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The Blood Plasma

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Contents of the Volume

i. Copyright Notice
ii. Editorial Board Members
iii. Chief Author and Dean
iv. Table of Contents
v. From the Chief Editor's Desk
vi. Research and Review Papers

1. The Application of Simulation Teaching Methods in Clinical Teaching of Surgery of Chinese Medicine. *1-4*
2. A Survey of Bancroftian Filariasis by Detecting Microfilaria and Circulating Antigenaemia in Biase Cross River State, Nigeria. *5-10*
3. Therapeutic Drug Monitoring and Evaluation of Therapeutic Effectiveness and Adverse Effects in Iraqi Epileptic Patients. *11-33*
4. Effects of Blood Transfusion in Outcome of Elective Bowel Anastomosis. *35-37*
5. Spatiotemporal and Joint Kinematic Analyses in Hemiparetic Cerebral Palsy Children During Stance Phase. *39-45*
6. Curative Effect of Extracts of Sapindus Mukorossi and Rheum Emodi in CCl4 Induced Liver Cirrhosis in Male Rats. *47-52*
7. Correlates of Impaired Renal Function in Highly Active Antiretroviral Therapy (HAART) Naive HIV Infected Patients in Maiduguri, Nigeria. *53-58*
8. Toxic of Effects of Heavy Metals (Cu, Ni, Fe, Co, Mn, Cr, Zn) to Hematology of Mastacembelus Armatus Thriving in Harduaganj Reservoir, Aligarh, India. *59-64*

vii. Auxiliary Memberships
viii. Process of Submission of Research Paper
ix. Preferred Author Guidelines
x. Index

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The Application of Simulation Teaching Methods in Clinical Teaching of Surgery of Chinese Medicine

By Li Jiehui, Tang Qianli, Zhang Li, Di Jiaqi, Feng Jing, Fu Jun, Yu Yuan & Yang Changmou

The First Affiliated Hospital of Guangxi University of Chinese Medicine, Nanning, Guangxi

Abstract - Objective : To evaluate the application effects of simulation teaching methods in clinical teaching of surgery of Chinese medicine.

Methods : Questionnaire surveys were conducted to collect information about how teachers and students appraised different models of teaching; then the students were randomly selected into two groups, one taught with simulation teaching methods and the other with conventional teaching methods; the teaching quality was evaluated when the teaching was over.

Results : Students in the group taught with simulation teaching methods got a better academic record than those in the group taught with conventional teaching methods, the difference was significant ($P < 0.05$).

Conclusion : In clinical teaching of surgery of Chinese medicine, simulation teaching methods are significantly better than conventional ones and worth spreading.

Keywords : *Simulation teaching methods; Surgery of Chinese medicine; Clinical teaching.*

GJMR-L Classification : *NLMC Code: W 88, WO 21, QY 50, WO 20*

Strictly as per the compliance and regulations of:

The Application of Simulation Teaching Methods in Clinical Teaching of Surgery of Chinese Medicine

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Keywords : Simulation teaching methods; Surgery of Chinese medicine; Clinical teaching.

I. Introduction

Medical simulation teaching is a process in which real clinical situations are simulated and teaching methods highly according to medical ethics are adopted; while it is performed, all simulative and virtual devices that can be available are used to design teaching conditions, including patients, scenes, laboratories for subject skill training and assessment, wards, operating rooms and hospitals, all of which are effective aids for theoretical teaching and clinical practice; it can promote clinical diagnostic ability and clinical operation skills of medical college students across-the-board, foster their quicken and correct clinical thinking, help reduce the occurrence of medical negligence and tangle in clinical practice and enable the students to go smoothly through the following three stages: study of theory, being permitted to become a doctor and clinical practice [1]. In addition, simulation teaching methods can help the students learn a basic knowledge of clinical work and facilitate them learning the ropes of their professions in the future [2]. In this study, we collected information about how teachers and students appraised different models of teaching via questionnaire surveys, performed clinical simulation teaching of surgery of Chinese medicine in hospitals and evaluated teaching quality by examinations.

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II. Methods

a) Questionnaire surveys

i. *Questionnaire design*

Questionnaires were to be filled out by informants and fell into two sorts, one for teachers and the other for students. We preserved the anonymity of all informants so as to respect their privacies.

Questionnaires for students: Collecting information about how much students from Guangxi University of Chinese Medicine were satisfied with teachers' teaching methods, the requirements for satisfaction including ① If teachers used teaching methods flexibly; ② If teachers used simulation teaching methods; ③If teachers placed emphases upon communication between themselves and students; ④ If teachers offered students guidance on how to study. The students were to be asked: How many teachers have met the requirements. The numbers of such teachers were classified into five levels: all (100% teachers used a certain method), majority (75% teachers used a certain method), half (50% teachers used a certain method), minority (25% teachers used a certain method) and none (0% teachers used a certain method).

a. *Questionnaires for teachers (Part I) :*

This part was to collect information about where teachers from Guangxi University of Chinese Medicine got knowledge sources of simulation teaching. To explore the feasibility of simulation teaching, we needed to learn about how much teachers were familiar with it and about what teachers thought if it were introduced. Only by gathering this information could we do better in simulation teaching. We supplied solutions to every question about simulation teaching trial put forward by teachers and tried to clear away obstacles to the trial. In this part, we designed a question in which the knowledge sources of simulation teaching were classified into four categories: ① Foreign literature; ②

Domestic literature; ③ Others (broadcast, television, newspaper, oral message, etc.); ④ I don't know.

b. *Questionnaires for teachers (Part II) :*

This part was to collect information about what attitude teachers from Guangxi University of Chinese Medicine maintained towards the introduction of simulation teaching methods. For this purpose, we designed another question: What's your attitude towards the introduction of simulation teaching methods? There were four answer choices: completely agree; partially approve; I don't care; disagree.

ii. *Informants*

The informants of our surveys were teachers and students from Guangxi University of Chinese Medicine in 2009. All the teachers were engaged in teaching, and all the students were undergraduates receiving a five-year education program.

iii. *Sampling methods*

Student informants were selected by numerically random sampling from the roster of the dean's office, and the questionnaires for them were distributed and collected by the class manager; teacher informants were chosen in technical title order by numerically random sampling.

b) Quality evaluation of simulation teaching methods

i. *Objects and grouping*

Objects of this part of research were 120 full-time undergraduate interns of Year 2005 majoring in surgery of Chinese medicine from the First Affiliated Hospital of Guangxi University of Chinese Medicine. They were randomly divided into two groups, experiment group (52) and control group (68).

ii. *Curriculum, class hour and teaching method*

Curriculum and class hour : The lessons were given according to teaching program and plan when the objects were studying at No. 5 Surgical Department of the First Affiliated Hospital of Guangxi University of Chinese Medicine.

Teaching methods : The experiment group was taught with the following methods.

① Teachers should give priority to heuristic mode of teaching, and they should perform teaching in various ways. They should guide students to teaching themselves the textbook, organize focus discussions on cases, treatment and other related problems and hold simulative consultations in class so as to bring students' enthusiasm into full play and optimize classroom atmosphere and promote students' ability.

② Starting from subjective complaint of patients, students rehearsed diagnostic work following "analysis – obtaining evidence – exclusion – confirmation" step by step. Then they received the following standard treatment training: selection of optimal therapeutic regimen – observation of new developments – adjustment of measures – diagnosis confirmation – analysis of prognostic factors – plan for recovery. By doing so, the students may develop a systematic structure of knowledge based on the textbook.

③ Teachers should focus on the instruction of focal and doubtful points and try to reduce or avoid repetition in content of the textbook so that the students could deepen cognition and comprehension of the knowledge they have learnt and memorize them.

The control group was taught with conventional teaching methods – teachers played the lead and made summaries at the end of class.

iii. *Evaluation of teaching quality*

Examination : Both groups took closed-book examinations; examination room and timetable and invigilation were arranged by the Section of Teaching Affairs.

Examination questions : Questions were selected from the question bank or set independently. They fell into two types, namely objective and subjective. Objective questions accounted for 60% of all the questions in an exam, including multiple-choice, gap-fill, true/false and matching questions; the design of these questions was to measure how much students have understood and memorized the basic concepts and theory of surgery of Chinese medicine. Subjective questions (40%) included essay questions and case analysis; they were designed to measure students' ability to understand and make analysis and judgement and apply theory to practice. The reference key to all questions was offered before both were sent to the Section of Teaching Affairs.

Marking : The examination papers were enveloped with paper bags by invigilators and submitted to paper markers.

iv. *Statistical analysis*

Results of the questionnaire surveys were analyzed with Ridit test.

III. Results

a) About how much students were satisfied with teachers' teaching methods

A total of 1477 questionnaires were distributed, 1470 being valid. From them, we learnt that the students were more satisfied with that the teachers attached importance to communication and taught in a flexible way, and less satisfied with the use of simulation methods and guidance on how to study (Table 1).

b) About where teachers got knowledge sources of simulation teaching

A total of 256 questionnaires were distributed. As regards the knowledge source of simulation teaching, the results show that 55 teachers got it from foreign literature, 136 teachers from domestic literature,

40 teachers from others, and 25 teachers were uncertain about it. This indicates that teachers mostly acquired knowledge of simulation teaching from domestic literature.

Table : 1 Evaluation of how much students were satisfied with teachers' teaching methods. All, majority, half, minority and none are levels to show how many teachers apply a certain method.

Teaching method	Evaluation level and informant number				
	All	Majority	Half	Minority	None
Flexibility	319（22%）	591（40%）	356（24%）	204（14%）	0（0）
Simulation	208（14%）	313（21%）	344（23%）	300（20%）	305（22%）
Communication	699（48%）	627（42%）	105（7%）	39（3%）	0（0）
Guidance	376（26%）	432（29%）	315（21%）	150（10%）	197（14%）

c) About teachers' attitude toward introduction of simulation teaching methods

From the 256 questionnaires we distributed, we knew that 129 teachers were in full agreement with the introduction of simulation teaching methods, 86 teachers partially approved it, 32 teachers were indifferent to it and 9 teachers were against it. That is to say, most teachers were in favor of the introduction of simulation teaching methods.

d) Evaluation of teaching quality of simulation teaching methods

The academic record of students in both groups (experiment group and control group) was classified into four levels: ≥85 – excellent; 70-84 – good; 60-69 – pass; <60 – fail. The result reveals that, students in the experiment group got a better academic record than those in the control group, and the difference between both groups was significant ($P < 0.05$), indicating that simulation teaching methods were better than the conventional ones and deserved to be spread clinically. (Table 2).

Table 2 : Evaluation of teaching quality of simulation teaching methods.

Group	Number	Evaluation level			
		Excellent	Good	Pass	Fail
Experiment group	52	18	26	7	1
Control group	68	12	32	21	3

Note : Analyzed with Radit test, u=2.5739, P<0.05.

IV. Discussion

Medical college students should receive as much basic clinical training as possible, and strengthen the ability to do logical reasoning and integration by practice so as to know how to summarize all the information of patients step by step and interpret patients' performances using relevant theory. However, teachers play the lead and give instruction whereas students play a passive role as an audience during conventional teaching [3]. Moreover, the clinical teaching under a conventional teaching environment is hard to meet the demand of current teaching characterized by being systematic, large-scale and comprehensive. So it is difficult for conventional clinical teaching to produce results that reaching the objective of current clinical teaching.

Good teaching methods are a prerequisite to the promotion of teaching quality, and the basis for ability enhancement as well [4]. As a new teaching model, simulation teaching is in essence a teaching activity centering on patients and questions; it can to a great extent motivate the students, thoroughly acquaint them with what they have learned, increase their capacity for problem analysis and solving, enhance their awareness of that patients are the focus of clinical work and help them establish a relatively solid foundation for clinical practice [5]. In the whole course of simulation teaching, students play the leading role and experience the processes of hard reading, information searching, plan designing, discussion, simulated operation, summing up and evaluation; they are in the state of active learning and exploration from beginning to end.

Practice is the basis on which traditional Chinese medicine came into being, and clinical teaching of traditional Chinese medicine is an important part of the talent development in higher education of traditional Chinese medicine and a key teaching stage in which students integrate the theory of traditional Chinese medicine with clinical practice. In clinical teaching, many practices are arranged; students are generally organized to learn by watching clinical practices after they have received a part of medical theoretical lessons and go on a field trip to a hospital when all lessons are finished. In addition, clinical teaching is a significant step to the cultivation of practical talents characteristic of high medical ethics, good work style, full grasp of basic knowledge and strong hands-on capability; it conditions a smooth transition from medical student to junior doctor and the development of high-quality practical clinical talents of surgery of Chinese medicine[6-8]. With the system reform and enrollment expansion in colleges and universities, change in medical environments, increase in employment pressure and other similar factors, the traditional clinical teaching model has failed to satisfy the need of the great majority of students and even has become an obstacle to intern doctors' learning. Therefore, great importance must be attached to clinical teaching, and a new model suitable for clinical teaching of surgery of Chinese medicine should be explored.

Our Questionnaire surveys show that students were dissatisfied with teachers employing the conventional teaching model, and that the overwhelming majority of teachers were in favor of the introduction of a simulation teaching model, so it is of significance to popularize simulation teaching methods. Based on the fact that the conventional teaching methods have been out of date, we probed into the use of a new teaching model, and introduced simulation teaching methods to clinical teaching of surgery of Chinese medicine. This may offers a new idea for the reform of the traditional teaching model. From the results of our study, we know that simulation teaching methods are significantly better than the conventional ones, and that simulation teaching methods can help improve hands-on ability, facilitate grasp of autonomic learning ways, develop the ability to think in a scientific way, broaden the ken, arouse students' interest in science and cultivate the spirit of scientific exploration in students. Simulation teaching methods can also help build closer relations between teachers and students, make the assessment of students simpler, reduce the cost of teaching with practice and promote teaching quality. They are valuable methods for clinical teaching, and deserve to be popularized clinically.

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A Survey of Bancroftian Filariasis by Detecting Microfilaria and Circulating Antigenaemia in Biase Cross River State, Nigeria

By Mbah. M., Ogban. G.I., Okafor.I.M., Ejezie.G.C., Alaribe.A. A. A.
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Abstract - The estimation of filariasis prevalence in Biase Local Government has previously relied upon clinical evaluation and examination of night blood smears. However, night blood smears examination fail to detect the infection in individuals having low parasitaemia and cryptic filarial infection. The present study was undertaken to evaluate the prevalence of filarialsis in nine wards of Biase local government by immunochromatographic test (ICT).

Methods : Clinical examination was performed according to WHO criteria to classify filarial disease. Night blood smears collected between 21.00 to 00.00h were examined to detect microfilaria (MF). For estimation of circulating filarial antigen (CFA) by Binax Now filarialsis, 2ml of blood was collected from each individual by venepuncture at any time of the day.

Results : A total of 425 participants made up of 260 males and 165 females were examined randomly from the community with particular emphasis on those with suspected cases of infection such as elephantiasis of the leg.

Keywords : *Circulating filarial antigen-flariasis-biase-microflariaemia.*

GJMR-L Classification : *NLMC Code: WC 880*

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Results : A total of 425 participants made up of 260 males and 165 females were examined randomly from the community with particular emphasis on those with suspected cases of infection such as elephantiasis of the leg. The result shows that 56 (13.2 percent) of subjects had microfilaria of wuchereria bancrofti from night samples collected, while 207 (48,7 percent) of the population studied had positive result with ICT cards. There was a statistically significant difference in the prevalence of W. bancrofti microfilaria and circulating filarial antigenaemia by method of detection(X^2=11.004, P<0.05).We found out that there was no correlation between the two methods of detection of filarial infection (r=0.967, P>0.05).

Interpretation and conclusion : The study emphasizes the use of CFA estimation being a more sensitive and specific diagnostic tool for the evaluation of the true prevalence of the disease. The high CFA prevalence in the study area necessitates intervention measures to check its transmission.

Keywords : Circulating filarial antigen-flariasis-biase-microflariaemia.

I. Introduction

Lymphatic filariasis (LF) caused by the filarial nematode wuchereria bancrofti affects more than 120million people worldwide(1).

In Africa, the Prevalence of lymphatic filariasis is especially striking, affecting over 40 million people in the sub-Saharan region alone(2). Overall, Africa is thought to account for 40 percent of all cases of lymphatic filariasis in the world(3).

The third most endemic country in the world for this disease (after India and Indonesia) is Nigeria, where it is caused by W. bancrofti, and 22,1 percent of the population is thought to be infected(4).

In 2003, a survey was carried out in Plateau and Nassarawa state in Nigeria where the prevalence of lymphatic filariasis determined by ICT test was 22.5 percent and 22.4 percent respectively(5). The diagnosis of filarial infection by clinical examination and parasitological methods was the mainstay in detecting filarial infection up to early nineties. These methods though correctly assess the clinical cases and microfilaraemic subjects with high microfilariae MF count, but fail to identify low MF count and cryptic filarial infection in asymptomatic amicrofilariaemic individuals(6). In recent years, with the introduction of new diagnostic methods such as rapid diagnostic tests(RDTS) , the prevalence of filarial disease was redefined in many parts of the globe. The antigen and antiboby assays have several advantages over microscopic identitification of MF in blood, which is the traditional method of diagnosing Lf infection(14,15,16).They are more sensitive (i.e., MF-negative persons with positive antigen or antiboby test are frequently identified)(17) and both overcome the logistical constraint of obtaining blood at night, which is necessary in the many endemic areas where MF have nocturnal periodicity. The purpose of this study was to Study the infection status of the human population and finally to access the impact of mectizan distribution for Onchocerciasis control on lymphatic filariasis in area where the two diseases are co-endemic. To date such study has not been done in Biase thus ,the results of the present study may be relevant to determine the geographic distribution of lymphatic filariasis and the location of communities that requires treatment beyond Biase Local Government area, Cross River State, Nigeria.

II. Materials and Methods

Study area : The study was carried out in nine word namely Abayong, Akpet/Abini, Etono/Ikum, Adim, Ehom, Mbiakpan, °Agwagune, Umon and Ekei. (Total population 89737 males and 79446 females, census

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2007) of Biase Local Government Area, Cross River State Nigeria. Biase Local Government is bordered in the north east by Yakurr and Obubra Local government, in the south by Akamkpa and Odukpani local governments and in the West by Abia State.

The study was approved by the Ethical Committe of the Cross River Ministry of Health, Calabar. Informed consent was obtained from study individuals (parents in case of minor). Children less than 16 years old and individuals who did not give their consent were not part of the study.

Sample collection, a door-to-door survey was carried out from October 2008 to November 2009 in the local government to include individuals (adults and children aged 16 years and above in the study. History suggestive of filariasis and diethyl-carbamazine citrate (DEC) or mectizan consumption was recorded.

Mf dectection; Mf was detected by making two thick blood smears of 20μl each on a clean glass slide from 21.00 to 00.00h. The smears were air dried, dehaemoglobinised and stained with Giemsa stain to detect Mf.

Antigenaemia detection : About 2ml of blood was collected from all the individuals (n=425; 260 males and 165 females) enrolled in the study. Sera were separated in the field and brought to the laboratory and stored at -20^{0}c until tested. The binax now flilariasis was used for detecting and quantifying w. bancrofti antigen. The test card was removed from the pouch just prior to use. The card was laid flat on the work surface . The capillary tube was filled to the 100μl mark using capillary action with venous blood. The 100μl of sample was added Slowly from the capillary or pipette onto the top of the pink and white pad. The 100μl of sample was added slowly from the capillary or pipette onto the top of the pink and white pad.

