-type natriuretic peptide (BNP) is a member of a four natriuretic peptide family that shares a common 17-peptide ring structure. The N-terminal fragment (NT-pro-BNP) is biologically inert, but both are secreted in the plasma in equimolar quantities and both have been evaluated for use in the management of CHF **[9]**. BNP stimulates natriuresis and vasodilation with consequent afterload reduction, inhibits renin- angiotensin-aldosterone release and sympathetic nervous activity, and reduces fibrosis. BNP and NT-pro-BNP are frequently used in the diagnosis of CHF and distinguishing between patients with dyspnea of cardiac or pulmonary origin. 'Normal' values of these peptides vary depending on the type of test used. The performance characteristics of these tests vary depending on the patients on whom they are used and the manufacturer. For this reason, the determination of reference values for this peptide represents such a challenge **[9]**.

SUBJECTS AND METHODS

This case control study was carried out at Pediatric Cardiology Unit, Pediatric Intensive Care Unit and Medical Biochemistry Department in Zagazig University Hospitals during the period from March 2012 to September 2014.

Subjects :
The study covered 60 subjects that will be divided into the following groups:

Group I : (CHF patients):
Forty five patients in pediatric age groups ranging between 1.5 months and 12 years, 21 (46.7%) males and 24 (53.3%) females. All the

patients in group I have the inclusion criterion of having clinical CHF signs and symptoms, and they will be subdivided into 3 groups:

Group I-1: Fifteen patients having DCM.

Group I-2: Fifteen patients having CHD : 7 cases of common atrio-ventricular canal (CAVC) (47%), 3 cases of combined atrial septal defect (ASD) and ventricular septal defect (VSD) (20%), 2 cases of combined ASD, VSD and pulmonary stenosis (PS) (13%), one case VSD (6.7 %), one case of double inlet left ventricle (DILV) (6.7%) and one case of tricuspid atresia (6.7%).

Group I-3: Fifteen patients that developed CHF secondary to non cardiac causes involving: 6 cases of severe pneumonia (40%), 4 cases of acute severe asthma (27%), one case of severe bronchiolitis (6.7%), one case of severe bronchopneumonia (6.7%), one case of Acute Respiratory distress Syndrome(ARDS) (6.7%), one case of right lung collapse (6.7%) and one case of anemic heart failure on top of acute severe hemolysis(6.7%).

Exclusion criteria including:
1- Recent cardiopulmonary surgery.
2- Current hemodialysis.

Group II (control group):
Fifteen healthy individual as a control group, the subjects of this group are matched in age and gender to the patients groups.

All children in this study were subjected to complete history taking, general examination including vital signs and anthropometric measures, cardiac examination, chest examination, abdominal examination and clinical assessment of CHF with grading of severity according to Modified Ross Score.

X-ray chest and heart and standard 12 lead Electrocardiography (ECG) were performed. Echocardiographic study was performed in all patients using Ultrasound Machine, Vivid7 (GE medical system, Horten, Norway). Echocardiographic examination included LVEDD, LVESD, and LV systolic function in the form of left ventricular EF and FS using two-dimensional echocardiography and M-mode echocardiography. Also mitral valve E/A ratio was performed by continuous wave (CW) Doppler. By echocardiography, EF % <50% was systolic heart failure and mitral valve E/A ratio <1 was diastolic heart failure [(10)].

Blood for NT-proBNP assay was taken from peripheral venous puncture (3 ml) and collected in serum separator tubes (SST) within 3 hours of

the echocardiography and allowed samples to clot for 30 minutes before centrifugation for 15 minutes. Serum was removed and stored at -20°C until the time of analysis. All reagents were brought to room temperature before use. NT-proBNP was analyzed using a research NT-proBNP ELISA Kit (EIAAB and USCN Life Company, China).

Statistical analysis:
All data were collected, tabulated and statistically analyzed using Statistical Package for the Social Sciences (SPSS version 18). Quantitative data were expressed as the mean ± SD & median (range). Continuous data were checked for normality by using Shapiro Walk test. Independent Student t-test was used to compare two groups of normally distributed data. Mann-Whitney test was used to compare two groups of non normally distributed data. ANOVA (Analysis of variance) was used to test the difference about mean values of parameters of normally distributed data among the groups of study. Kruskal-Wallis test was used to compare more than two groups of non normally distributed data. Spearman's coefficient was calculated to assess relationship between study parameters, (+) sign indicate direct correlation and (-) sign indicate inverse correlation, also values near to 1 indicate strong correlation & values near 0 indicate weak correlation. All tests were two sided, $P<0.05$ was considered statistically significant (S), $P<0.001$ was considered highly statistically significant (HS), and $P\geq0.05$ was considered non statistically significant (NS).