Important : Each drop was allowed to soak in before adding the next drop onto the pad. Incorrect addition of sample may result in device failure.

It was allowed until the sample has flown into the pink area and was completely wet (this should take about 30 seconds to 1 minute).

The adhesive liner was removed and discarded and the adhesive of the test card was exposed.

The card was closed. To ensure good test flow, the card was pressed very firmly along the entire area to the right of the window.

The timing started.

The result was read through the viewing window after 10 minutes.

III. Result Interpretation

a) Positive result

The test was positive if two lines (TandC) were seen in the viewing window. Any pink line in the T area indicated a positive result. The test was positive even when the T line appeared lighter or darker than the C line.

b) Negative result

The test was negative if only the C line was seen. To ensure that low positive samples had sufficient time to develop, a negative result was not to be recorded until 10 minutes have elapsed from when the card was closed.

Statistical analysis : The Pearson correlation coefficient, student T-test and chi-square test were used to analyse the data. X2 for trend was used to find the relation of age with Mf and CFA prevalence while the Pearson Correlation was used to find out if there is any relationship between Knotts concentration method and the antigen detection.

IV. Results

A total of 425 individuals were examined from Biase local government. The prevalence of Mf and CFA was 13.2 percent and 48.7 percent respectively There was a statistically significant difference in the prevalence of W. bancrofti microfilaria and circulating filarial antigenaemia by method of detection($X2=11.004$, $P<0.05$). The correlation analysis showed that there is no relationship between the two methods of detection of filarial infection ($r=0.967$, $P>0.05$). The percentage of Mf and CFA positive individual increased steadily with age reaching a peak in the 16-26 year age group. The prevalence of Mf and CFA decrease steadily between 49-70 year age group. Beyond 70 year there was a fall in CFA prevalence while no individual was positive for Mf. There was a statistically significant difference in the distribution of circulating antigen of lymphatic filariasis in the blood of subjects by age ($P<0.05$) table 1 (1).

The Relationship between circulating filarial antigen (CFA) and microfilaria (MF) detection with clinical status is presented in table 2. it was observed that in asymptomatic individuals (n =399), ICT Now Filariasis Kits could detect infections in 203 (53.4%) individuals while night blood smear had 48 (12.6%) positive cases only. In symptomatic individuals (n=26), the prevalence was 61.5 and 46.1 percent by ICT and night blood smear respectively. Infection rate detected by CFA was significantly ($P<0.05$) higher compared to that by night blood smear examination.

Of the 425 individuals included in the study, 26 had clinical symptoms of filariasis (elephantiasis and hanging groin). Among the 24 individuals presenting with elephantiasis, Mf was present in 10 (38.5%) and CFA in 14 (53.9%) cases. All the 2 individuals presenting with hanging groin were microfilaraemic and were also found positive for CFA (Table 3). It was observed that all the microfilaraemic individuals were CFA positive but all the CFA positive individuals were not microfilaraemic. A total of 159 individuals were CFA positive but having no circulating Mf. From the 159 amicrofilaraemic antigen

positive individuals, 151 were asymptomatic and amicrofilaraemic having cryptic infection detected by ICT now filariasis test kits.

Table 4 shows the prevalence of lymphatic filariasis according to the knotts concentration methods and ICT. Among participants who had meaningful results, 56 (13.2 per cent) were positive for the thick blood film technique and 207 (48.7 per cent) by ICT card test. Out of 56 mf positive persons by the Knotts concentration method, only 2(3.6 per cent) were negative by the card test, whereas 151(41.1 per cent) individuals were negative by the Knotts concentration method. 216(58.8 per cent) were negative according to both Knotts concentration and ICT card test whereas 218(51.3 per cent) were negative for ICT card test alone. The overall sensitivity of the whole blood ICT card test was 96.5 per cent (56/58) while the specificity of the test was 58.8 per cent (216/367). The two false negative were males in the 37-47 year of age group.

V. Discussion

Filariasis is a major public health problem in Nigeria. With the continuous change in environmental factors, urbanization and availability of newer diagnostic tools (8), the estimation of 22,1 percent of the population thought to be infected is bound to be increased (4). With the widespread availability of the CFA assay which reflects adult worm burden (9), it can now be demonstrated that a majority of the earlier studies underestimated the prevalence of filariasis in endemic communities (7).

The prevalence of CFA was considerably higher than Mf prevalence in all the age groups (10),(11),(12). In the present study, the prevalence of filarial infection in the population was approximately four times higher when determined by CFA positivity compared to Mf examination in all the age groups expect 49-59 and 60-70 year age groups that the infection was one against sixteen and two against nineteen for Mf and CFA respectively (table 1). In the context of filariasis elimination programme, use of antigen detection in the diagnosis of filarsisis, particularly in young children is important as treatment at an earlier age may prevent subsequent development of clinical disease.

The average CFA prevalence was about 4 times higher than the Mf prevalence indicating that majority of infection was antigen positive but Mf negative. In this study, the prevalence of lymphatic filariasis was 3.56 times higher when determined by ICT compared to microfilaria examination in all age groups. This also confirms the work done by Cynthia et al., (2003) in Sao Paulo Brazil where the ICT test was 5.2 times higher than the Knotts concentration method. The present study found a high sensitivity (96 per cent) of the ICT card test compared with the Knotts concentration method. The prevalence of microfilaraemia and antigenaemia were slightly higher in males than in females: reasons being that male subjects (61.2 per cent) were more in number than the females (38.8 per cent). Females had euphorbia of vein puncture and also most of them were engaged in farm work during the period of blood collection. Cynthia et al. (2003) had similar results where the prevalence of microfilaremia and antigenaemia was slightly higher in males than in females in Brazil. In this case however, Mf prevalence was estimated by a relatively less sensitive 20µl blood smear and the present CFA+/Mf-might include low density Mf carriers. The prevalence of microfilaraemia and antigenaemia seemed to decrease with age (table 1). This is contrary to the work done in Cook Islands where the percentage of CFA positive subjects increased steadily with age reaching a peak in the 30-40 year age group (7).

A cost analysis of the ICT card test was carried out during the research. The Knotts concentration method was shown to have lower price (ICT cost per unit US$8 vs. Knotts concentration cost per unit US $0.3). However, certain features of the ICT card test proved to be extremely advantageous high sensitivity, the ability to offer prompt diagnosis, no need for complicated laboratory procedures, and no need for specialized technicians. These combined characteristics overcame the low price of the Knotts concentration making to be the overall more cost effective option, thereby justifying its use as a diagnostic tool in screening in endemic areas.

In conclusion, about 60 percent antigenaemia in the study population is a matter of concern and necessary control programme is needed to check the transmission of filariasis in the local government and neighbouring local government.

Table 1 : Prevalence of *Wuchereria bancrofti* microfilaraemia and circulating filarial antigenaemia by age.

Age Group (Year)	No Examined	No(%) positive for Mf	No(%) Positive for CFA
27 – 37	105	18 (17.1)	44 (41.9)
16 – 26	178	29 (16.3)	90 (50.6)
38-48	67	6(8.9)	33 (49.2)
49-59	25	1 (4)	16 (64)
60-70	37	2(5.4)	19 (54.3)
71-81	13	-(0)	5 (38.5)
Total	425	56 (13.2)	207 (48.7)

Mf- Microfilaria
No- number.
-denotes absence of positive cased use in results section.

Table 2 : Relationship between circulating filarial antigen (CFA) and microfilaria (Mf) detection with clinical status.

Symptoms	Test Results				
	CFA + Ve/ Mf + Ve	CFA – Ve/ Mf – Ve	CFA – Ve/ Mf + Ve	CFA – Ve Mf – Ve	Total
Asymptomatic	52	151	0	196	399
Symptomatic	13	8	0	5	26
Total	65	159	0	201	425

Table 3 : Prevalence of microfilaria of *W. bancrofti* and circulating filarial antigeneamia among subjects with elephantiasis and hanging groin.

	Number examined	No. (%) + For CFA	No (%) for microfilaria
Elephantasis of the leg	24	14 (58.3)	10 (41.6)
Hanging groin	2	2 (100)	2 (100)
Total	26	16 (61.5)	12 (46.1)

Table : 4 Prevalence of lymphatic filariasis according to the Knotts concentration method and ICT card test.

ICT Card Test		Knotts concentration	
	(percent)Positive	Negative (percent)	Total
Positive	56(TP)	151(FP)	207
Negative	2(FN)	216(TN)	218
Total	58	367	425

TP = True positive=number positive according to both knotts concentration and ICT test.
FN = False negative=number positive for knotts concentration and negative by the ICT card test.
FP = False positive = number positive for the ICT and negative for the knotts concentration.
TN = True negative = number negative according to both knotts and ICT card test.
Sensitivity =TP/ (TP+FN) = 56/(2+56) = 0.96=96 per cent.
Specificity=TN/ (FP+TN) =216/ (151+216) = 0.59=59 per cent.
Positive predictive value (PPV) = TP/ (TP+FP) = 56/(56+151) = 0.27=27 per cent.
Negative predictive value (NPV) =TN/ (TN+FN) = 216/ (216+2) = 0.99=99 per cent.
False Discovery rate (FDR) = FP/ (FP+TP) = 151/(151+56) = 0.73=73 per cent.

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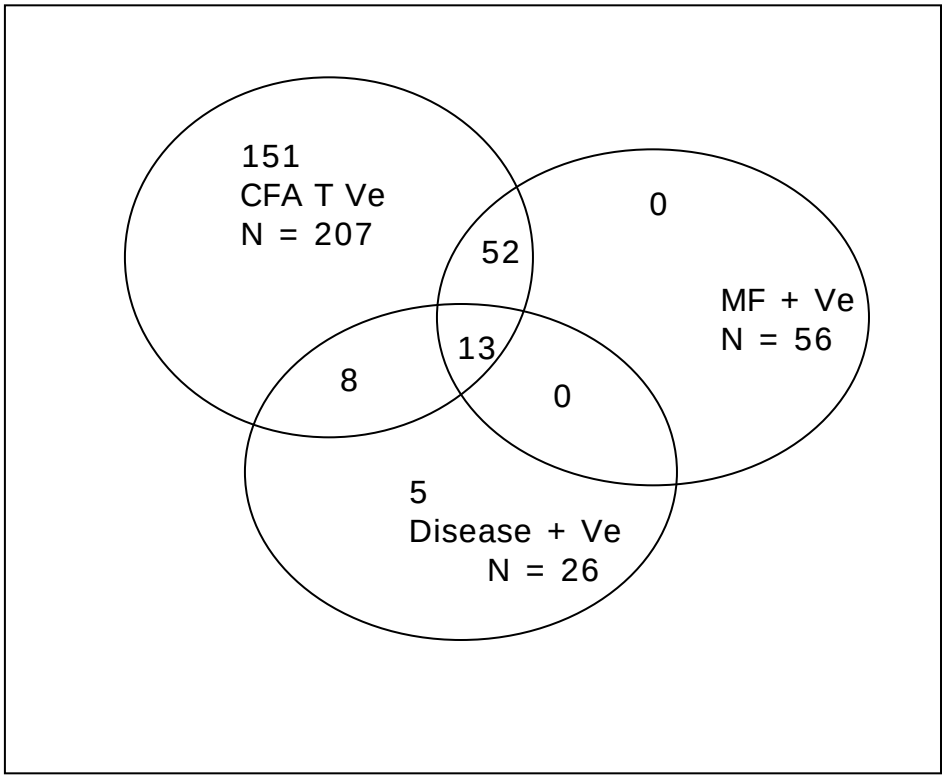

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Therapeutic Drug Monitoring and Evaluation of Therapeutic Effectiveness and Adverse Effects of Antiepileptic Drugs in Iraqi Epileptic Patients

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Abstract - This study was designed to evaluate the therapeutic effectiveness and adverse effects of carbamazepine, valproic acid, topiramate, and their combination in Iraqi epileptic patients. Ninety epileptic patients were participated in this study, their age ranged from (1-45) years. Seventy patients were previously diagnosed with epilepsy and received antiepileptic drugs for at least six months before this study (retrospective groups). The remaining patients were newly diagnosed with epilepsy (prospective groups). Twenty healthy subjects were selected to be a normal group for the purpose of comparison. The results showed that 90%, 75%, and 60% of patients in retrospective groups were seizure free after 3 months of treatment with carbamazepine, valproic acid, and topiramate respectively. On the other hand, only 45% of patients on combination therapy were seizure free. Whereas in prospective groups, 80% and 100% of the patients were seizure free after treatment with carbamazepine and valproic acid respectively. Serum levels of carbamazepine and valproic acid within the therapeutic range were found in about half of patients. While the remaining patients had their serum levels either in sub-therapeutic or in toxic level.

Keywords : *TDM, carbamazepine, valproic acid, topiramate, combination therapy, effectiveness, adverse effects, liver function tests.*

GJMR-L Classification : *NLMC Code: QT 162.U4, WB 300*

Strictly as per the compliance and regulations of:

Therapeutic Drug Monitoring and Evaluation of Therapeutic Effectiveness and Adverse Effects of Antiepileptic Drugs in Iraqi Epileptic Patients

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Abstract - This study was designed to evaluate the therapeutic effectiveness and adverse effects of carbamazepine, valproic acid, topiramate, and their combination in Iraqi epileptic patients. Ninety epileptic patients were participated in this study, their age ranged from (1-45) years. Seventy patients were previously diagnosed with epilepsy and received antiepileptic drugs for at least six months before this study (retrospective groups). The remaining patients were newly diagnosed with epilepsy (prospective groups). Twenty healthy subjects were selected to be a normal group for the purpose of comparison. The results showed that 90%, 75%, and 60% of patients in retrospective groups were seizure free after 3 months of treatment with carbamazepine, valproic acid, and topiramate respectively. On the other hand, only 45% of patients on combination therapy were seizure free. Whereas in prospective groups, 80% and 100% of the patients were seizure free after treatment with carbamazepine and valproic acid respectively. Serum levels of carbamazepine and valproic acid within the therapeutic range were found in about half of patients. While the remaining patients had their serum levels either in sub-therapeutic or in toxic level. The treatment was associated with a significant elevation in hepatic serum enzyme levels that was usually mild and asymptomatic and less than twice the upper limit of normal in all groups. The adverse effects developed were mild to moderate in nature. In conclusion, carbamazepine was more effective in retrospective groups; while, valproic acid in prospective groups was slightly more effective than carbamazepine in controlling seizures; moreover, mono therapy was more effective than combination therapy. TDM showed a poor correlation between the serum concentration of carbamazepine and valproic acid and their therapeutic and adverse effects.

Keywords : TDM, carbamazepine, valproic acid, topiramate, combination therapy, effectiveness, adverse effects, liver function tests.

I. Introduction

Epilepsy is a disorder that is best viewed as a symptom of disturbed electrical activity in the brain, which may be caused by a wide variety of etiologies. It is a collection of many different types of seizures that vary widely in severity, appearance, cause, consequence, and management. Seizures that are prolonged or repetitive can be life-threatening. The effect of epilepsy on patients' lives can be significant and extremely frustrating [(1)]. Of note is that seizures in many patients do not remit despite appropriate medication, and lifelong antiepileptic drugs (AEDs) therapy is usually required for those with refractory epilepsy. This practice poses a medical dilemma because prolonged AEDs therapy is often associated with a wide range of chronic adverse effects, including metabolic and endocrine disturbances, behavioral or psychiatric problems, idiosyncratic reactions, negative cognitive effects, and drug interactions [(2)]. Since AEDs have a narrow therapeutic index and complex pharmacokinetic properties, wide fluctuations in their plasma concentration can lead to either toxic effects or loss of therapeutic efficacy [(3)].

Therapeutic Drug Monitoring (TDM) is a concept of individualization of therapy based on drug concentration data, and application of pharmacokinetic and pharmacodynamic principles. It is not only a process of measuring drug concentration levels in biological fluids, but putting them into service of an optimized individual pharmacotherapy. The aim of TDM is to accomplish the optimal therapeutic drug response with minimal adverse drug effects e.g. better pharmaceutical care of patients [(4,5)].

This study was designed to evaluate the therapeutic effectiveness and adverse effects profile of AEDs carbamazepine, valproic acid, topiramate, and their combination through the assessment of the effect of these drugs on the frequency of seizure attack and on liver function tests in Iraqi epileptic patients. Also the present study was conducted to monitor and compare the serum levels of carbamazepine and valproic acid and to relate these levels to therapeutic effectiveness and adverse effects profile.

II. Subjects & Methods

a) Patients

This study was carried out at Tikrit teaching hospital in Salah Al-Deen governorate from November 2011 until June 2012. Ninety patients completed the

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courses of the study successfully. Seventy patients were previously diagnosed with epilepsy and received AEDs for at least six months before this study (retrospective groups) and these patients had poorly controlled epilepsy. Their age ranged from 1 - 45 years (mean ± SEM = 18.85 ± 1.25), of them 32 (45.71%) patients were male and 38 (54.28%) patients were female. The remaining patients were newly diagnosed with epilepsy and did not receive any AED before this study (prospective groups). Their age ranged from 2 - 32 years (14.95 ± 2.11), of them 9 (45%) patients were male and 11 (55%) patients were female.

The previously diagnosed patients were recruited into the following retrospective groups:

Group 1 : Includes 20 epileptic patients tested at baseline and after three months of treatment with carbamazepine (at dose 431.57 ± 16.75 mg/day) (mean ± SEM).

Group 2 : Includes 20 epileptic patients tested at baseline and after three months of treatment with valproic acid (at dose 492.10 ± 35.01 mg/day).

Group 3 : Includes 10 epileptic patients tested at baseline and after three months of treatment with topiramate (at dose 57.50 ± 7.49 mg/day).

Group 4 : Includes 20 epileptic patients tested at baseline and after three months of treatment with combination therapy as following:

i. Sixteen patients receiving carbamazepine and topiramate (at dose 787.5 ± 67.00 mg/day and 73.43 ± 12.80 mg/day respectively).
ii. Two patients receiving carbamazepine and valproic acid (at dose 600.00 ± 199.99 mg/day and 800.0 ± 0.0 mg/day respectively).
iii. Two patients receiving valproic acid and topiramate (at dose 800.00 ± 0.0 mg/day and 50.0 ± 0.0 mg/day respectively).

The newly diagnosed patients were recruited into the following prospective groups:

Group 1 : Includes 10 epileptic patients tested at baseline and after three months of treatment with carbamazepine (at dose 400 ± 29.81 mg/day) (mean ± SEM).

Group 2 : Includes 10 epileptic patients tested at baseline and after three months of treatment with valproic acid (at dose 430 ± 29.99 mg/day).

b) Healthy Subjects

Twenty subjects who were apparently healthy selected for the purpose of comparison. These subjects were selected from the medical staff and some relative volunteers, of them 9 were male (45%) and 11 were female (55%). Their ages were ranged from 1 – 49 years (19.55 ± 4.50).

c) Exclusion Criteria :

- Diabetic patients.
- Hypertensive patients.
- Patients with IHD, CHF, arrhythmias, or dyslipidemia.
- Hepatic impaired patients.
- Patients with thyroid dysfunction.
- Pregnancy, whether confirmed or suspected.
- Alcohol abusers.

d) Sample Collection And Preparation

Six milliliters of venous blood sample were drawn from each patient in the morning at 8:30 – 9:30 AM after 8-12 hours fasting by vein puncture, before starting drug treatment (as baseline sample) and then after 3 months of treatment. Serum was used for the measurement of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyl transferase (GGT), and concentration of carbamazepine and valproic acid. One blood sample was drawn from each healthy subject.