RESULTS

Group I involved our CHF cases who showed different stages of CHF either acute or chronic or resolving CHF. Group II consisted of 15 healthy control subjects matched in age and sex with the groups of CHF cases. There are statistically non significant differences in our study between the groups of cases and the group of control regarding age, gender, body weight and length. There are statistically non-significant difference between the three groups of cases regarding symptoms and signs (Hepatomegaly, dyspnea, edema, orthopnea and cyanosis) . According to clinical severity, CHF cases (45cases of group I) were classified according to Modified Ross Score into : Five cases (11.1%) as Ross IA (mild CHF), 11 cases (24.4%) as Ross IB (moderate CHF) and 29 cases (64.4%) as Ross IC (severe CHF) . There is statistically non-significant difference between the three groups of CHF cases (Group I-1, I-2 and I-3) regarding distribution of cases clinical severity of CHF according to Modified Ross Score (Table 1).

Table (1): Comparison of Modified Ross Score among the cases groups

Variable	GroupI-1 (DCM) (n=15)		Group I-2 (CHD) (n=15)		Group I-3 (NCCHF) (n=15)		χ^2	P
	N	%	N	%	N	%		
Modified Ross:								
Ross IA	0	0	1	7	4	27	8.61	0.072
Ross IB	4	27	2	13	5	33		NS
Ross IC	11	73	12	80	6	40		

DCM= Dilated cardiomyopathy
CHD= Congenital heart diseases
NCCHF= non cardiac causes of CHF
χ^2= Chi-square test
NS= Non Significant

Some echocardiographic diameters left ventricular EF, FS and mitral valve E/A ratio) showed statistically highly significant decrease in the three groups of cases (groups I-1, I-2 and I-3) compared to the control group (group II) with p value < 0.001, while LVEDD and LVESD showed statistically highly significant increase in the three groups of cases (groups I-1, I-2 and I-3) compared to the control group (group II) with p value <0.001 (Table 2, Fig. 1), but there is statistically non significant difference between groups of cases (groups I-1, I-2 and I-3) regarding the echocardiographic parameters (EF, FS, LVEDD, LVESD and mitral valve E/A).

Table (2): Comparison of ECHO findings of the studied groups cases and control

Variable	Group I-1 (DCM) (n=15)	Group I -2 (CHD) (n=15)	Group I-3 (NCCHF) (n=15)	Group II (Control) (n=15)	F	p
EF (%): Mean ± SD	40.27 ± 5.43	45.2 ± 10.05	44.8 ± 9.6	71.33 ± 4.59	49.16	<0.001 HS
FS (%): Mean ± SD	20.2 ± 4.14	23.27 ± 5.08	23 ± 6.07	39.47 ± 4.24	47.15	<0.001 HS
LVEDD(mm) Mean ± SD	46.33± 4.25	41.80± 8.86	40.93± 9.37	32.33± 6.38	9.11	<0.001 HS
LVESD(mm) Mean ± SD	35.27 ± 5.09	31.73± 7.9	30.6± 8.19	20.0± 4.7	14.57	<0.001 HS
E/A: Mean ± SD	1.04 ± 0.25	0.97 ± 0.11	1.08 ± 0.18	1.54 ± 0.20	26.8	<0.001 HS

EF = Ejection fraction   FS = Fractional shortening
HS=High Significance   LVEDD= Left ventricular end diastolic dimensions
LVESD = Left ventricular end systolic dimensions   E/A ratio= Mitral valve E/A ratio
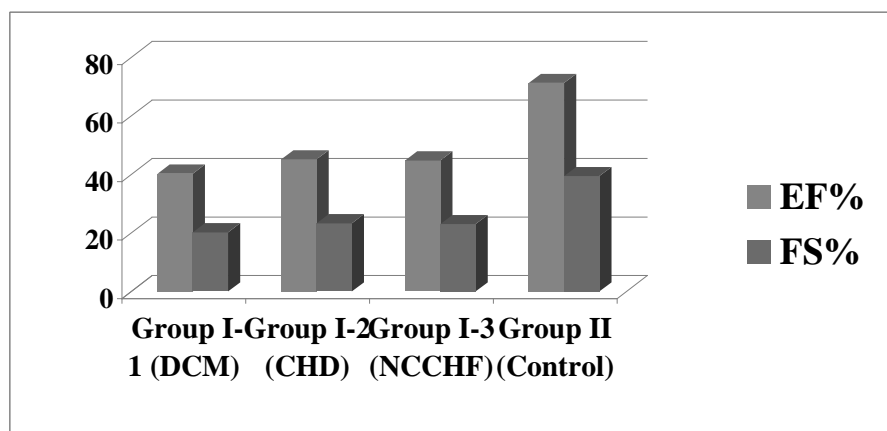
Figure (1): Comparison between groups of CHF cases and control group as regard EF and FS.