Liver enzymes were measured in serum by colorimetric method using the kit from Randox, (UK) for ALT & AST, Biomérieux, (France) for ALP, and Human, (Germany) for GGT. All the assays were performed on spectrophotometer.

e) Serum Drug Determination

Concentrations of carbamazepine and valproic acid in this study were determined by using high performance liquid chromatography with ultra violet detector (HPLC-UV). The HPLC system comprised the following: Waters 1500 series HPLC pump (USA), Waters 2487 dual λ absorbance detector (USA), and a computer with Waters Breeze software as data collecting system.

i. *Determination of serum carbamazepine concentration:*

- *Chromatographic condition:*

The chromatographic column C18 (4.6 mm × 250 mm, 5 µm) was used. Mobile phase was water, methanol, and acetonitrile (45:45:10). The system operated at ambient temperature. The flow rate was 1.0 $ml.min^{-1}$. An aliquot of 20 µl was injected for HPLC analysis. Monitoring was performed at 254nm [6].

- *Solutions preparation:*

Stock solution of carbamazepine (200µg.ml^{-1}) was prepared in methanol in a 25ml brown glass flask volumetric and stored at -20°C. The carbamazepine working solutions at concentrations of (1.6 µg.ml^{-1}, 3.125 µg.ml^{-1}, 6.25 µg.ml^{-1}, 10.0 µg.ml^{-1}, 12.5 µg.ml^{-1}, 15.0 µg.ml^{-1}, and 25.0 µg.ml^{-1}) were prepared by serial dilution of carbamazepine stock solution with methanol

from high to low. They were all stored away from light at 4°C [6].

- *Sample processing and extraction:*

An accurately sucked test serum 0.2 ml and the extract (chloroform: ethyl acetate = 50:50) 2 ml was successively set into a centrifuge tube on a vortex mixer for 5 min. After centrifuged at 4000 r.min^{-1} for 10 min, the organic layer 1.5 ml was transferred to another 5 ml centrifuge tube, and blow-dried with cold air in water bath at 60°C. At last, 200 µl mobile phase was added into the centrifuge tube and 20 µl solution was injected for HPLC analysis [6].

- *Standard curve drawing :*

Standard solutions (1.6 µg.ml^{-1}, 3.125 µg.ml^{-1}, 6.25 µg.ml^{-1}, 10.0 µg.ml^{-1}, 12.5 µg.ml^{-1}, 15.0 µg.ml^{-1}, 25.0 µg.ml^{-1}) 200 µl were took into a centrifuge tube with plug respectively, and then they were blow-dried with cold air in water bath at 60°C. Blank serum 200 µl was added in the centrifuge tube above respectively, making to the corresponding concentrations of standard serum. Serum was extracted according to the performance of the sample processing and extraction. Take the concentration of standard solution as the abscissa, and take the peak area value of standard substance as the vertical axis [6]. Drug concentration in the patient serum can be calculated by this method. The standard curve of carbamazepine is shown in figure 1.

ii. *Determination of serum valproic acid concentration:*

- *Chromatographic condition*

The chromatographic column C18 (4.6 mm × 250 mm, 5 µm) was used. Mobile phase consisting of acetonitrile and 0.05 M potassium dihydrogen ortho phosphate (pH adjusted to 3 with ortho phosphoric acid) (45:55 v/v) was used. The system operated at ambient temperature. The flow rate was 1.2 ml.min^{-1}. An aliquot of 50 µl was injected for HPLC analysis. The eluate was monitored at dual wavelength of UV detector at 210 nm from 0 to 10min [7].

- *Solutions preparation:*

Stock solution of valproic acid (1000µg.ml^{-1}) and diazepam (1000µg.ml^{-1}) was prepared in methanol and acetonitrile respectively in a 25ml brown glass flask volumetric and stored at -4°C. The valproic acid working solutions at concentrations of (20.0 µg.ml^{-1}, 50.0 µg.ml^{-1}, 80.0 µg.ml^{-1}, 120.0 µg.ml^{-1}, and 150.0 µg.ml^{-1}) were prepared by serial dilution of valproic acid stock solution with methanol from high to low. They were all stored away from light at 4°C [7].

- *Sample processing and extraction:*

To 250µl serum sample, acetonitrile solution of diazepam equivalent to 2.5 µg was added as internal standard and shaken well. Then equivalent amount of (250µl) acetonitrile was added for protein precipitation and mixed on a vortex mixer for 1 minutes and centrifuged at 4000 rpm for 20 min. 50 µl of the supernatant was injected on to HPLC column [7] .

- *Standard curve drawing :*

Standard solutions (20.0 µg.ml^{-1}, 50.0 µg.ml^{-1}, 80.0 µg.ml^{-1}, 120.0 µg.ml^{-1}, and 150.0 µg.ml^{-1}) 250 µl were taken into a centrifuge tube with plug respectively, and then they were blow-dried with cold air. Blank serum 250 µl was added in the centrifuge tube above respectively, making to the corresponding concentrations of standard serum. 250 µl of acetonitrile with 2.5 µg diazepam solution was then set into each centrifuge tube on a vortex mixer for 1 min, operated according to the performance of the sample processing and extraction. Plot the peak height ratio between valproic acid and diazepam vs. concentration of the drug to construct the calibration curve using the results from serum standard and serum blank [7]. Drug concentration in the patient serum can be calculated by this method. The standard curve of valproic acid is shown in figure 2.

f) Statistical Analysis

All data were expressed as mean ± standard error means (SEM). Statistical analyses were carried out using paired t-test to compare between mean values of parameters. P value < 0.05 was considered statistically significant. Descriptive analysis was carried out by Microsoft Office Excel 2007 software.

III. Results

a) Efficacy Of Treatment With Carbamazepine, Valproic Acid, Topiramate, And Combination Therapy :

i. *Retrospective groups:*

Table (1) shows the frequency of seizure in retrospective groups receiving carbamazepine, valproic acid, topiramate, and combination therapy after three months of treatment.

The data show that (90%) of the patients treated with carbamazepine mono-therapy did not have any seizure attack after treatment, whereas, (75%) of patients treated with valproic acid mono-therapy did not suffer from any seizure attack after treatment, and (60%) of the patients treated with the topiramate mono-therapy had an excellent control of their seizures after treatment.

On the other hand, only (45%) of patients receiving combination therapy were seizure free, whereas the remaining (55%) patients had poor seizure control after treatment.

ii. *Prospective groups:*

Table (2) shows the frequency of seizure in prospective groups receiving carbamazepine and valproic acid after three months of treatment.

The data in this table show that all the patients treated with valproic acid did not suffer from any seizure

attack after treatment, whereas only two patients exhibited one attack per month after treatment with carbamazepine.

b) Therapeutic Drug Monitoring:

i. *Serum carbamazepine:*

Table (3) shows serum carbamazepine concentration in prospective and retrospective groups receiving carbamazepine as mono-therapy or in combination therapy after three months of treatment.

The data show that the mean of the values of serum carbamazepine after treatment was within normal therapeutic range (10.75 ± 2.96, 10.24 ± 1.82, and 8.47 ± 0.99 μg/ml in prospective, retrospective, and retrospective with combination therapy respectively). No significant differences were observed among these groups.

Table (4) shows serum carbamazepine range in prospective and retrospective groups receiving carbamazepine as mono-therapy or in combination therapy after three months of treatment.

About half of the patients (50%, 65%, and 50% in prospective, retrospective, and retrospective with combination therapy respectively) had steady state serum concentration of carbamazepine within therapeutic range of (4 – 12 μg/ml) when the usual daily dose of carbamazepine was given (400.0 ± 29.81 mg/day prospectively alone, 431.57 ± 16.75 mg/day retrospectively alone, or 777.77 ± 60.65 mg/day retrospectively combined with another drug); however, this daily dose was sub-therapeutic (<4 μg/ml) for at least (20%, 15%, and 27.77% in prospective, retrospective, and retrospective with combination therapy respectively). On the other hand, this dose produced excessive serum concentrations (>12 μg/ml) in (30%, 20%, and 22.22% in prospective, retrospective, and retrospective with combination therapy respectively). Figures (3 – 5) show the chromatograms of drug-free serum (blank), serum spiked by standard of carbamazepine, and serum spiked by carbamazepine in patient's sample respectively. Retention time was 6.034 minutes for carbamazepine.

ii. *Serum valproic acid:*

Table (3) shows serum valproic acid concentration in prospective and retrospective groups receiving valproic acid as mono-therapy or in combination therapy after three months of treatment.

The data show that the mean of the values of serum valproic acid after three months of treatment was within normal therapeutic range (74.47 ± 17.11, 71.01 ± 12.36, and 67.27 ± 8.67 μg/ml in prospective, retrospective, and retrospective with combination therapy respectively). No significant differences were observed among these groups.

Table (5) shows serum valproic acid range in prospective and retrospective groups receiving valproic acid as mono-therapy or in combination therapy after three months of treatment.

About half of the patients (40%, 50%, and 75% in prospective, retrospective, and retrospective with combination therapy respectively) had steady state serum concentration of valproic acid within therapeutic range of (50 – 100 μg/ml) when the usual daily dose of valproic acid was given (430.0 ± 29.99 mg/day prospectively alone, 492.10 ± 35.01 mg/day retrospectively alone, or 800.0 ± 0 mg/day retrospectively combined with another drug); however, this daily dose was sub-therapeutic (<50 μg/ml) for at least (30%, 30%, and 25% in prospective, retrospective, and retrospective with combination therapy respectively). On the other hand, this dose produced toxic serum concentrations of valproic acid (>100 μg/ml) in (30% and 20% in prospective and retrospective groups respectively), while no patient developed toxic concentration when valproic acid administered with another AED. Figures (6 – 8) show the chromatograms of drug-free serum (blank), serum spiked by standard of valproic acid, and serum spiked by valproic acid in patient's sample respectively. Retention time was 1.344 and 7.093 minutes for valproic acid and internal standard of diazepam respectively.

c) Effect Of Treatment With Carbamazepine, Valproic Acid, Topiramate, And Combination Therapy On Liver Function:

i. *Serum (ALT) :*

Table (6) shows the serum ALT in retrospective groups. The baseline values of ALT in all groups were significantly higher than the healthy subjects' values. These values after three months of treatment were significantly higher than the baseline values and the healthy subjects' values. The percent increase between the baseline values of serum ALT and after three months values were ranged from 19.28% to 28.81%.

Table (7) shows the serum ALT in prospective groups. The values of ALT were significantly increased in patients receiving carbamazepine for three months when compared with their baseline values and also with the healthy subjects' values. While, no significant increase in ALT values after treatment with valproic acid as compared with their values at baseline. However, there were significant increases in values of serum ALT at baseline and after three months values when compared with the values of healthy subjects. The percent change between the baseline values of serum ALT and after three months values was 85.07% for carbamazepine and 17.88% for valproic acid.

ii. *Serum (AST):*

Table (8) shows the serum AST in retrospective groups. The baseline values of AST in all groups were significantly higher than the healthy subjects' values. These values after three months of treatment were

significantly higher than the baseline values and the healthy subjects' values. The percent increase between the baseline values of serum AST and after three months values were ranged from 29.72% to 43.91%.

Table (9) shows the serum AST in prospective groups. The values of AST were significantly increased in patients receiving valproic acid for 3 months as compared with the baseline values and with the healthy subjects' values. While, the carbamazepine's group of patients showed a significant increase in values of serum AST when compared with the healthy subjects' values only without significant increase in these values when compared with their baseline values. Both groups showed a significant increase in the value of AST at baseline level when compared with the healthy subjects' values.

The percent change between the baseline values of serum AST and after three months values was 25.17% for carbamazepine and 42.58% for valproic acid.

iii. *Serum (ALP) :*

Table (10) shows the serum ALP in retrospective groups. The baseline values of ALP in patients treated with valproic acid and in those treated with combination therapy showed significant increases when compared with the values of healthy subjects; whereas, no significant differences in the those values were existed between carbamazepine and topiramate groups of patients when compared with the values of the healthy subjects. However, these values after three months of treatment in all groups were significantly higher than the healthy subjects and baseline values. The percent increase between the baseline values of serum ALP and after three months values were ranged from 17.68% to 21.23%.

Table (11) shows the serum ALP in prospective groups. There were no significant increases in the serum level of ALP in both groups between baseline values and after three months values. However, there were significant increases in these values at baseline and after three months of treatment in both groups when compared with the values of the healthy subjects. The percent change between the baseline values of serum ALP and after three months values was 27.99% for carbamazepine and 24.00% for valproic acid.

iv. *Serum (GGT):*

Table (12) shows the serum GGT in retrospective groups. The GGT values after three months of treatment with carbamazepine, valproic acid, and combination therapy were significantly higher than their values at baseline level; whereas, no significant increase in GGT values in patients receiving topiramate therapy was observed.

These values after three months of treatment with carbamazepine, topiramate, and combination therapy showed significant increase as compared with the values of the healthy subjects; whereas, no significant increase in GGT values was observed in patients receiving valproic acid therapy.

At baseline, the values of GGT in the group of patients receiving carbamazepine and the group receiving combination therapy showed a significant increase when compared with the healthy subjects' values. On the other hand, no significant increases in the level of GGT in patients receiving valproic acid and in those receiving topiramate therapies were found. The percent increase between the baseline values of serum GGT and after three months values were ranged from 7.82% to 29.71%.

Table (13) shows the serum GGT in prospective groups. The values of GGT after three months of treatment with carbamazepine were increased significantly when compared with these values at baseline and with the values of the healthy subjects. While, no significant changes in the values of GGT were observed in valproic acid treated patients when compared with baseline and with the healthy subjects' values. The percent change between the baseline values of serum GGT and after three months values was 56.74% for carbamazepine and 33.14% for valproic acid.

d) Adverse Effects Associated With The Treatment With Carbamazepine, Valproic Acid, Topiramate, And Combination Therapy

i. *Retrospective Groups*

Table (14) shows the adverse effects associated with the treatment in retrospective groups after three months of treatment. Different types of adverse effects were observed in all groups and the incidence of these effects was varied among these groups. The combination therapy had the higher rate of incidence of these effects than the other groups; whereas, topiramate had the lower rate of incidence. The following adverse effects were reported more frequently among the groups: headache, fatigue, loss of appetite, weight gain, and weight loss.

ii. *Prospective Groups*

Table (15) shows the adverse effects associated with the treatment in prospective groups after three months of treatment. Different types of adverse effects were observed in both groups and their incidences were varied between these groups. The following adverse effects were reported more frequently in both groups: headache, fatigue, and loss of appetite.

IV. Discussion

Efficacy Of Treatment

Controlling seizures with minimal adverse effects and maintaining the patient's ability to perform daily activities are the critical measures of treatment efficacy. In this study, the efficacy of the AED therapy was measured in terms of the number of seizures

experienced by the patients throughout the follow up period of three months. Seizure counts are the only reasonable and standard way to evaluate efficacy of treatment [8].

As shown in tables (1), 48 (68.57%) patients in retrospective groups became seizure free, while 22 (31.42%) experienced different rate of seizure attack during that period. In group treated with carbamazepine, 90% of patients were seizure free after three months of therapy, whereas 75% of patients on valproic acid were seizure free. 60% of patients received topiramate became seizure free after treatment. On the other hand, only 45% of patients on combination therapy were seizure free. Thus, in retrospective groups, carbamazepine showed an excellent control of seizures followed by valproic acid and topiramate mono-therapy, whereas the combination therapy was associated with poor control of seizures.

Whereas in table (2), 18 patients (90%) in prospective groups became seizure free and only two (10%) patients experienced a single seizure attack during the study period. In group treated with carbamazepine, 80% of patients were seizure free and only 20% of patients had a single seizure attack per month, whereas all the patients (100%) on valproic acid became seizure free after treatment. Thus, in prospective groups, valproic acid has a slightly better control of seizures than carbamazepine.

In general, mono-therapy is the ideal strategy for seizure control, and approximately 50% to 70% of all patients with newly diagnosed epilepsy can be maintained on one drug [9,10]. After failure of the first mono-therapy, only 14 to 20% of patients with seizures will be successfully controlled with any alternative single drug; however, many less respond if the first drug was ineffective [11]. This later group represents part of the approximately 20% to 30% of people with persistent seizures and chronic epilepsy even with medical treatment. Overall, persistent seizures are more common in patients with frequent seizures, multiple types of seizures, abnormal neurologic findings, a brain lesion, onset in the first year of life, or abnormal EEG findings [12]. Combining AEDs with different mechanisms of action to achieve freedom from seizures may be advantageous (perhaps allowing synergistic drug effects), although this approach is as yet unproven and typically results in complex and additive side effects [13]. Unfortunately, after failing mono-therapy trials, less than 10% of patients have complete control of seizures with dual therapy [14,1].

The results gained in this study are in agreement with the results of the other studies. In a study conducted by Ripple T. et al. (2011) to evaluate the effectiveness and safety of AEDs in patients with epilepsy. They reported that carbamazepine had advantages in epilepsy control over newer AEDs as a class, and valproic acid provided epilepsy control similar to newer AEDs [15]. Kowalik A. et al. (2008) studied the effect produced by the conversion from carbamazepine or oxcarbamazepine to topiramate in 140 adolescents and adults with epilepsy. They reported that a seizure reduction of ≥ 50% was achieved in 91% of patients in the last scheduled period (week 12-26); 62% of patients entering that period remained seizure free [16]. A retrospective review of 1,617 seizure free patients revealed that 21% were on poly-therapy and the remaining patients were on mono-therapy [17].

Therapeutic Drug Monitoring

The data in table (3) showed that the mean of the values of the serum concentrations of carbamazepine and valproic acid after 3 months of treatment in all groups were within normal therapeutic range when the usual daily doses of carbamazepine and valproic acid were given. However, and as shown in table (4), about half of patients taking usual daily doses of carbamazepine had the therapeutic level of drug. While, the remaining patients had their serum levels of carbamazepine either in sub-therapeutic or in toxic level. The lowest concentration of carbamazepine in these patients was 1.8 μg/ml whereas the highest concentration was 27.6 μg/ml.

Carbamazepine has complex physicochemical properties, short half life and narrow therapeutic index. A variety of drugs could inhibit its metabolism and increasing the risk of accumulation. Erythromycin and other macrolides were well recognized to cause significant elevation of carbamazepine concentration [18]. Large inter-individual differences in apparent plasma half life linked to auto induction and narrow therapeutic range make this drug suitable for monitoring [19].

As shown in table (5), therapeutic level of valproic acid was also found in about half of patients taking usual daily doses of valproic acid. The remaining patients had their serum levels of valproic acid either in sub-therapeutic or in toxic level. The lowest concentration of valproic acid in these patients was 22.6 μg/ml whereas the highest concentration was 162.3 μg/ml.

Valproic acid is an inhibitor of certain CYP enzymes and as such can cause drug-drug interactions, including with other AEDs such as carbamazepine. However, valproic acid was devoid of enzyme inducing properties, but a risk of interaction still existed as an inhibitor of oxidative and non oxidative drug metabolism. As a result plasma level of it fluctuates during chronic treatment. Metabolites of valproic acid contribute to both antiepileptic and toxic effects. Considering all these effects, therapeutic monitoring of valproic acid is also quite useful [19,20].

So, TDM of carbamazepine and valproic acid in this study did not show a wide fluctuation in the serum level of each drug as higher proportion of patients taking these drugs individually or combined with other AEDs

had their serum drug level within therapeutic range, and the toxic and sub-therapeutic levels were not quite high. However, poor correlation was found between the serum concentration of carbamazepine and valproic acid and their therapeutic effects. It is suggested that monitoring of both drugs would be helpful when their toxicity and efficacy are doubtful.

Studies on the effect of TDM on outcome in terms of complete seizure control and/or best compromise between improved seizure control and adverse effects are scarce [21]. In randomized controlled trial conducted by Jannuzzi et al. (2000) on the impact of TDM included 180 newly diagnosed patients with epilepsy who were about to start treatment with carbamazepine, valproic acid, phenytoin, phenobarbital, or primidone. Patients were randomized to either treatment with dosage adjusted on clinical grounds alone, or treatment with dosage adjusted to achieve serum concentrations within predefined target ranges. After a follow-up of up to 24 months, there were no significant differences between the two groups with respect to patients achieving 12-month remission (60% in the TDM group vs. 61% in the control group), patients were remaining seizure-free since initiation of treatment, time to first seizure or to 12-month remission, or frequency of adverse effects. Hence, this study could not demonstrate an effect of routine use of TDM on the clinical outcome of early treatment of patients with epilepsy [22].

Subash V. et al. (2011) were reported that there was poor correlation between daily dose and therapeutic levels of valproic acid after six months of treatment of epileptic children with valproic acid [7]. Imad A. (1992) tried to find the relationship between serum carbamazepine concentration and clinical effect in 111 epileptic patients. He reported that the therapeutic monitoring did not make management of epilepsy easy, but it could improve its therapeutic effect with avoidance of toxicity [23].

TDM is particularly useful in determination of drug levels and identification of therapeutic failure due to under dosage, and "even in the presence of optimal dosage" for identification of serious toxicity, inter individual pharmacokinetic variability (rapid or slow metabolism of drug) and detection of pharmacokinetic interactions [24].

One of the most common cause of lower concentration of drug than expected for the prescribed dose in this study is poor patient compliances. Poor compliance is a bigger issue in this set up, which mainly belongs to rural population, due to poor socioeconomic conditions, illiteracy and dependence on free supply of drugs from public hospitals. Assessing compliance on clinical grounds alone can be difficult especially in patients with infrequent seizures or easy to treat epilepsy [25]. Compliance can be improved by limiting to a minimum the number of daily doses and by regular monitoring of the drug level [19].

Toxic levels of carbamazepine and valproic acid were documented in this study and they may be attributed to the significant intra- and inter-individual pharmacokinetic variability of both agents [26,27]. Also, drug levels may be found within the toxic range in patients with uncontrolled seizures as such patients tend to be prescribed increased doses.

Effect On Liver Function

As shown in tables (6 and 8), there was a significant elevation in the activity of ALT and AST in all retrospective groups after three months of treatment. Such elevation where also observed in prospective groups as data in tables (7 and 9) showed that the activity of ALT showed a significant elevation after treatment with carbamazepine with a non significant elevation in those treated with valproic acid, and there was a significant elevation in AST activity after treatment with valproic acid with a non significant elevation with carbamazepine. However, these elevations are usually less than twice the upper limit of normal in all groups.

ALT and AST are an excellent marker of hepatocellular injury. Several drugs may cause raised aminotransferase enzymes, and among them are the AEDs [28], and mild alterations of aminotransferases can occur without clinical significance [29].

In table (10), the activity of ALP in all retrospective groups showed a significant elevation after three months of treatment, whereas in table (11), the activity of ALP in prospective groups showed a non significant elevation after treatment as compared with baseline values. The results regarding GGT activity showed that there was a significant elevation in all retrospective groups after treatment except the group treated with topiramate which showed a non significant elevation {table (12)}. While in table (13), the activity of GGT in prospective groups showed a significant elevation after treatment with carbamazepine and a non significant elevation after treatment with valproic acid when compared with baseline values. Again, these elevations are usually less than twice the upper limit of normal in all groups.

It has been mentioned that ALP is the most frequently used biochemical marker of bone formation, and increased values were documented both in adults and in children receiving AED therapy in most studies. The reported incidence of this elevation ranges from 19-56% [30]. Carbamazepine is considered to increase vitamin D metabolism, and risk of bone disease. Decreased vitamin D levels in subjects on carbamazepine might result in increased blood levels of ALP [31]. GGT is a sensitive test of hepatobiliary disease; its usefulness is limited by lack of specificity. Medications like carbamazepine may also cause a mild rise in GGT [28]. GGT would confirm hepatic source for a

raised ALP. However, hepatic enzymes (GGT and ALP) elevations are frequent and do not have necessarily a pathological meaning [29].

There is controversy regarding the exact mechanism for increased enzyme activities in treatment with AEDs. Some studies conclude that increase occurs due to enzyme induction along with liver cell damage [32], while other studies maintain that increase is due to enzyme induction and is mostly mild and clinically insignificant [33]. The results in this study indicate that the AEDs used in this study may cause an asymptomatic rise in liver function tests in both retrospective and prospective groups that does not signify liver dysfunction and does not require action, in addition to that, none of the patients suffered from liver disease, thus, mild increase (less than five times the upper limit of normal [34]) found in enzyme levels may only reflect enzyme induction and not hepatocellular damage. Also the results indicate that the short duration of treatment in prospective groups produced approximately the same effect on liver enzyme activities as the long duration of treatment in retrospective groups. The change in enzyme activities produced by the combination therapy is not significantly different from those produced by mono-therapy.

Enzyme induction is one reported iatrogenic effect leading to elevated hepatic serum enzyme levels in patient populations that are not directly indicative of hepatic injury. This has been well documented, especially for AEDs [35]. Hepatic enzyme induction by AEDs in asymptomatic patients was cited by Wall et al. (1992) in a study of 206 adults and children. Of these, serum GGT was elevated in 74.6%, ALP in 29.7%, and ALT in 25.2% [36]. Of 242 patients administered AEDs, 40 exhibited high levels of serum GGT and nearly all cases indicated hepatic microsomal enzyme induction as measured by antipyrine half-life, leading Hirayanagi et al. (1991) to conclude that, in these patients, elevated serum GGT did not necessarily indicate hepatocellular damage [37]. Similar studies with AED therapy indicated ALT elevations up to three times and AST elevations up to two times the upper limit of normal in more than one-quarter of the patient population. These were not considered clinically significant but instead were attributed to enzyme induction. Liver biopsies in similar patients undergoing long-term antiepileptic therapy showed no signs of chronic liver damage [35].

Adverse Effects

As shown in tables (14 and 15) carbamazepine was responsible for the incidence of adverse effects in 80% of patients in retrospective group and 50% of patients in prospective group, and the number of types of these effects occurred in retrospective group was higher than that types occurred in prospective group. This may be probably due to long duration of treatment in retrospective group (i.e. more than six months versus only three months in prospective group). The common adverse effects documented in both groups were headache, blurred vision, fatigue, and loss of appetite. Most of the patients (8 out of 11) in both groups with carbamazepine level in toxic range showed these adverse effects. Neurological adverse effects are common with high doses of carbamazepine, particularly when the plasma concentration exceeds 9 μg/ml [38].

In retrospective group treated with valproic acid mono-therapy, 75% of patients had adverse effects, whereas, 60% of patients in prospective group receiving valproic acid showed adverse effects and the number of types of these effects occurred in retrospective group was higher than the types that occurred in prospective group. Again, the duration of treatment may be responsible for this. The most common adverse effects documented in both groups were headache, fatigue, ataxia, and loss of appetite. Only 4 out of 7 patients in this study with valproic acid level in toxic range showed these adverse effect. CNS adverse effects are more common when plasma concentrations of valproic acid exceed 100 μg/ml although some patients may have plasma concentrations of 150 μg/ml or higher without adverse effects [38].

80% of patients were suffered from adverse effects after three months of treatment with topiramate mono-therapy; however, it was associated with the incidence of the lower number of adverse effects when compared with other treatment options. The most common adverse effects documented were CNS-related effects including headache, fatigue, and loss of appetite. Whereas combination therapy was responsible for the incidence of a wide range of adverse effects in 85% of patients in retrospective group that received combined AEDs. This is due to the fact that when seizures are poorly controlled; AEDs are used in combination, leading to potential pharmacokinetic or pharmacodynamic interactions, causing more adverse effects than might occur when the AED is taken as mono-therapy. Combination therapy can result in additive or sometimes supra-additive adverse effects [39]. The most common adverse effects documented were headache, fatigue, loss of appetite, blurred vision, and weight loss.

However, these adverse effects were considered to be mild to moderate in nature and did not require discontinuation of the medications and the patients can tolerate them. Ripple T. et al. (2011) reported that carbamazepine had more adverse effects than newer AEDs, and there were adverse events that occurred more commonly with valproic acid. However, these effects did not significantly increase the risk of withdrawals [15]. Also, like what was reported by other researchers, the results from this study showed that there was no relationship between serum levels of carbamazepine or valproic acid and their adverse

effects as these effects occurred over a wide range of serum drug level [40-42].

A few number of patients in this study did not show any adverse effects after treatment especially in children. There is a fact that some adverse effects, such as diplopia or dizziness, may be difficult in children or nonverbal children and adults who are unable to describe their symptoms to caregivers [43]; in addition, some adverse effects (like weight change) are insidious because of the slow and incremental increase in severity or impact over time [39].

V. Conclusions

- Carbamazepine was more effective in mono-therapy in retrospective groups than other treatment options; whereas, valproic acid in prospective groups was slightly more effective than carbamazepine in controlling seizures.
- Mono-therapy with AED should always be attempted first in treatment-naive patients as the advantages include excellent control of seizure, fewer adverse drug reactions, easier administration and decreased cost, while the combination therapy was associated with a poorer seizure control and higher incidence of adverse drug reactions.
- Poor correlation was found between the serum concentration of carbamazepine and valproic acid and their therapeutic effects; therefore, TDM of both drugs will be useful only when individuals are non-responsive to treatment or vulnerable to adverse reactions with standard doses.
- AEDs significantly increase levels of liver enzymes activity; however, these alterations are mostly mild and clinically insignificant and do not justify routine testing.
- AEDs in this study have been shown to be well tolerated with mild to moderate adverse effects in nature. However, no relationship between serum levels of carbamazepine or valproic acid and their adverse effects was observed as these effects occurred over a wide range of serum drug level.

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Table 1 : *Frequency of seizure* in retrospective groups after three months of treatment. The data were expressed as number (n) and percentage (%).

Frequency of seizure	Carbamazepine n=20	Valproic acid n=20	Topiramate n=10	Combination therapy n=20
Seizure free	18 (90%)	15 (75%)	6 (60%)	9 (45%)
Once / month	1 (5%)	3 (15%)	0 (0%)	3 (15%)
Twice / month	0 (0%)	0 (0%)	0 (0%)	2 (10%)
Thrice / month	0 (0%)	0 (0%)	0 (0%)	2 (10%)
Once / two months	1 (5%)	0 (0%)	2 (20%)	2 (10%)
Once / three months	0 (0%)	2 (10%)	2 (20%)	2 (10%)

Table 2 : *Frequency of seizure* in prospective groups after three months of treatment. The data were expressed as number (n) and percentage (%).

Frequency of seizure	Carbamazepine n=10	Valproic acid n=10
Seizure free	8 (80%)	10 (100%)
Once / month	2 (20%)	0 (0%)
once / two month	0 (0%)	0 (0%)
Once / three months	0 (0%)	0 (0%)

Table 3 : Serum drug concentration in prospective and retrospective groups receiving carbamazepine and valproic acid (as mono-therapy or in combination therapy) after three months of treatment.

Groups	SERUM DRUG CONCENTRATION (μg/ml)		
	Prospective n=10	Retrospective n=20	Retrospective combination n=20†
Healthy subjects	----------	----------	----------
Carbamazepine	10.75 ± 2.96	10.24 ± 1.82	8.47 ± 0.99
Valproic acid	74.47 ± 17.11	71.01 ± 12.36	67.27 ± 8.67

Each value represents the mean ± standard error of mean.
n= number of patients.
† = 18 patients received carbamazepine.
4 patients received valproic acid.

Table 4 : Serum carbamazepine range in prospective and retrospective groups receiving carbamazepine (as mono-therapy or in combination therapy) after three months of treatment. The data were expressed as number (n) and percentage (%).

Range	CARBAMAZEPINE		
	Prospective n=10	Retrospective n=20	Retrospective Combination n=18
Sub-therapeutic level (<4 μg/ml)	2 (20%)	3 (15%)	5 (27.77%)
Therapeutic level (4 – 12 μg/ml)	5 (50%)	13 (65%)	9 (50%)
Toxic level (>12 μg/ml)	3 (30%)	4 (20%)	4 (22.22%)

Table 5 : Serum valproic acid range in prospective and retrospective groups receiving valproic acid (as mono-therapy or in combination therapy) after three months of treatment. The data were expressed as number (n) and percentage (%).

Range	VALPROIC ACID		
	Prospective n=10	Retrospective n=20	Retrospective Combination n=4
Sub-therapeutic level (<50 μg/ml)	3 (30%)	6 (30%)	1 (25%)
Therapeutic level (50 - 100 μg/ml)	4 (40%)	10 (50%)	3 (75%)
Toxic level (>100 μg/ml)	3 (30%)	4 (20%)	0 (0%)

Table 6 : Serum *ALT* in retrospective groups at baseline and after three months of treatment.

Retrospective groups	Number of patients	SERUM ALT (U/L)		
		Baseline	After 3 months	% change
Healthy subjects	20	7.00 ± 0.51	----------	----------
Carbamazepine	20	8.95 ± 0.84 b	10.75 ± 0.80 *a	20.11%
Valproic acid	20	9.85 ± 0.71 b	11.75 ± 1.06 *a	19.28%
Topiramate	10	10.50 ± 0.54 b	13.20 ± 1.08 *a	25.71%
Combination therapy	20	9.82 ± 0.79 b	12.65 ± 1.29 *a	28.81%

Each value represents the mean ± standard error of mean.
* P < 0.05 significant difference from baseline values.
a P < 0.05 significant difference from healthy subjects values.
b P < 0.05 significant difference from healthy subjects values.

Table 7 : Serum *ALT* in prospective groups at baseline and after three months of treatment.

Prospective groups	Number of patients	SERUM ALT (U/L)		
		Baseline	After 3 months	% change
Healthy subjects	20	7.00 ± 0.51	----------	----------
Carbamazepine	10	6.70 ± 1.25	12.40 ± 1.57 *a	85.07%
Valproic acid	10	12.30 ± 1.11 b	14.50 ± 2.05 a	17.88%

Each value represents the mean ± standard error of mean.
* P < 0.05 significant difference from baseline values.
a P < 0.05 significant difference from healthy subjects values.
b P < 0.05 significant difference from healthy subjects values.

Table 8 : Serum *AST* in retrospective groups at baseline and after three months of treatment.

Retrospective groups	Number of patients	SERUM AST (U/L)		
		Baseline	After 3 months	% change
Healthy subjects	20	8.25 ± 1.45	----------	----------
Carbamazepine	20	12.95 ± 1.10 b	16.80 ± 0.96 *a	29.72%
Valproic acid	20	12.45 ± 1.44 b	16.95 ± 1.54 *a	36.14%
Topiramate	10	14.80 ± 1.23 b	21.30 ± 1.54 *a	43.91%
Combination therapy	20	12.91 ± 0.83 b	17.55 ± 1.47 *a	35.94%

Each value represents the mean ± standard error of mean.
* P < 0.05 significant difference from baseline values.
a P < 0.05 significant difference from healthy subjects values.
b P < 0.05 significant difference from healthy subjects values.

Table 9 : Serum *AST* in prospective groups at baseline and after three months of treatment.

Prospective groups	Number of patients	SERUM AST (U/L)		
		Baseline	After 3 months	% change
Healthy subjects	20	8.25 ± 1.45	----------	----------
Carbamazepine	10	14.70 ± 2.43 b	18.40 ± 1.83 a	25.17%
Valproic acid	10	15.50 ± 1.52 b	22.10 ± 1.82 *a	42.58%

Each value represents the mean ± standard error of mean.
* P < 0.05 significant difference from baseline and values.
a P < 0.05 significant difference from healthy subjects values.
b P < 0.05 significant difference from healthy subjects values.

Table 10 : Serum *ALP* in retrospective groups at baseline and after three months of treatment.

Retrospective groups	Number of patients	SERUM ALP (U/L)		
		Baseline	After 3 months	% change
Healthy subjects	20	63.96 ± 5.02	----------	----------
Carbamazepine	20	77.06 ± 8.00	90.69 ± 11.49 *a	17.68%
Valproic acid	20	84.98 ± 6.60 b	100.32±10.15 *a	18.05%
Topiramate	10	77.62 ± 8.73	93.14±14.53 *a	19.94%
Combination therapy	20	81.06 ± 7.25 b	98.27 ± 12.67*a	21.23%

Each value represents the mean ± standard error of mean.
* P < 0.05 significant difference from baseline and values.
a P < 0.05 significant difference from healthy subjects values.
b P < 0.05 significant difference from healthy subjects values.

Table 11 : Serum *ALP* in prospective groups at baseline and after three months of treatment.

Prospective groups	Number of patients	SERUM ALP (U/L)		
		Baseline	After 3 months	% change
Healthy subjects	20	63.96 ± 5.02	----------	----------
Carbamazepine	10	95.80 ± 13.19 b	122.62 ± 13.95 a	27.99%
Valproic acid	10	89.27 ± 11.76 b	110.70 ± 15.12 a	24.00%

Each value represents the mean ± standard error of mean.
a P < 0.05 significant difference from healthy subjects values.
b P < 0.05 significant difference from healthy subjects values.

Table 12 : Serum *GGT* in retrospective groups at baseline and after three months of treatment.

Retrospective groups	Number of patients	SERUM GGT (U/L)		
		Baseline	After 3 months	% change
Healthy subjects	20	5.71 ± 0.31	----------	----------
Carbamazepine	20	7.61 ± 0.61 b	9.48 ± 1.02 *a	24.57%
Valproic acid	20	6.01 ± 0.59	6.48 ± 0.69 *	7.82%
Topiramate	10	6.20 ± 0.83	6.88 ± 1.02 a	10.96%
Combination therapy	20	8.11 ± 0.73 b	10.52 ± 1.34 *a	29.71%

Each value represents the mean ± standard error of mean.
* P < 0.05 significant difference from baseline and values.
a P < 0.05 significant difference from healthy subjects values.
b P < 0.05 significant difference from healthy subjects values.

Table 13 : Serum *GGT* in prospective groups at baseline and after three months of treatment.