The serum level of NT-proBNP showed statistically highly significant statistical increase in groups of cases (I-1, I-2 and I-3) compared to the control group (group II) with p value < 0.001.(Table 3, Fig. 2), but there is statistically non significant difference between the three groups of cases regarding the serum level of NT-proBNP with p value > 0.05 (Table 4).

Table (3): Comparison of NT-proBNP level of the studied groups (cases and control)

| Variable	Group I-1 (DCM) (n=15)	Group I-2 (CHD) (n=15)	Group I-3 (NCCHF) (n=15)	Group II (Control) (n=15)	Kw	p
NT- ProBNP (pg/ml) Median	6200	6300	4800	179		<0.001
Range	4800 - 10800	530 - 6800	1900 - 6800	89 - 1100	36.18	HS

KW= Kruskal Wallis test
HS= Highly Significant

Table (4): Comparison of NT- ProBNP level of cases groups

Variable	Group I-1 (DCM) (n=15	Group I-2 (CHD) (n=15)	Group I-3 (NCCHF) (n=15)	KW	p
NT- ProBNP (pg/ml) Median Range	6200 4800 - 10800	6300 530 - 6800	4800 1900 - 6800	5.59	0.06 NS

KW= Kruskal Wallis test
NS= Non Significant
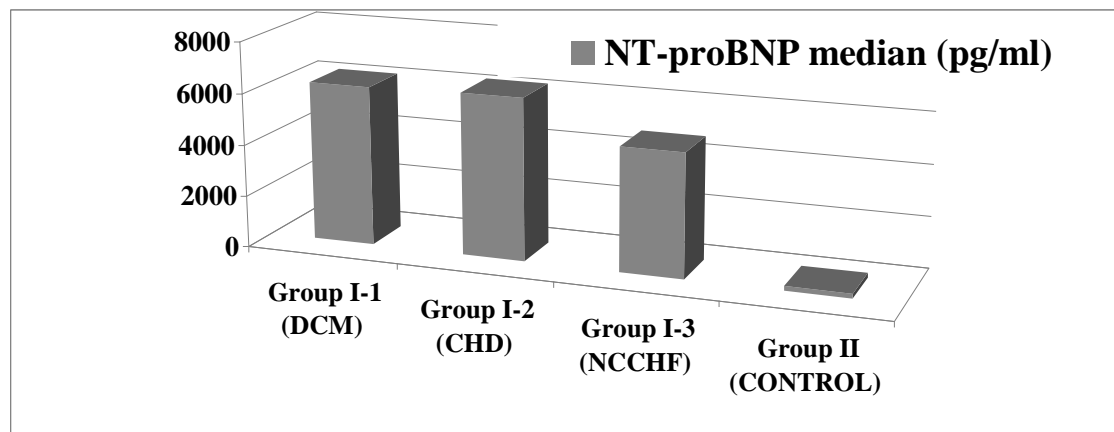
Figure (2): Comparison between groups of CHF cases and control group as regard the serum level of NT-proBNP

There is statistically highly significant difference between the three classes of Modified Ross Score in CHF cases regarding the serum level of NT-proBNP (Table5).

Table (5): Relation between NT-ProBNP level of Group I (45 cases of CHF) and Modified Ross scoring

Variable	Ross IA (n=5)	Ross IB (n=11)	Ross IC (n=29)	KW	p
NT- ProBNP (pg/ml)					
-Median -Range	1900 530- 3800	4800 4200 – 5500	6300 5800 – 10800	31.47	<0.001 HS

KW= Kruskal Wallis test
HS= Highly Significant

The serum level of NT-proBNP showed statistically significant increase in cases of HFrEF compared to cases of HFpEF with p value < 0.05 (Table 6, Fig. 3). Also, The serum level of NT-proBNP showed statistically highly significant increase in cases of HFpEF compared to control group with p value <0.001 (Table 7, Fig. 3).