Prospective groups	Number of patients	SERUM GGT (U/L)		
		Baseline	After 3 months	% change
Healthy subjects	20	5.71 ± 0.31	----------	----------
Carbamazepine	10	6.89 ± 1.59	10.80 ± 0.75 *a	56.74%
Valproic acid	10	5.28 ± 1.07	7.03 ± 1.42	33.14%

Each value represents the mean ± standard error of mean.
* P < 0.05 significant difference from baseline and values.
a P < 0.05 significant difference from healthy subjects values.

Table 14 : *Adverse effects* in retrospective groups after three months of treatment. The data were expressed as number (n) and percentage (%).

Retrospective / Adverse effects	CARBAMAZEPINE n=20	VALPROIC ACID n=20	TOPIRAMATE n=10	COMBINATION THERAPY n=20
Weight loss	2 (10%)	1 (5%)	1 (10%)	4 (20%)
Weight gain	1 (5%)	7 (35%)	1 (10%)	3 (15%)
Loss of appetite	3 (15%)	1 (5%)	2 (20%)	7 (35%)
Gastric pain	----------	2 (10%)	----------	----------
Constipation	1 (5%)	1 (5%)	----------	2 (10%)
Blurred vision	3 (15%)	----------	----------	4 (20%)
Diplopia	1 (5%)	----------	----------	2 (10%)
Ataxia	1 (5%)	2 (10%)	1 (10%)	1 (5%)
Headache	14 (70%)	6 (30%)	7 (70%)	12 (60%)
Chest pain	----------	----------	1 (10%)	2 (10%)
Muscle cramp	----------	----------	----------	3 (15%)
Fatigue	7 (35%)	6 (30%)	4 (40%)	8 (40%)
Insomnia	1 (5%)	---------	----------	----------
Shortness of breath	----------	2 (10%)	----------	----------
Parasthesia	----------	----------	2 (20%)	----------

Table 15 : Adverse effects in prospective groups after three months of treatment. The data were expressed as number (n) and percentage (%).

Prospective / Adverse effects	CARBAMAZEPINE n=10	VALPROIC ACID n=10
Weight loss	1 (10%)	----------
Weight gain	1 (10%)	----------
Loss of appetite	2 (20%)	1 (10%)
Constipation	1 (10%)	----------
Blurred vision	2 (20%)	----------
Ataxia	----------	2 (20%)
Headache	5 (50%)	2 (20%)
Fatigue	1 (10%)	3 (30%)

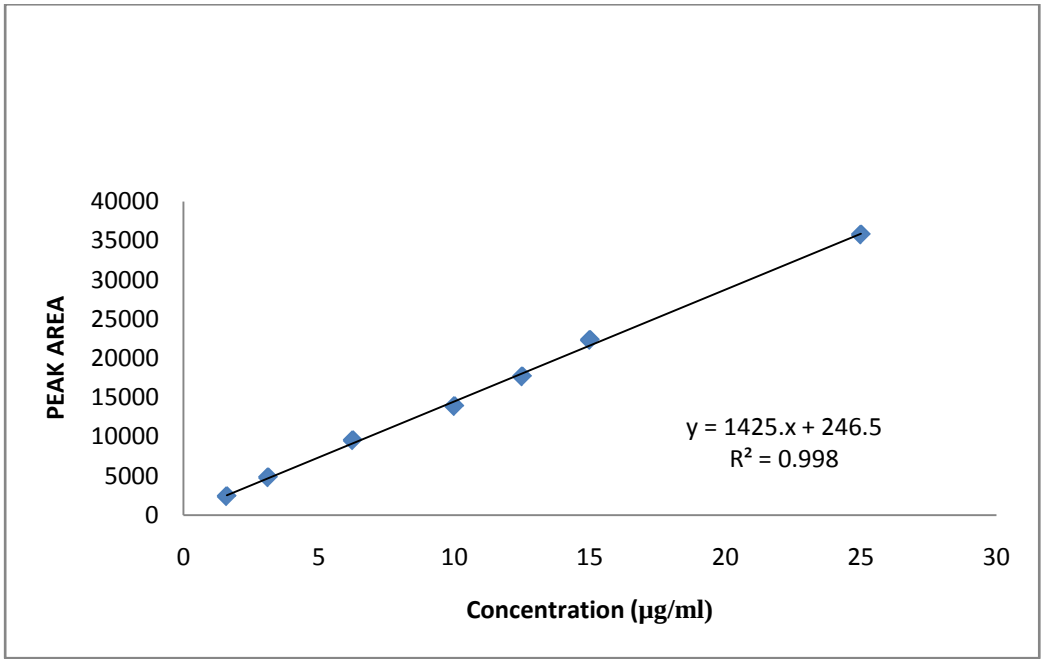

Figure 1 : Carbamazepine standard curve.

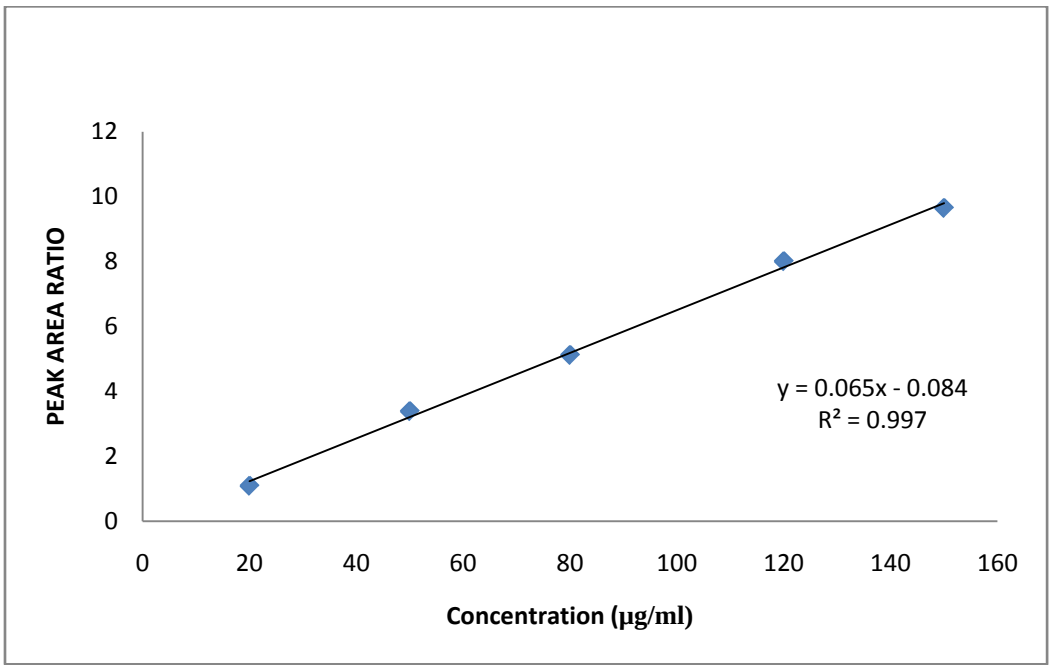

Figure 2 : Valproic acid standard curve.

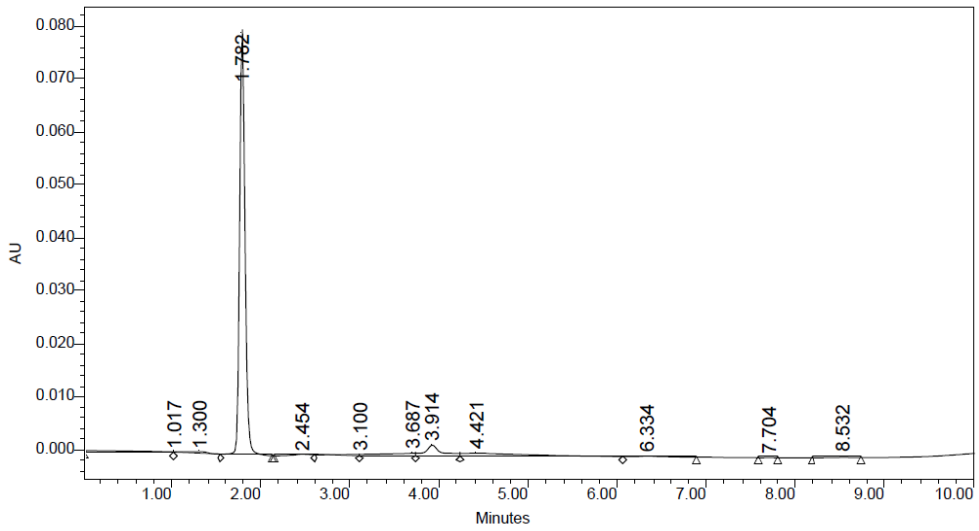

Figure 3 : Chromatogram of blank serum.

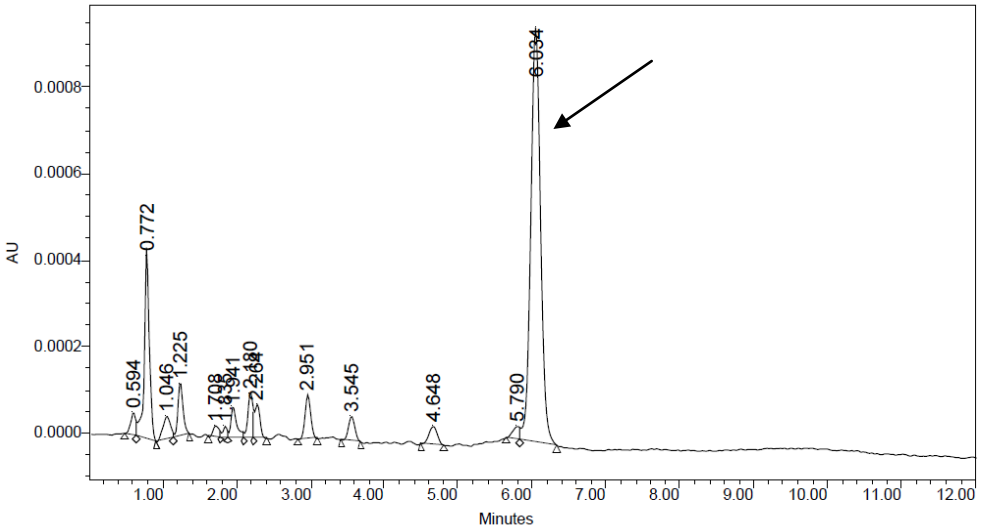

Figure 4 : Chromatogram of standard of carbamazepine.

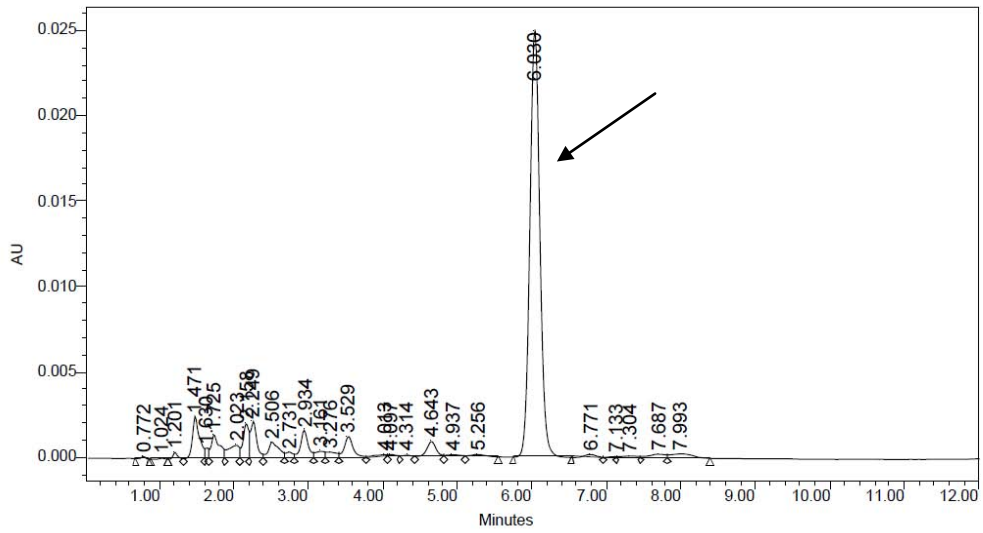

Figure 5 : Chromatogram of carbamazepine in patient's sample.

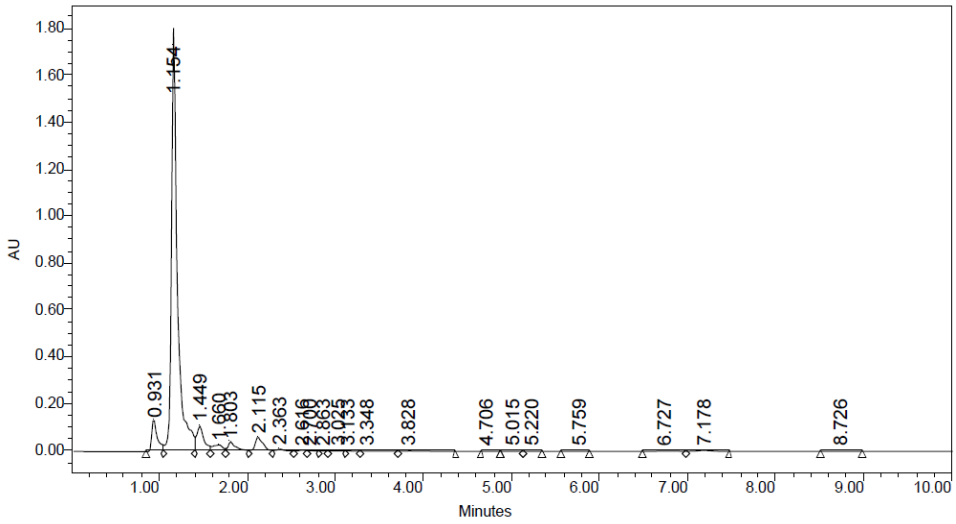

Figure 6 : Chromatogram of blank serum.

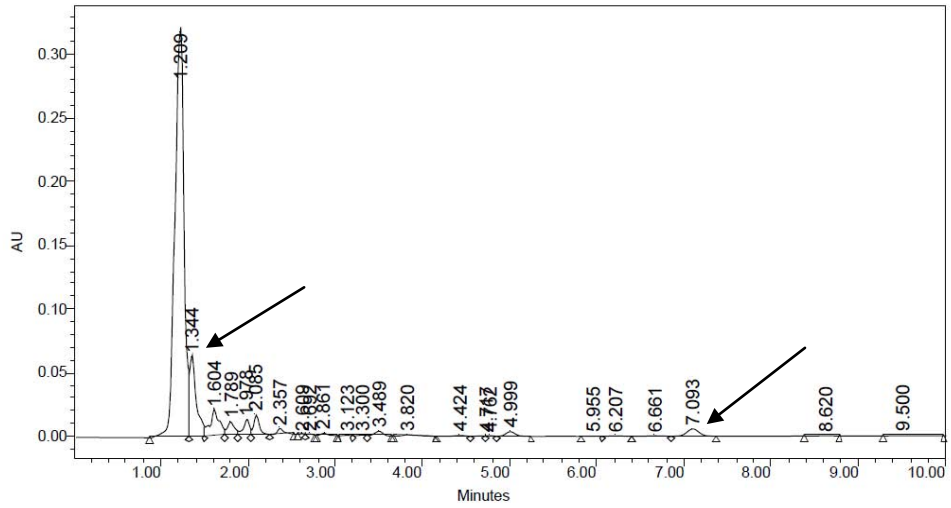

Figure 7 : Chromatogram of standard of valproic acid and internal standard of diazepam.

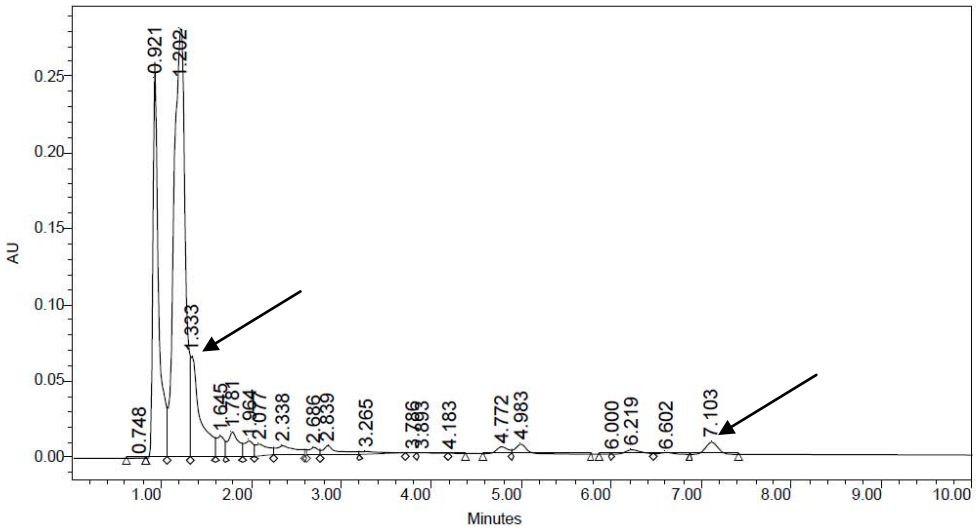

Figure 8 : Chromatogram of valproic acid and internal standard of diazepam in patient's sample.

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Effects of Blood Transfusion in Outcome of Elective Bowel Anastomosis

By Abdallah Y.A. Ahmed

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Abstract - *Background :* Bowel anastomosis is one of the commonest procedure done in surgical practice, and its outcome influence by many factors include; patients, technical, and operation basis, but intraoperative blood transfusion (BT) is discriminated from other risk factors in that; its intentionally added risk factor.

Objective : To evaluate the effect of blood transfusion in outcome of elective bowel anastomosis in Khartoum teaching hospital.

Patient And Method : Twenty eight patients admitted into Khartoum teaching hospital, and underwent elective bowel anastomosis were enrolled in the study. Data was collected by questionnaire for each patient.

Result : The total number of patients was 28, 18 were male and 10 were female, mean age was 50 years, 14.3% were underwent small bowel anastomosis, 85.7% were underwent large bowel anstomosis, and 35.7% were transfused intraoperatively. Regardless other risk factors the incidence of surgical site infection (SSI) was significantly high in transfused patients, in comparison to nontransfued 30% vs 0.0%, also there was a high rates of other complications in transfused patients than those weren't.

Conclusion : Intraoperative blood transfusion is a good predictor for development of complications in elective bowel anastomosis.

GJMR-L Classification : NLMC Code: WB 356

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Conclusion: Intraoperative blood transfusion is a good predictor for development of complications in elective bowel anastomosis.

I. Introduction

After discovery of ABO group, blood transfusion have become a common treatment for anemia and acute blood loss, its adverse effects had been notice in last decades, especially the immunosuprresion one.[1]

Blood transfusion alter both local and systemic immune response to injury, it impair lymphocyte and macrophage function, blastogenesis and interaction with other cells. Allogeneic leukocytes have a critical role in the induction of transfusion-induced immunosuppression, also BT decrease the production of interleukin -2 (IL2) which found to be an important factor in cell immunity and stimulation of healing.[1,2,3] These effects can be reverse by administration of exogenous IL2 in chronic healing, also some studies found that leucocytes-depleted blood doesn't impair healing. [3,4,5]

In bowel anastomosis blood transfusion increase incidence of abscess formation, reduce collagen synthesis result in impair anastomotic strength, and high rate of anastomotic leak (AL).[1,2,3,4,6,7,8] Some studies reported that this effect related to intra and postoperative BT rather than preoperative transfusion, implies that its effect might be at least partially surrogated by other intraoperative factors, such as contamination and shock. [2,9,10]

Testini et al study found that; preoperative blood transfusion a causative factor in development of AL.[11,15]Also some study found that; high rate of complications related to massive blood transfusion in emergencies.[12,13,14]

Nevertheless blood transfusions are frequently required in gastrointestinal surgery to correct anemia or because of excessive blood loss from associated trauma or operative procedures. Thus, it is important to establish the effect of such transfusions on intestinal repair.