Table (6): Comparison between HFpEF and HFrEF regarding the serum level of NT-proBNP

Variable	HFpEF (EF>50%) (n=6)	HFrEF (EF<50%) (n=39)	MW	p
NT- ProBNP (pg/ml)				
Median Range	4900 530 - 6300	6200 1900– 10800	59	0.049 S

HFpEF = heart failure with preserved ejection fraction
S= Significant
HFrEF= heart failure with reduced ejection fraction
MW = Mann-Whitney test.

Table (7): Comparison between HFpEF and controls regarding the serum level of NT-proBNP

Variable	CHFpEF (EF>50%)(n= 6)	Control (n= 15)	MW	p
NT- ProBNP (pg/ml)				
Median Range	4900 530 - 6300	179 89 - 1100	1.000	<0.001 HS

HFpEF = heart failure with preserved ejection fraction
HS= highly significant
HFrEF= heart failure with reduced ejection fraction
MW = Mann-Whitney test.
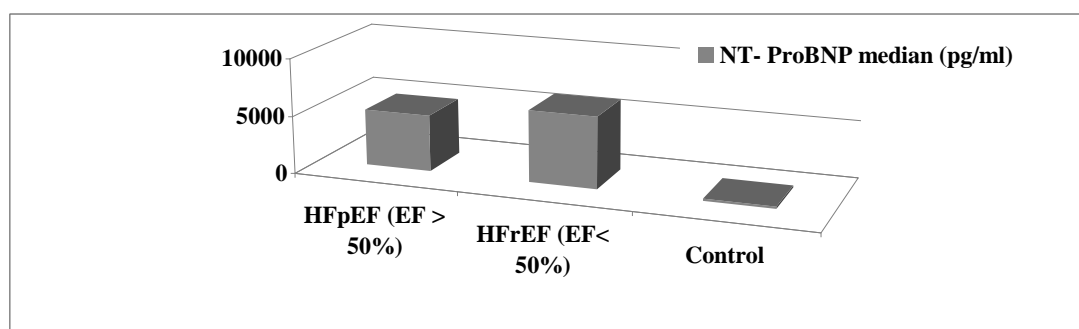
Figure (3): Comparison between cases of HFpEF
and cases of HFrEF regarding the erusm level of NT-p roBNP

There is highly significant statistical positive correlation between serum NT-proBNP and Modified Ross Score in the 45 cases of CHF with p value <0.001. NT-proBNP showed statistically highly significant positive correlations with LVEDD and LVESD with p value < 0.001 for each, and highly significant negative correlations with left ventricular EF and FS with p value <0.001 for each item, and statistically significant negative correlation with mitral valve E/A ratio with p value = 0.001. Serum NT-proBNP showed non statistically significant correlations with age, body weight, length, heart rate, respiratory rate and CBC parameters in the 45 cases of CHF(Table 8, Fig. 4-8).

Table (8): Correlation between NT- ProBNP level in patients of CHF and age, body weight, length, vital signs, Modified Ross Score, CBC and ECHO findings

Variable	r (n = 45)	P (n = 45)
Age	-0.017	0.913NS
Body weight	-0.056	0.714NS
Length	-0.099	0.529 NS
Heart rate	0.039	0.804 NS
Respiratotry rate	-0.05	0.746 NS
Modified Ross score	**0.848**	**< 0.001 HS**
Hb (gm /dL)	-0.02	0.898 NS
RBCs (x 1000)	0.097	0.524 NS
WBCs (x 1000)	-0.052	0.733 NS
Platelets (x 1000)	-0.040	0.792 NS
LVEDD (mm)	**0.634**	**< 0.001 HS**
LVESD (mm)	**0.742**	**< 0.001 HS**
EF%	**-0.634**	**< 0.001 HS**
FS%	**-0.568**	**< 0.001 HS**
E/A ratio	**- 0.585**	**0.001 HS**