II. Patients and Methods

This a prospective descriptive hospital base clinical study, conducted in Khartoum teaching hospital (KTH), which is the biggest tertiary hospital in Khartoum, the capital of the Sudan, in the period from 15th Sep 2011 to Aug 1st 2012.

A total of twenty eight patients underwent elective bowel anastomosis, by general surgical units in (KTH) were enrolled in the study after consented. All large bowel anastomosis were preceded by bowel preparation, and all patients received single prophylactic dose of antibiotic, followed by a therapeutic course in postoperative period. Data was collected by structure questionnaire for each patient, from the date of operation till discharge from the hospital and for outpatient follow up for presence of complications, for at least first outpatient visit, variables included were; personal data, diagnosis, operative details, BT regardless the amount, presence of complications, postoperative bowel rest and hospital stay periods. Patients aged below twelve years, or had severe comorbidies were excluded from the study. Data was analyzed by computer using Statistical Package for Social Science (SPSS) program, version (16).

III. Results

Data analysis of twenty eight patients was done, all patients were underwent open, hand sewn elective bowel anastomosis, 62.3% were male and 37.3% were

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female, mean age was 50.39 years (±15.17) and rang from 22 to 75 years.

The indication of anastomosis was resection of bowel tumors in 64.3% (n=18) of patients; one was small bowel and 17 were large bowel tumors, in 28.5% (n=10) the indication was reversal of stoma; eight were colostomies and two were ileostomies, in one patient the indication was Crohns disease and in another one was chronic small bowel fistula.

Enteroenteric anastomosis was done in 14.3% of patients, enterocolonic in 50% and colocolonic in 35.7% of anastomosis, 85.7%(n=24) of anastomosis were accomplished by double layer technique and 14.3%(n=4) by single layer, sutures material was polyglycolic acid and needle was round bodied in all anastomosis.

Regarding blood transfusion 35.7% of patients were received whole blood intraoperatively, and 64.3% weren't transfused, we weren't considered the amount of blood. Contamination was presented in 21.4% of patients during operation.

Considering complications, the incidence of SSI was 10.7% (n=3), AL 3.6% (n=1), fistula 3.6%, and death 3.6%. All intraabdonial complications were developed in patients who were transfused intraoperatively.

Drainage of peritoneal cavity was done in 62.9% of operations, and 50% had nasogastric tube (NGT) decompression.

IV. Discussion

The effect of blood transfusion (BT) in outcome of bowel anastomosis has been investigated by many studies, which proved its adverse impact in healing process and immunity of the host, that result in high rate of infectious complications and leakage, but it's necessary in certain circumstances such as shock and massive resection etc.[1-14] In our study all three SSI were developed in transfused group, with rate of 30% among this group (p=0.014), which found to be a strong association, also in this group AL rate was 10% , fistula 10%, and mortality rate was 10%, and all other complications were developed in this group, which cited blood transfusion a cause in this group. (table2)

As other risk factors for complications such as malnutrition, cardiovascular and respiratory diseases weren't showed any significant difference in their rates between two groups, even some risk factors showed a higher rate in nontransfused group such as chemotherapy, smoking and alcohol abuse.(table1) In some studies the amount of blood found to be the risk (massive BT), rather than transfusion itself, unfortunately in our study we weren't considered neither the amount of blood nor intra operative hemorrhage degree as a separate risk factors for development of complications, and how much they affect the outcome was not provided in our results and analysis. [12,13,14]

Intraoperative contamination was found to be high in transfused group than nontransfued 30% vs 16.7%, which added a burden into this group, also our analysis revealed a strong association between BT and peritoneal drainage (p=0.007), all patients received blood also were had a drain, but there was no significant association between peritoneal drainage and development of complications, it looks as surgeons were anticipated the development of AL in those patients received intraoperative whole blood. In Ketan et al study all AL were developed in transfused patients.[3] Lujan et al study found that; SSI and intraoperative blood transfusions were also associated with significantly higher rates of AL.[7]

Enterocolonic and colocolonic anastomosis followed by all SSI, fistula and leak in this study, and enteroenteric anastomosis wasn't developed complications, concluded the adverse effect of BT is more obvious in large bowel procedures than small bowel, this evidence was supported by Reiping et al study in large bowel anastomosis, also this study concluded that; BT is risk factor for SSI regardless the site of anastomosis in large bowel procedures.[2]

Considering postoperative fasting period there was no significant difference between two groups, the mean periods were four day in transfused patients, and five days in nontransfused, and hospital stay period was prolonged in transfused group, which was 11.56 days vs 8.22 days in nontrasfused group, which found to be increased proportionally with development of complications.

There were some limitations in this study, as other risk factors might change the outcome by adding some burden into one group, and the effect of blood amount wasn't considered, so more precise studies have to be done to give more support to our results.

V. In Conclusion

Intraoperative blood transfusion has adverse effects in elective bowel anastomosis, significantly increase rate of SSI; also it's a good predictor for development of other complications.

VI. Aknownlagment

My thanks to all member of general surgical uints in Khartoum Teaching Hospital, who help me in data collection,and to my boss Mr. Salah Nori who supported me throughout the study.

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Spatiotemporal and Joint Kinematic Analyses in Hemiparetic Cerebral Palsy Children During Stance Phase

By Abbas Sikina, Nong Xiao, Yuxia Chen & Ling Hu

Rehabilitation Center, Chongqing Medical University, Chongqing

Abstract - The aim of this study is to identify and quantify spatiotemporal and joint kinematics in hemiparetic cerebral palsy children by three dimensional gait analysis (3DGA). Gait strategy of 36 Hemiparetic and 31 healthy children was quantified by the new anatomically based protocol of 3DGA. Spatiotemporal and joint kinematics of lower limbs were identified and calculated. Results revealed that, the gait pattern of the paretic and non paretic sides of hemiparetic children were different compared to healthy subjects. Shorter stance phase was noted on the paretic side compared to non paretic and healthy subjects ($P<0.05$). Hemiparetic children walked with significantly reduced velocity, stride length, step length and cadence compared to healthy subjects. However step width increased considerably in the hemiparetics compared to healthy children. Joint kinematics during stance indicated that hemiparetic children walked with significantly increased anterior trunk tilt, pelvic tilt and pelvic retraction compared to healthy subjects ($P<0.05$).

Keywords : *Hemiparetic cerebral Palsy; Gait analysis; Spatiotemporal; Joint kinematics.*

GJMR-L Classification : *NLMC Code: WS 342*

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Abbas Sikina [α], Nong Xiao [σ], Yuxia Chen [ρ] & Ling Hu [ω]

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Keywords : Hemiparetic cerebral Palsy; Gait analysis; Spatiotemporal; Joint kinematics.

I. Introduction

Hemiparetic cerebral palsy (CP) is a form of spastic cerebral palsy in which one arm and leg on either the right or left side of the body is affected. It is the most common syndrome in children born at term and is second in frequency only to spastic diplegia among preterm infants (Kulak and Sobaniec, 2004). Patients with spastic hemiplegia have unilateral prehensile dysfunction as a consequence of lesions within sensorimotor cortex and corticospinal tract. Children whose hemiparesis involves the upper limb to a greater extent than the lower (arm-dominant hemiparesis) are much more likely to experience learning difficulties than those whose clinical pattern is leg-dominant (Galli et al., 2010).

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Three dimensional Gait analysis can provide a more objective evaluation including kinematic, kinetic, and dynamic electromyographic assessment. Hence enabling clinicians to differentiate gait deviations objectively and understand the primary problem behind a complex disorder more accurately.

In literature some studies examined quantitatively the spatiotemporal and joint kinematics of hemiparetic cerebral palsy children, these studies mainly focused on comparing functional motor evaluations of the right and left hemiplegic gaits. Galli et al. (2010) compared right and left hemiplegic gaits using 3DGA to analyze the difference in patterns, the results demonstrated that right hemiplegic gait walked with higher velocity than left hemiplegic gait. Wheelwright et al. (1993) assessed spatiotemporal parameters of gait in hemiparetic children and reported that, hemiparetic children walked more slowly with shorter step length, decreased cadence and longer swing time than normal children. Motor functions of right versus left hemiplegic children together with other intellectual, verbal and non-verbal functions were investigated. The results revealed that both groups showed overall slight or moderate impairments in motor function but the left hemiplegic group had more severe motor limitation than the right hemiplegic group (Carlsson et al., 1994). Cimolin et al. (2007) analyzed gait strategy of uninvolved limb in children with spastic hemiplegia and reported that uninvolved limbs had significant longer stance phase, knee joint more flexed, hip joint presented high flexion at the beginning of gait cycle and ankle kinematics presented values closed to normal. It appears evident that literature did not point out works on distinguishing quantitatively spatiotemporal and joint kinematics in hemiparetic cerebral palsy children during stance phase. A deeper understanding of their motor disability may generate rehabilitative strategies and treatment on improvement of gait. 3DGA is nowadays the most accurate tool in defining peculiar motor characteristic in children with CP.

The aim of this study is furthermore to identify and quantify gait pattern of hemiparetic CP children and compare their results with those obtained in a group of healthy children.

II. Methods

a) Subjects

Thirty six hemiparetic CP children participated in the study with age range of 2-15years, among them 27 were right hemiparetic and 9 left hemiparetic .The age, weight and height of hemiplegic children were 7.8 ± 3.8 years, 26.2 ± 13.5 kg and 122.1 ± 22.5 cm respectively. According to (Arguelles et al., 1995) in terms of the assessment of degree of CP severity, all children had a mild severity (can walk unaided); in addition all patients were leg-dominant lower limb primarily involved with relative sparing of the upper limb. They had no history of functional lower limbs surgery and absence of pharmacological treatments in the last year.

A control group of thirty one healthy children were investigated; their age, weight and height were 8.4 ± 4.1 years, 28.9 ± 13.2 kg and 126.9 ± 22.5 respectively. Selection criteria for this group included no prior history of cardiovascular, neurological or musculoskeletal disorders. They exhibited normal range of motion, muscle strength, and had no apparent postural or motor deficits.

All subjects were volunteers and their parents gave written consent to the children's participation in this study. This study was approved by Ethics Committee of the Children's Hospital of Chongqing Medical University in China.

b) Data collection

The assessment composed of three dimensional gait analysis which was conducted in a laboratory equipped with 9m linear walkway and 6 infrared cameras operating at 60 HZ frequency. 2 Force plates embedded at the centre of the walkway used to determine foot contact and foot-off events synchronized with the system made from motion Analysis Company (Helen Hayes model). Reflective markers (10mm in diameter) were placed according to anatomical landmarks as shown in fig 1. (Motion analysis version 11 user's manual).

Anthropometric measures were taken and preparation of patient followed by inserting 26 markers directly on the subject's skin for measurement of static phase. The walking phase involved removal of 4 markers named (R. ankle medial, L. ankle medial, R. knee medial and L knee medial) from the subject's body leaving 22 markers as the new anatomically based protocol suggests (Leardini et al., 2007).

Subjects were allowed to walk barefoot at their self-selected speed along 9m walkway. Seven trials were recorded for each child in order to guarantee the consistency of the results. The following parameters were identified and calculated for each subject.

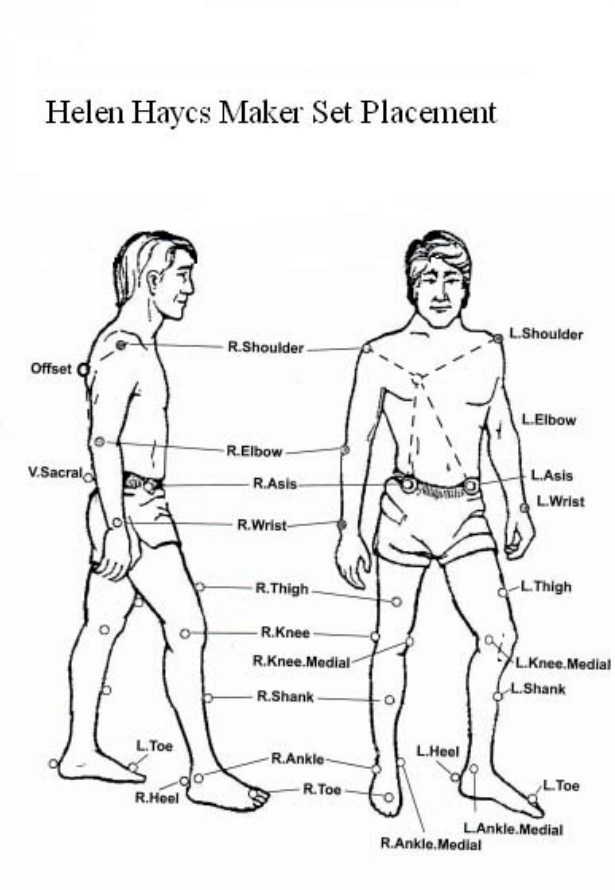

Fig. 1 : R - Right L - Left

Spatiotemporal parameters

Step length, step width, stride length, cadence, velocity, support time, non support time and double support time.

Kinematics : joint angles during stance phase

Trunk and Pelvis : Lateral tilt, forward tilt and rotation. Hip, knee and ankle: Flexion, extension, abduction, adduction and rotation.

c) Data analysis

Cortex1.1.4 software and orthotrack software version 6.6.1 were used to define gait parameters and estimate kinematics.

All previously defined spatiotemporal parameters and joint kinematics were computed for each subject. Comparison was made between non paretic and paretic sides of hemiparetic children with the right limb of healthy subjects.

d) Statistical analysis

SPSS version 19 was used for statistical analysis and data was analyzed by student's T-test. Statistical significance difference was defined as $P < 0.05$.

III. Results

Age, body weight and height were not significantly different among hemiparetic and healthy children. Table 1 displays the mean (standard deviation) of the spatiotemporal, ankle, knee and hip kinematics for hemiparetic group with the distinction between paretic and non paretic sides and those for healthy subjects. Table 2 displays trunk and pelvic kinematics.

a) Spatiotemporal parameters

Shorter stance phase was noted on the paretic side compared to non paretic and healthy subjects, Hemiparetic children walked with significant reduced velocity compared to healthy. Cadence, step length and stride length revealed significant lower values in comparison to healthy subjects ($P < 0.05$). For double support time, there was no significant difference in the two groups. Step width increased considerably in the hemiparetics compared to healthy children.

Table 1 : Summary of Gait parameters for hemiparetic children and healthy subjects.

	Hemiparetics N=36		
	Non paretic side	Paretic side	Healthy N=31
spatial temporal parameters			
Stance (%gait cycle)	62.24 (4.15) †	57.72 (2.82) •†	60.29 (2.20)
Swing ((%gait cycle	37.76 (4.15) †	42.28 (2.82) •†	39.71 (2.20)
Velocity (cm/s)	84.23 (24.20)	82.88 (24.90) •†	94.13 (21.28)
Stride length	81.59 (21.37) †	80.01 (21.71) †	92.45 (17.32)
Cadence	113.95 (17.18)	112.18 (16.06) †	122.39 (18.53)
Step length	39.44 (11.02) †	41.83 (12.11) †	47.13 (9.21)
Double support time	11.58 (3.88)	10.45 (4.54)	10.70 (1.82)
Step width		15.24 (4.68) †	12.87 (3.00)
Kinematics			
Ankle joint			
dorsal flexion	27.22 (21.91)	17.70 (15.04) •†	25.63 (11.88)
plantar flexion	-4.26 (5.20)	-6.95 (5.18) •	-7.57 (7.96)
Ankle abduction	11.81 (11.94)	17.07 (15.31) †	10.29 (10.57)
Ankle adduction	-7.46 (10.14)	-3.67 (18.09)	-5.63 (9.26)
Ankle internal rotation	10.83 (33.64)	12.14 (26.86)	8.58 (10.87)
Ankle external rotation	-35.97 (30.87)	-34.37 (31.70)	-31.21 (21.30)
Knee joint			
Knee flexion	38.70 (8.62) †	33.93 (10.38)•	31.47 (10.92)
Knee extension	11.05 (8.97) †	7.00 (9.12) •	4.88 (7.60)
Knee valgus	7.68 (5.09)	7.84 (5.39)	5.66 (5.70)
Knee varus	-5.90 (5.57)	-5.01 (4.82)	-5.40 (6.89)
Knee internal rotation	22.57 (32.57)	17.48 (34.96)	19.35 (23.65)
Knee external rotation	-21.81 (26.91)	-28.02 (32.89)	-22.79 (16.02)
Hip joint			
Hip flexion	47.60 (10.69) †	44.24 (11.12) •†	37.63 (9.39)
Hip extension	-9.76 (10.10)	-7.87 (12.05)	-8.55 (6.88)
Hip abduction	5.17 (5.59)	4.15 (6.45)	5.50 (4.40)
Hip adduction	-6.10 (6.33)	-6.50 (6.21)	-4.95 (3.92)

Hip internal rotation	18.24 (9.42)	22.97 (11.69) •	16.56 (13.87)
Hip external rotation	-6.38 (11.91)	-1.01 (11.99) †	-8.25 (15.42)

•p value < 0.05, compared between paretic and non paretic sides of hemiparetic patients, †p value < 0.05, compared with healthy subjects.

Table 2 : Summary of Pelvic and Trunk kinematics for hemiparetic children and healthy subjects.

		Hemiparetics N=36	Healthy N=31
Pelvic kinematics			
Pelvic lateral tilt (pelvic obliquity)	Hip up	7.07 (9.67)	4.67 (2.63)
	Hip down	-7.60 (6.85)	-5.66 (3.17)
Pelvic forward tilt (pelvic tilt)	Hip anterior	20.23 (8.77) †	13.06 (5.10)
	Hip posterior	9.38 (9.19)	8.36 (4.60)
Pelvic rotation	Hip forward	9.44 (8.86)	8.66 (3.60)
	Hip trailing	-7.61 (3.77) †	-11.14 (7.06)
Trunk kinematics			
Trunk lateral tilt	Shoulder up	3.55 (2.54)	3.03 (2.05)
	Shoulder down	-4.35 (3.42)	-3.32 (2.01)
Trunk forward tilt	Shoulder anterior	8.90 (4.71) †	5.30 (3.11)
	Shoulder posterior	-0.60 (4.11)	-2.14 (3.45)
Trunk Rotation	Shoulder forward	7.98 (8.49)	7.23 (5.13)
	Shoulder trailing	-9.31 (7.68)	-7.50 (6.45)

† *p* value < 0.05, compared between Hemiparetic children with healthy subjects.

b) Kinematics : joint angles during stance phase

Ankle joint, Reduced dorsal flexion ability was generally present on the paretic side compared to non paretic and healthy ($P < 0.05$) with excessive plantar flexion on the non paretic side than paretic. The paretic side displayed comparatively higher values of ankle abduction than healthy subjects. No significant difference was observed in the other parameters (adduction and rotation).