NS = non-significant, HS = highly significant, HB= Hemoglobin, RBCs= Red Blood Cells, WBCs = White Blood Cells, EF = Ejection fraction, FS = Fractional shortening, LVEDD= Left ventricular end diastolic dimensions, LVESD = Left ventricular end systolic dimensions, E/A ratio= Mitral valve E/A ratio.
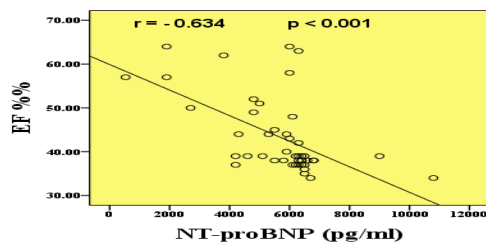
Figure (4): Correlation between NT- ProBNP level and left ventricular EF%
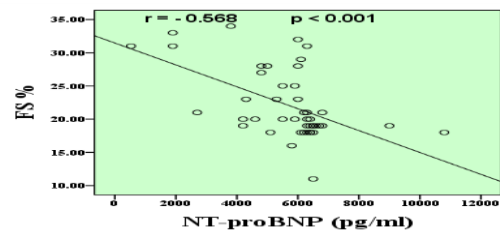
Figure (5): Correlation between NT- ProBNP and left ventricular FS%
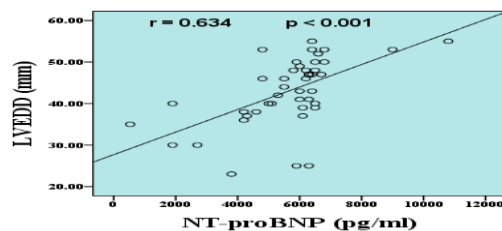
Figure (6): Correlation between NT- ProBNP level and LVEDD
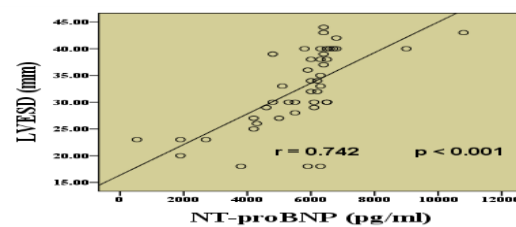
Figure (7): Correlation between NT- ProBNP level and LVESD
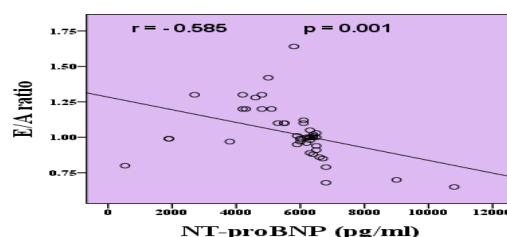
Figure (8): Correlation between NT- ProBNP level and mitral valve E/A ratio

The best cutoff value of NT-proBNP in diagnosis of CHF was 1500 pg/ml with 98% sensitivity and 100% specificity and the p value <0.001 (Table 9).

Table (9): Validity of NT- ProBNP in diagnosis of CHF

Cutoff	AUC	Sensitivity	Specificity	p-value
1500	0.99	98%	%100	<0.001 HS

AUC= Area under the curve
HS= Highly Significant

DISCUSSION

Brain natriuretic peptide (BNP) is one of the cardiac markers for CHF. It correlates with symptoms of CHF and may indicate LV volume and pressure overload in the presence of shunt [11]. The N-terminal fragment of proB-type natriuretic peptide (NT-proBNP) is secreted from cardiac myocytes together with BNP. Both BNP and NT- proBNP have been used to identify the presence and to determine the severity of CHF in children [12,13]. Most of the pediatric studies demonstrate an increase in natriuretic peptide levels in proportion to the symptomatic severity and the degree of remodeling in diverse pediatric cardiac diseases [14]. In this study, we hypothesize that changes in NT-proBNP serum levels are associated with changes in echocardiographic indices of LV systolic and diastolic function in children in CHF in cases of DCM, CHD and CHF of non cardiac origin, so we studied whether the rapid bedside determination of NT-proBNP level could be used for diagnosis of CHF and to predict the severity.

There was no statistically significant difference between the groups of cases (DCM, CHD and cases of non cardiac origin of CHF in our study regarding the proportion of CHF classes of clinical severity according to modified Ross score. Among whole cases, Ross IA (Mild CHF) represented 11.1% (5 cases), Ross IB (Moderate CHF) represented 24.4% (11 cases), and Ross IC (Severe CHF) represented 64.4% (29 cases).

With respect to the echocardiographic parameters in our study, there was statistically highly significant decrease in EF% and FS % (representing systolic dysfunction) in CHF cases of group I (1, 2 and 3) as compared with the control group (group II) with p value <0.001. In addition, there was statistically highly significant increase in LVEDD and LVESD in the patient groups as compared with the control group with p value <0.001. There is statistically significant decrease in E/A ratio (representing diastolic dysfunction) in the CHF cases groups as compared to the control group with p value <0.001. This has agreement with Zoair et al., who studied 20 cases of DCM and 20 healthy controls, they found that there is statistically significant difference between the group DCM cases and the control group regarding EF%, FS%, LVEED and LVESD, with p value of 0.001 for each parameter. The study did not include E/A ratio [15]. Also, Elwan et al. who studied 42 patients (24 ASD and 18 VSD, 11 of them in CHF) and 15 healthy controls found statistically significant difference between cases of CHD (ASD, VSD with and without CHF) and control group regarding LVEED and LVESD [16].

In our patients of CHF, there was a highly statistically significant increase in the level of NT-proBNP in CHF cases in group I (1, 2 and 3) compared to the control group (group II) with p value <0.001. While there is no significant statistical difference among the three groups of cases (I-1, I-2 and I-3) regarding NT-proBNP level. There is statistically significant difference between the grades of clinical severity in CHF cases classified according to Modified Ross Score (RossIA, Ross IB and Ross IC) regarding the level of NT-proBNP with p value <0.001. Koura et al. (17) supported our study as they had a cross sectional (comparative) study where 30 children divided into 11 cases of DCM and 19 cases of LRS (left to right shunt). They conclude that the NT-ProBNP level is elevated in both LRS and DCM in pediatric age. This elevation is more remarkable with heart failure and increased pulmonary artery pressure (PAP) in both diseased groups. Also, Narin et al. agreed with our study, as they found a statistically significant difference between NT-ProBNP levels in each Ross clinical group not only before treatment but also on assessment on the 7th day of treatment in the patient group (p < 0.001) [18].

In our study, there was statistically highly significant increase in the level of serum NT-proBNP in cases of CHFpEF in comparison to control group with p value <0.001. Also, there was statistically significant decrease in the level of serum NT-proBNP in cases of HFpEF in comparison to cases of HFrEF with p value <0.05. This has agreement with Masutani et al. who studied 18 pediatric patients with HFpEF and 22 patients with HFrEF; as they found plasma BNP levels were elevated in both CHF groups, but to a significantly smaller degree in HFpEF than in systolic heart failure (SHF) patients **[19]**.

Our results showed there is no correlation between serum NT-proBNP and patients' age, body weight, length, heart rate, respiratory rate and CBC parameters (HB, RBCs, WBCs and platelets) in group I (45 CHF patients) . But we found statistically highly significant positive correlation between serum NT-proBNP and the class of clinical severity according to Modified Ross Score with p value <0.001. Regarding echocardiographic parameters NT-proBNP showed highly significant positive correlation with LVEDD and LVESD, and highly significant negative correlation with ejection fraction and fractional shortening with p value <0.001. Also, NT-proBNP showed significant negative correlation with mitral valve E/A ratio with p value = 0.001. Elwan et al. supported our study as they found significant positive correlations between NT-proBNP concentration with LVEDD, LVESD, systolic pulmonary artery pressure (SPAP), and shunt size, and there was significant negative correlation with EF and FS, in cases of CHD (ASD, VSD with and without CHF), but in our study we did not examine SPAP and their study did not include mitral valve E/A ratio **[16]**.

The results of our study showed that, using a cutoff point of NT-proBNP as 1500 pg/ml, the sensitivity of NT-proBNP as a diagnostic biomarker in children with CHF was 98% and the specificity was 100%. This has agreement with Zoair et al. who used a cutoff point of NT-proBNP as 1500 pg/ml as a diagnostic biomarker in children with DCM, the sensitivity was 85% and the specificity was 100% **[15]**. However, Rusconi et al. who studied CHF in 36 pediatric patients with DCM-found that NT-proBNP level above 1000 pg/ml clearly identified the sickest patients. NT-proBNP levels between 450 and 1000 pg/ml did not distinguish between symptomatic and asymptomatic patients **[20]**. With a marked difference from our

study, Narin et al. used the NT-proBNP cut off value of 174.3 pg/ml to distinguish healthy children from the patients with left ventricular systolic dysfunction caused by cardiomyopathy **[18]**, this may be due to the difference in properties of the kit used.

NT-ProBNP level is significantly elevated in CHF with different causes (DCM, CHD and non cardiac causes of CHF) and in cases of HFpEF in pediatric age. So, we recommend the use of NT-ProBNP as a routine marker for diagnosing suspected patients with symptoms and signs suggesting CHF for rapid evaluation of cardiac functions, especially in absence of reachable echocardiographic examination.

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