The knee joint displayed quite significant differences in flexion and extension ability. The paretic side showed lower flexion ability compared to non paretic ($P< 0.05$) with significant hyperextension on the non paretic side than paretic and healthy subjects. However both paretic and non paretic sides highlighted mean values of rotation, varus and valgus closed to healthy subject's data.

Regarding the hip joint, Hemiparetic children showed significant increased values of flexion ability compared to healthy subjects. Significant differences were found in terms of the hip rotation, the paretic side revealed high values of external rotation compared to healthy subjects ($P<0.05$) with slight increase in internal rotation compared to non paretic side. No significant differences were observed in abduction and adduction ability in the two groups.

As concerns the pelvic and trunk kinematics, Hemiparetic children walked with significant increased anterior pelvic and trunk tilt compared to healthy subjects ($P<0.05$). Pelvic rotation with hip trailing (pelvic external rotation) revealed comparatively higher values in the hemiparetics than healthy subjects. No significant differences were observed in pelvic obliquity, lateral trunk tilt and trunk rotation between the two groups.

IV. Discussion and Conclusion

Hemiparetic cerebral palsy has functional consequences that are varied and can potentially affect all activity of daily living. About 33% of CP children have hemiplegia with weakness and spasticity predominantly affecting one side of the body and the deficit concerns the motor ability of the body's side opposite to the site of cerebral lesion (Hagberg et al., 2001; Liptak and Accardo, 2004; Nashner et al., 1983).

Although the term "hemiplegia" connotes involvement of only one side, hemiparetic children often have motor involvement not only on affected side, but also on the non affected side as well, particularly in those cases with more severe types of hemiplegia which demonstrates an altered gait pattern of lower limb (Gage, 2004). In literature, few studies have examined quantitatively some aspects of motor control during gait in hemiplegic children (Carlsson et al., 1994; Cimolin et al., 2007; Galli et al., 2010; Wheelwright et al., 1993). Gait analysis focused mainly on comparing functional motor evaluations in right and left gait types. No studies have investigated quantitatively spatiotemporal and joint kinematics in hemiparetic cerebral palsy children during stance phase. Nevertheless; the non affected side (non paretic) was neglected. Hence there is clinical need to identify and investigate both sides of hemiparetic children for developing either deficit-specific or rehabilitative strategies. The aim of this study was the quantification of spatiotemporal and joint kinematics in hemiparetic children during stance phase.

With regard to spatiotemporal parameters hemiparetic children walked more slowly than healthy children with shorter step length, decreased cadence and longer step width. Walking velocity is the product of step length and cadence, hence reduction in either one parameter may account for gait slowing and it might be considered a strategy in order to obtain a better stability and equilibrium during walking. The shorter stance phase on the paretic side compared to non paretic and healthy children is related to the deficient ability to load and transfer weight through their affected leg. It has been proposed that improving weight transfer through the affected leg during progressive training with the feet of the patients placed in a variety of diagonal position may improve gait symmetry in hemiplegics (Olney et al., 1991). Ankle joint showed an asymmetry pattern, the paretic side revealed reduced dorsal flexion ability and increased abduction during stance phase compared to non paretic side. This pattern is common in hemiplegic patients with equinovarus foot deformity. The deformity can be explained by the premature onset of the gastrocnemius medialis muscle (Boulay et al., 2012). As for pelvic, hip and knee kinematics, the significant reduced knee flexion during stance may necessitate such compensatory maneuvers as hip circumduction, hip hiking, and contra lateral vaulting with excessive elevation of the pelvis to avoid toe drag (Kim et al., 1994; Perry, 1969). Hemiparetic children walked with significant increased anterior pelvic tilt with increased pelvic external rotation compared to healthy subjects. The external pelvic rotation is also known as pelvic retraction. Hemiparetic children often walk with abnormal pelvic motion patterns including increased anterior pelvic tilt (Saunders et al., 1953; Winters et al., 1987) and retraction of the affected side (Aminian et al., 2003; O'Sullivan et al., 2007; Park et al., 2006). These alterations can occur as a result of one or a combination of different variables such as weakness, skeletal deformities, abnormal muscle activation pattern and compensatory mechanisms. The significant higher values of hip flexion displayed by hemiparetic children compared to healthy subjects with slight increased internal hip rotation on the paretic side than non paretic is due to increased protraction of the pelvis. Pelvis is a single segment; increase protraction may result in internal hip rotation. The most prominent feature observed in trunk kinematics was significant increased anterior trunk tilt on the hemiparetics compared to healthy subjects. Hemiparetic children walk with

increased anterior trunk tilt as a compensatory mechanism to maintain balance and forward progression. Cerebral palsy children frequently show impaired trunk control and stability, which can affect performances of activities of daily life such as sitting, reaching and walking. In contrast, literature on trunk control in children with hemiparetic cerebral palsy (leg dominant) is scarce (Hadders-Algra and Brogren, 2008; Prosser et al., 2010; van der Heide et al., 2005).

A potential weakness of this study may be; lack of classification of the patients according to (Winters et al., 1987) into 4 gait strategies based on saggital plane kinematics, even though the use of classification system resulted in small subject numbers being allocated to some gait types.

However our results support previous observations which showed that analysis of gait pattern of hemiparetic CP children generally presents a unique motor strategy different from healthy subjects (Cimolin et al., 2007).

From clinical perspective, the identification and precise quantification of gait pattern in hemiparetic CP children is important for development of effective and specific rehabilitative programs.

V. Acknowledgements

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VI. Conflicts of Interest

None

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Curative Effect of Extracts of Sapindus Mukorossi and Rheum Emodi in CCl4 Induced Liver Cirrhosis in Male Rats

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Abstract - *Aim :* To study the curative effect of Sapindus mukorossi and Rheum emodi extracts in CCl4 induced liver cirrhosis in male rats.

Methods : The dried powder of S. mukorossi & R. emodi was extracted successively with petroleum ether, benzene, chloroform and ethanol and concentrated in vacuum. The curative effect of the extracts of the fruit pericarp of S. mukorossi and rhizomes of R. emodi was studied using CCl4 induced liver cirrhosis in male rats. Biochemical parameters including serum transaminases [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)] and alkaline phosphatase (ALP) in serum were analyzed. The biochemical findings were supplemented with histopathological examination of rat liver sections.

Results : Extracts of the fruit pericarp of S. mukorossi (2.5mg/mL) and rhizomes of R. emodi (3.0 mg/mL) protected the rats from CCl4 induced liver cirrhosis as judged from histopathological evidences and serum marker enzyme activities.

Keywords : *Cirrhosis, Sapindus mukorossi, Rheum emodi.*

GJMR-L Classification : *NLMC Code: WI 25, WI 700, QY 140*

Strictly as per the compliance and regulations of:

Curative Effect of Extracts of *Sapindus Mukorossi* and *Rheum Emodi* in CCl4 Induced Liver Cirrhosis in Male Rats

Mohammed Ibrahim [α], Anjum A [σ] & Mohammed Abdul Waheed [ρ]

Abstract – Aim : To study the curative effect of *Sapindus mukorossi* and *Rheum emodi* extracts in CCl4 induced liver cirrhosis in male rats.

Methods : The dried powder of *S. mukorossi & R. emodi* was extracted successively with petroleum ether, benzene, chloroform and ethanol and concentrated in vacuum. The curative effect of the extracts of the fruit pericarp of *S. mukorossi* and rhizomes of *R. emodi* was studied using CCl4 induced liver cirrhosis in male rats. Biochemical parameters including serum transaminases [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)] and alkaline phosphatase (ALP) in serum were analyzed. The biochemical findings were supplemented with histopathological examination of rat liver sections.

Results: Extracts of the fruit pericarp of *S. mukorossi* (2.5mg/mL) and rhizomes of *R. emodi* (3.0 mg/mL) protected the rats from CCl4 induced liver cirrhosis as judged from histopathological evidences and serum marker enzyme activities.

Conclusion: We can conclude from this study, that the extracts' of *S. mukorossi* and *R. emodi* can cure the CCl4 induced liver cirrhosis in male rats.

Keywords : Cirrhosis, Sapindus mukorossi, Rheum emodi.

I. Introduction

The liver is an organ of paramount importance. Due to its unique and considerable regenerative capacity, even a moderate cell injury is not reflected by measurable change in its metabolic functions. However, some of its functions are so sensitive that abnormalities start appearing depending upon the nature and the degree of initial damage. The etiology of the liver disorders depends on various factors as nutritional, biochemical, bacteriological, viral, or environmental aberration. The liver plays a significant role not only in the metabolism and disposition of the chemicals to which it is exposed directly or indirectly, but also in the metabolism of fats, carbohydrates, proteins, and immune-modulation. The impairment of the liver function is generally caused by xenobiotics, excessive exposure to various pharmacological and chemical agents, and protozoal or viral infections. Depending upon the severity of cellular injury, acute hepatitis can lead to chronic hepatitis, which is finally terminated to cirrhosis or malignant lesions in untreated cases. Alcoholic liver disease (ALD) is one of the most serious consequences of chronic alcohol abuse. Liver cirrhosis, the culmination of the illness, is one of the leading causes of death in western countries.[1, 2]

Hepatic fibrosis occurs in the advanced liver disease, where normal hepatic tissue is replaced with collagen rich extracellular matrix (ECM) and, if left untreated, result in cirrohsis.[3, 4] Cirrhosis is a complication of many liver diseases that is characterized by abnormal structure and function of the liver. The diseases that lead to cirrhosis do so because they injure and kill liver cells and the inflammation and repair that is associated with the dying liver cells causes scar tissue to form. The liver cells that do not die multiply in an attempt to replace the cells that have died. This results in clusters of newly-formed liver cells (regenerative nodules) within the scar tissue. There are many causes of cirrhosis; they include chemicals (such as alcohol, fat, and certain medications), viruses, toxic metals (such as iron and copper that accumulate in the liver as a result of genetic diseases), and autoimmune liver disease in which the body's immune system attacks the liver. The magnitude of derangement of liver by disease or hepatotoxins are generally measured by the level of glutamate pyruvate transaminase (ALT), glutamate oxaloacetate transaminase (AST), alkaline phosphatase (ALP), bilirubin, albumin, and whole liver homogenate.

Medicines that are used today are not definitely the same as those that were used in ancient times or even in the recent past. India has a wealth of medicinal plants most of which have been traditionally used in Ayurveda, Unani systems of medicine and by tribal healers for generation. In ancient Indian literature, it is

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mentioned that every plant on this earth is useful for human beings, animals and other plants. The liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, fight against diseases, nutrient supply, energy provision and reproduction [5]. The liver is expected not only to perform physiological functions but also to protect the hazards of harmful drugs and chemicals. In spite of tremendous scientific advancement in the field of hematology in recent years, liver problems are on the rise. Jaundice and hepatitis are two major hepatic disorders that account for a high death rate. [6]

Herbal drugs are playing an important role in health care programs worldwide, and there is a resurgence of interest in herbal medicines for treatment of various ailments including hepatopathy. India, the abode of Ayurvedic system of medicine, assigns much importance to the pharmacological aspects of many plants. Hepatoprotective effect of some plants like *Spirulina maxima* [7], *Eclipta alba* [8], *Boehmeria nivea* [9], *Cichorium intybus* [10], and *Picrorhiza kurroa* [11] has been well established. Nearly 150 phytoconstituents from 101 plants have been claimed to possess liver protecting activity[12]. At the same time, surprisingly, we do not have satisfactory plant drugs/formulations to treat severe liver diseases. Most of the studies on hepatoprotective plants are carried out using chemical induced liver damage in rodents as models. A few excellent reviews have appeared on this subject in the recent past[13]. This study is based on the natural products responsible for repairing and healing of adversely affected liver cells. In the present study, we selected two plants namely *S. mukorossi* and *R. emodi* and investigated the hepatoprotective effect of these plant extracts against CCl4 induced hepatocyte damage *in vitro* and liver injury *in vivo.*

S. mukorossi Gaerten (Sapindaceae), commonly known as Ritha or Aritha is found throughout India. The major constituents of its fruit are saponins (10%-11.5%), sugars (10%) and mucilage[14]. The fruit of the plant is reported to have expectorant, emetic, alexipharmic, and abortificiant effects. It is also used in excessive salivation, epilepsy and chlorosis[15], [16]. Saponins from this plant are known to be spermicidal *in vitro*[17]. This spermicidal property has been used in contraceptive cream[18]. The alcoholic extract (Sapindus trifoliatus Linn) is reported to possess antiimplantation activity.

R. emodi (Polygonaceae) commonly known as Indian or Himalayan Rhubarb is found in India. The major constituents of rhubarb rhizomes are anthraquinones. Rhubarb is used as a laxative, diuretic to treat kidney stones, gout, and liver diseases characterized by jaundice. Externally, it is used to heal skin sores and scabs. Paradoxically, although larger doses are used as laxative, small doses are used to treat dysenteric diarrhea[19]. Chinese use rhubarb as an ulcer remedy and consider it a bitter, cold, dry herb used to "clear heat" from the liver, stomach and blood, to expel helminthes and to treat cancer, fever, upper intestinal bleeding (ulcers), and headache[20],[21]. It is also used to treat toothache[22]. In Europe, rhubarb is a component of spring tonics or blood cleansing cures, including Swedish bitter[23]. Turkish or medicinal rhubarb is also one of the four major ingredients in the herbal cancer remedy. We isolated the extracts from both plants, and a study was designed using the extracts of *S. mukorossi* and *R. emodi* to assess the curative effect of *Sapindus mukorossi* and *Rheum emodi* extracts in CCl4 induced liver cirrhosis in male rats.

II. Materials and Methods

a) Plant materials

Authentic samples of *S. mukorossi* and *R. emodi* were obtained from authorized supplier M/s Munnalal Dawasas and Co. Hyderabad, Andhra Pradesh, India. The plants were previously identified and authenticated by experts in the Post Graduate and Research Department of Botany, Anwarul-loom College Hyderabad, Andhra Pradesh, India.

b) Animals

Male Wister rats weighing 175-200 g were obtained from the animal house of Deccan College of Medical Sciences, Hyderabad and housed in polycarbonate cages. The rats had free access to standard pellet chow and water *ad libitum* throughout the experiment with the exception of some experiments (see below) in which the animals were deprived of food, but not water, for 18-24 h before the experiments were performed. After procurement, all the animals were divided into different groups and were left for one week for acclimatization to experimentation room and were maintained on standard conditions (230, 60%-70% relative humidity and 12 h photo period). There were six animals in each group for observational screening and acute toxicity studies. All experimental protocols described below were approved by the ethical board.

c) Extraction, separation, and purification of the compounds

For phytochemical analysis, approximately 100 g of fruit pericarp of *S. mukorossi* and rhizomes of *R. emodi* was collected and materials were chopped, air dried at 35-40^0 and pulverized in electric grinder. The powder obtained was successively extracted with the following chemicals, petroleum ether (60-80)^0, benzene, chloroform, and ethanol, respectively. The extracts were then powdered by using rotary evaporator under reduced pressure. Fruit pericarp of *S. mukorossi* yielded 38 g, 28 g, 34 g, and 35 g and rhizomes of *R. emodi* yielded 19 g, 17 g, 21 g, and 22 g powdered extracts with petroleum ether, benzene, chloroform, and ethanol,

respectively. The extracts were obtained by percolation using 70% of ethanol as solvent at room temperature; according to process A of Farmacopeia dos Eastados Unidos do Brasil (1959) (AOAC 1990). The extracts were evaporated at 40^0 under vacuum and the residue was freeze-dried. The dry extracts of the fruit pericarp of S. mukorossi and rhizomes of R. emodi were tested for the presence of saponins and anthraquinones. Each extract of the fruit pericarp of *S. mukorossi* (SM) and rhizomes of *R. emodi* (RE) were column chromatographed over Silica gel (200 mesh), eluting with CHCl3-MeOH (70:30, 60:40, 50:50, 25:75) and compound fractions of (250 mL each) were collected and monitored by TLC. These column chromatographed compound fractions were further filtered to yield saponins from *S. mukorossi* and anthraquinones from *R. emodi*, which were separated by paper chromatography and preparative TLC to yield saponins [(SM-A (petroleum ether), SM-B (benzene), SM-C (chloroform) & SM-D (ethanol)], and anthraquinones [(RE-A (petroleum ether), RE-B (benzene), RE-C (chloroform) & RE-D (ethanol)], respectively. All the filtrates obtained were dried by evaporation (Rotometer, 40^0), the dried extracts were individually dissolved in 10 mL ethanol (95%) and then subjected to complete drying process and weighed according to the AOAC (1990) method [20].

d) Hepatotoxins

It is emphasized that hepatotoxins that cause acute hepatitis should have close resemblance with the viral hepatitis, clinically, biochemically, and histologically. Certain drugs are also responsible for chronic hepatic disease such as chronic hepatitis, fatty liver, cirrhosis, and several vascular lesions of the liver. In many instances drug induced hepatitis is indistinguishable from viral hepatitis. Chemically induced hepatic injury for experimental studies should be severe enough to cause cell death or to modify hepatic functions. The mechanism of acute hepatic injury depends upon the chemical compound and the species of animals used. We have studied hepatoprotective activity against carbon tetrachloride (CCl_4) induced hepatotoxicity. CCl_4 is one of the most powerful hepatotoxin in terms of severity of injury. It causes toxic necrosis leading to biochemical changes having clinical features similar to those of acute viral hepatitis [24], [25].

Induction of Liver Cirrhosis in Rats : Cirrhosis was induced by administering CCl4 intragastrically. The initial dose of CCl4 was 40µL/rat, and subsequent doses were adjusted based on the change in body weight as described.[26]

Estimation of Hydroxyproline : Hepatic hydroxyproline content was measured as described 27 (Table 1).

Detailed evaluation of Curative effect of *Sapindus mukorossi* and *Rheum emodi* in CCl_4 induced liver cirrhosis. The animals were divided into five groups of six animals each. Group 1 served as vehicle control and was administered with normal saline. Group 2 rats were given CCl4 40 µL/rat checking the biochemical parameters periodically for hepatotoxicity. Group 3 rats were given CCl_4 + extracts of *S. mukorossi* 2.5 g/kg, *p.o.* Group 4 rats were given CCl_4 + extracts of R. emodi 3.0 g/kg, p.o. Blood was collected from the orbital sinus in all animals and serum separated for different estimations (Table 1). The rats were anesthetized and sacrificed after the experimental period by cervical decapitation. The liver tissue was examined histopathologically.

e) Statistical analysis

The data obtained was subjected to statistical analysis using ANOVA for comparing different groups (Armitage, 1987) and Dunnett's *t* test for control and test groups (Dunnett, 1964). The two tailed unpaired student t test for comparing means before and after treatment and one tailed unpaired student *t* test for comparing control and drug treated group, ED50 value with 95% confidence limits (CL) by regression analysis using log dose response (Swinscow, 1980 & Ghosh, 1984) were used. $P < 0.05$ or less was taken as the criterion of significance.

III. Results

S. mukorossi and *R. emodi* extracts showed significant hepatoprotective activity against CCl4 induced liver injury in primary hepatocytes cultures [28]. The hepatotoxic effects of CCl_4 are attributed to its metabolism by P450 to yield toxic trichloromethyl radicals that can act as free radical initiators [29]. These radicals are believed to induce injury either by interacting with the unsaturated fatty acids of cell membranes, thereby causing lipid peroxidation, or by binding covalently to important macromolecules such as proteins, lipids, or DNA [30], [31]. The extracts of *S. mukorossi* and *R. emodi* reduced the levels of LDH and GPT released from CCl_4 injured rat hepatocytes into the medium in a concentration dependent manner, thus signifying their hepatoprotective activity [28].

In CCl_4 induced cirrhosis rats, serum activities of AST, ALT, ALP, and Bilirubin were increased significantly when compared to the control (Table 1).

The CCl_4 treated group showed a marked increase in serum Bilirubin (mg %) (1.82 ±0.08), ALT (IU/L) (1262.30 ± 1.97), AST (IU/L) (903.50 ± 30.00), and ALP (IU/L) (104.09 ± 3.00) activity indicating the injury caused by CCl_4. Treatment with the extracts of *S. mukorossi* and *R. emodi* significantly decreased the above elevated parameters and the normal architectural liver pattern was restored as given below. Slide of a control rat showing normal hepatocytes and architecture (Figure 1A). Slide of CCl_4 treated rat demonstrating the loss of hepatic architecture with formation of nodules of hepatocytes without lobular pattern and no central veins,

necrosis, thin fibrous bands encircling nodules of hepatocytes, micro-nodular cirrhosis of liver (Figure 1B). Slide of *S. mukorossi* treated rat showing normal lobular architecture no necrosis or fatty changes (Figure 1C). Slide of *R. emodi* treated rat showing normal lobular architecture. (Figure 1D). These histopathological findings demonstrate a Curative effect of Sapindus *mukorossi* and *Rheum emodi* in CCl_4 induced liver cirrhosis.

IV. Discussion

The purpose of this study was to explore the Curative effect of *Sapindus mukorossi* and *Rheum emodi* in CCl_4 induced liver cirrhosis. Administration of CCl_4 to normal rats increased serum levels of AST, ALT, ALP, and Bilirubin. The enzymes leaking out from damaged liver cells into circulating blood represent the damage to hepatic cells. It is well established that the toxic metabolite of CCl_4, a free radical CCl_3 is responsible for damage to liver cells. *S. mukorossi* and *R. emodi* extracts caused statistically significant decrease in all the above parameters at the dose of 2.5 mg/kg and 3.0 mg/kg given orally to CCl_4 treated rats. Histopathological examination of the liver sections of rats treated with CCl_4 Treatment with the extracts of *S. mukorossi* and *R. emodi* significantly decreased the above serum elevated parameters and the normal architectural liver pattern was restored. Slide of a control rat showing normal hepatocytes and architecture (Figure 1A). Slide of CCl_4 treated rat demonstrating the loss of hepatic architecture with formation of nodules of hepatocytes without lobular pattern and no central veins, necrosis, thin fibrous bands encircling nodules of hepatocytes, micro-nodular cirrhosis of liver (Figure 1B). Slide of *S. mukorossi* treated rat showing normal lobular architecture no necrosis or fatty changes (Figure 1C). Slide of *R. emodi* treated rat showing normal lobular architecture. (Figure 1D). Hepatic hydroxyproline content of the normal rats and the CCl_4 treated were compared with the treated extracts of *Sapindus mukorossi* and *Rheum emodi* it is an evident that the extracts of *Sapindus mukorossi* and *Rheum emodi* are having the curative effect on CCl_4 induced liver cirrhosis. Further it should be evaluate in the human studies in order to have the proper treatment for the liver diseases.

Table 1 : Effect of S. mukorossi and R. emodi extracts on serum biochemical parameters against CCl4 induced hepatic injury in rats (Curative study, mean ± SE).

Treatment	Dose(mg/kg, p.o .)	Serum Parameters				Liver weight
		ALT (IU/l)	AST (IU/l)	ALP (IU/l)	Bilirubin (mg)	Hepatic hydroxyproline content (µg/g)
Normal	--	127.73 ± 10.65	100.26 ± 11.50	40.11 ± 2.20	0.11 ± 0.02	235 ± 20
Vehicle + CCl4	--	1262.30 ± 1.97	903.50 ± 30.00	104.09 ± 3.00	1.82 ± 0.08	955 ± 13
S. mukorossi + CCl4	2.5	620.13 ± 6.20[b]	538.70 ± 3.07[a]	97.38 ± 1.02[b]	0.60 ± 0.02[b]	505 ± 03
R. emodi + CCl4	3.0	484.60 ± 4.09[b]	519.90 ± 3.41[b]	89.00 ± 2.19	0.62 ± 0.02[b]	485 ± 53

P<0.05, bP<0.01 vs control.

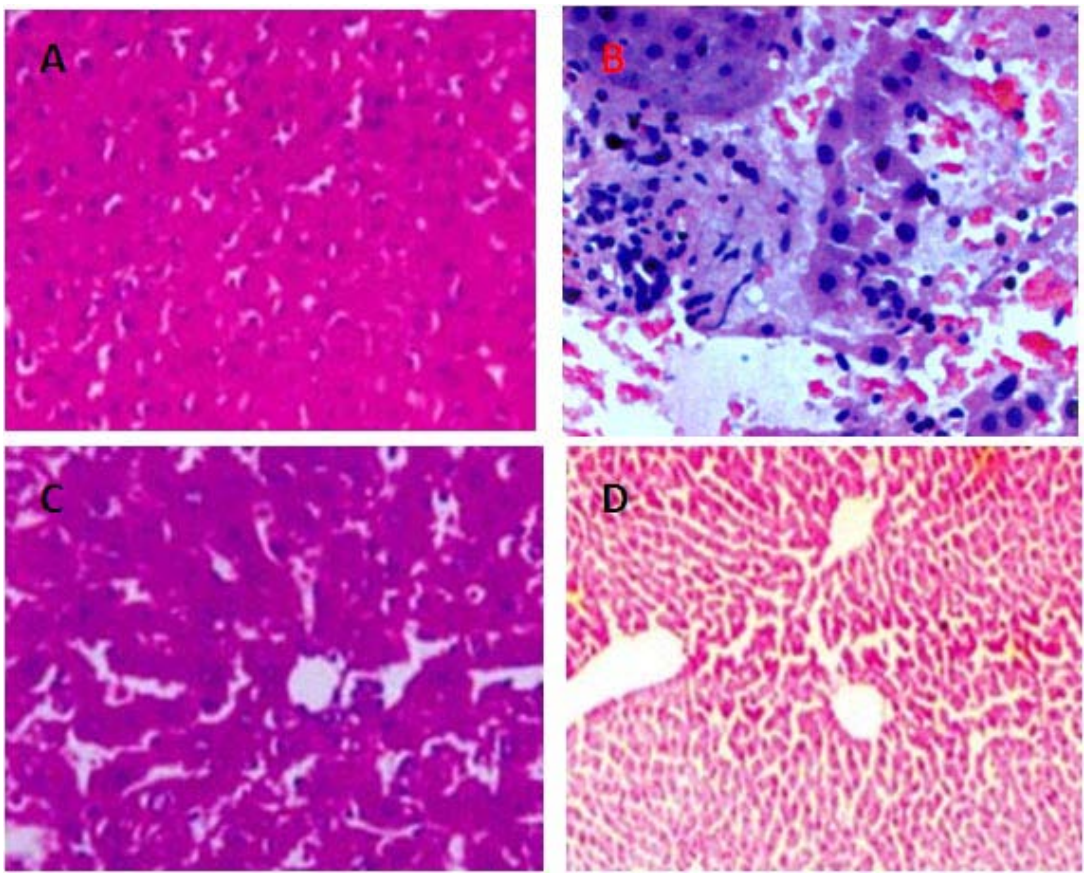

Figure 1 : Photograph of rat liver shows (HE, × 100). A: Slide of control rat showing normal hepatocytes and architecture; B: Slide of CCl4 treated rat demonstrating the loss of hepatic architecture with formation of nodules of hepatocytes without lobular pattern and no central veins, necrosis, thin fibrous bands encircling nodules of hepatocytes, micro-nodular cirrhosis of liver ; C: Slide of *S. mukorossi* treated rat showing normal lobular architecture no necrosis or fatty changes.; D: Slide of *R. emodi* treated rat showing normal lobular architecture.

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Correlates of Impaired Renal Function in Highly Active Antiretroviral Therapy (HAART) Naive HIV Infected Patients in Maiduguri, Nigeria

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Abstract - *Background :* Although renal function may be abnormal in as many as 30% of HIV -1 patients even in the era of highly active antiretroviral therapy, it may not be apparent at the initial stage and laboratory tests are needed to detect it. We determined the factors associated with impaired renal function in HIV infected patient initiating highly active antiretroviral therapy in North-eastern Nigeria. *Materials and Methods :* This was a retrospective study among HIV-1 infected patients that presented at infectious diseases clinic at the university of Maiduguri Teaching Hospital(UMTH) for care between July 2008- March 2009. Data were analysed for age, gender, weight, height, WHO clinical stage, HIV-1 RNA viral load, HBsAg and anti-HCV antibody status. Estimated glomerular filtration rate eGFR was calculated using the Cockcroft –Gault equation.

Keywords : *Highly active antiretroviral therapy, human immunodeficiency virus, correlates, serum creatinine.*

GJMR-L Classification : *NLMC Code: WC 503-503.7*

Strictly as per the compliance and regulations of:

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Materials and Methods : This was a retrospective study among HIV-1 infected patients that presented at infectious diseases clinic at the university of Maiduguri Teaching Hospital(UMTH) for care between July 2008- March 2009. Data were analysed for age, gender, weight, height, WHO clinical stage, HIV-1 RNA viral load, HBsAg and anti-HCV antibody status. Estimated glomerular filtration rate eGFR was calculated using the Cockcroft –Gault equation.

Results : A total of 415 participants with mean age of 43.65±9.70 (95% CI; 42.77 – 44.52), were considered for this study. Out of this 182 (43.6%) were males, with a mean age of 47.43±9.00, they were older than females with mean age of 40.54±9.08 ($p<0.05$). A total of 61(14.7%) had an eGFR<60mL/min, with disproportionately more males (17.0%vs 12.5%) having eGFR<60mL/min than females ($p<0.05$). On multivariate analysis, older age (≥50 years), anaemia (Hb<10g/dl), abnormal BMI (<18.5 kg/m² or >25.0 kg/m²) had significant associations with reduced GFR.

Conclusion : Older age, anaemia and abnormal weight are independently associated with risk of having impaired renal function in our cohort. We therefore recommend renal function tests to HIV infected patients at commencement of highly active antiretroviral therapy for effective and proper management.

Keywords : Highly active antiretroviral therapy, human immunodeficiency virus, correlates, serum creatinine.

I. Introduction

Despite the widespread use of highly active antiretroviral therapy (HAART), HIV disease remains associated with increased kidney disease risk (Phair and Palella,2011). Kidney disease is an often unrecognised problem as kidney function may be abnormal in up to 30% in HIV population (Gupta *et al.*,*2005*; Szczech*et al.*,2002). HIV-infected patients may undergo renal damage related to the HIV infection itself, to the presence of co-infection, arterial hypertension, diabetes or to the exposure to nephrotoxic drugs. Consequences of kidney disease in HIV-infected persons include increased risk of atherosclerosis and mortality, in addition immunosuppression that is known to be associated with development of opportunistic infections, malignancies and other organ diseases that affects kidney functions. (Choi A *et al.*,2010; Choi AI *et al.*,2010).

HIV-associated nephropathy (HIVAN) is traditionally the most common renal lesion affecting HIV-infected patients; it is the commonest cause of end stage renal disease (ESRD), often requiring renal replacement therapy. Although HIVAN has been documented in indigenous African patients, little is known about the prevalence or risk factors for renal disease in this population (Winston *et al.*, 1999; Cosgrove *et al.*, 2002; Szczech*et al.*,2004).

Kidney function can be measured by determining the glomerular filtration rate (GFR), the decrease in GFR has been shown to correlates with the severity of kidney disease. The Cockcroft-Gault equation, which estimates GFR using serum creatinine and anthropometric variables has been shown to predict renal function (Cockcroft and Gault,1976). The use of this equation in assessing GFR has been validated among black HIV positive patients (Chukwuonye,2007).

The aim of this study was to determine the factors associated with impaired renal function among the patients initiating highly active antiretroviral therapy.

II. Patients and Method

Design : Cross-sectional observational cohort study.

Study Area : The study was conducted in the Department of Medicine, University of Maiduguri Teaching Hospital, Borno State. This is a 500 bedded hospital designated as a Centre of Excellence for infectious diseases and provides primary, secondary and tertiary services for the North Eastern part of Nigeria. It also caters for the neighbouring Countries

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such as Cameroon, Niger and Chad Republics. Maiduguri the capital of Borno State is situated in the north eastern Nigeria and the largest settlement near the Lake Chad.

Study procedure : Cross-sectional data of 415 HIV positive patients were abstracted for the purpose of this study.

Variable abstracted included age, gender, weight and WHO clinical stage of HIV disease. Blood samples were collected for CD4 count using standardized flow cytometricCyflow machine (manufactured by Cytec, Partec, Germany 2005). While plasma HIV RNA levels was measured using freshly frozen specimen separated within 6 hours of phlebotomy utilizing the Amplicor HIV-1 Monitor Test, version 1.5 Manufactured by Roche® Germany, with a minimum cut off value of 200 copies per ml. Enzyme linked immunosorbentassay kits was used to detect the presence of HBsAg and HCV antibodies (DIA, PRO, DiagosticBioprobes Sri, via columella no 20128 milano-Italy).

The estimated Glomerular Filtration Rates (eGFRs) were calculated from serum creatinine measurements using the Cockcroft Gault equation (Cockroft and Gault, 1976; Chukwuonye, 2007) and graded according to the National Kidney Foundation grading (Leyvey*et al.*, 2003) of chronic kidney disease (CKD) as follows: Grade 1, 60-89mL/min; grade 2, 30-58mL/min, grade 3, 15-29mL/min; and grade 4, <15mL/min.

Ethical consideration : Permission was obtained from the University of Maiduguri Teaching Hospital (UMTH) Ethical Committee.

Statistical analysis : Data were analyzed using SPSS®, version 16.0 for Windows (SPSS Inc., Chicago, IL, USA). Categorical variables were compared using Chi-square test, group means were compared the students t-test. Mann Whitney test was used to compare variables that did not follow normal distribution.

Factors associated with reduced eGFR (defined as <60mL/min) were tested for inclusion in a multivariate logistic regression model. A P-value of < 0.05 was considered statistically significant.

III. Results

a) Stratification of participants based on gender

A total of 415 HIV positive, highly active antiretroviral therapy (HAART) naive patients with mean age of 43.65±9.70 (95% CI; 42.77 – 44.52), were considered for this study. Out of this 182 (43.6%) participants were males, with a mean age of 47.43±9.00, they were older than their female counterpart that had a mean age of 40.54±9.08 (p<0.05). Female gender was associated with significantly low haemoglobin, viral load, and proportion with renal impairment (eGFR). Male cohort had significantly high proportion of participants infected with hepatitis B virus, while the body mass index and AIDS status between the males and females were not comparable as shown in Table 1.

b) Stratification of participants based on renal function

Categorization of the participants based on renal function indicated that 61(14.7%) had an eGFR<60mL/min, with disproportionately more males (17.0%vs 12.5%) having eGFR<60mL/min. The overall mean eGFR was 95.65±39.09 (95%CI;91.90 – 99.40), with males being more likely to have lower eGFR (males: 88.82±36.46, females 100.82 ± 39.34; p=0.002). Other variables associated with reduced eGFR (<60ml/min) in comparison with normal eGFR (≥60ml/min) includes; older age (eGFR<60ml/min:49.87±11.07, eGFR ≥ 60ml/min: 42.46±8.90; p = 0.000), low CD4 count (eGFR<60ml/min: 182.21±1105.46, eGFR≥ 60ml/min: 222.04±152.03; p = 0.013), low haemoglobin (eGFR<60ml/min: 10.19±2.31, eGFR ≥ 60ml/min: 11.60±2.05; p = 0.000), low Body mass index (eGFR<60ml/min: 20.70±3.9, eGFR ≥ 60ml/min: 22.77±4.48; p = 0.013). AIDS cohort were more likely to have renal impairement (eGFR<60ml/min) than participants that had no features of AIDS (16.5% vs11.9%) respectively. No difference was observed in viral load, platelets numbers and total white cell count parameters with respect to participants renal function (p>0.05) as depicted in Table 2.

c) Multivariate analysis of factors associated with reducedeGFR (eGFR<60ml/min)

On multivariate analysis, with younger age(<50 years), Hb≥10g/dl,WBC≥3X10^9/l, platelets≥150x10^9/l, HIV-1RNA≥100000copies/ml, no AIDS status, Normal BMI(18.5-25.0kg/m2) as a referent, it shows that older age (≥50 years), anaemia (Hb<10g/dl), abnormal BMI (<18.5kg/m^2 or >25.0 kg/m^2) had significant associations with reducedeGFR (eGFR<60ml/min) as shown in Table 3.

Table 1 : Characteristics of patients stratified by sex.

	Males(n=182)	Females (n=233)	P-value
Age (years)	47.43±9.00	40.54±9.08	0.000*
Mean Hb (g/dl)	11.90±2.10	10.88±2.06	0.000*
Mean WBC	5.20±2.46	5.09±1.89	0.611
Mean platelets	246.43±101.72	275.02±103.39	0.005*
Mean CD4 count(cells/μl)	201.87±149.22	224.36±150.69	0.112
Mean viral load log10 (copies/ml)	4.79±5.13	4.09±5.51	0.009
eGFR(mL/min)	88.82±36.46	100.82±39.34	0.002
e GFR grade			
≥ 90	77(42.3%)	136(58.4%)	0.002*
60-89	74(40.7%)	68(29.2%)	0.019
30-59	25(13.7%)	27(11.6%)	0.623
15-29	06(03.3%)	01(0.42%)	0.057
<15	0(0%)	01(0.004%)	-
Hepatitis C	-	03(1.3%)	-
Hepatitic B	30(16.5%)	28(12.0%)	0.000*
AIDS status			
yes	104(57.1%)	127(54.5%)	0.668
no	78(42.9%)	106(45.5%)	0.668
BMI	22.65±4.39	22.13±4.58	0.338

BMI (body mass index).
*Statistically significant.

Table 2 : Characteristics of patients stratified by reduced eGFR.

	(eGFR≥60mL/min) N=356	(eGFR<60ml/min) N=61	P-value
Age (years)	42.46±8.90	49.87±11.07	0.000*
Gender			
Male, no (%)	161(83.0%)	31(17.0%)	0.000*
Female, no (%)	104(87.5%)	29(12.5%)	0.000*
Mean Hb (g/dl)	11.60±2.05	10.19±2.31	0.000*
Mean WBC	5.05±1.93	5.68±2.90	0.115
Mean platelets	262.12±103.00	251.32±113.34	0.471
Mean CD4 count(cells/μl)	222.04±152.03	182.21±105.46	0.013*
Mean viral load log10 (copies/ml)	4.96±5.44	4.97±5.27	0.958
Hepatitis C	3	0	
Hepatitic B	48(13.5%)	10(16.4%)	0.000*
BMI	22.77±4.48	20.70±3.90	0.013*
AIDS status, no=231			
yes	193(83.5%)	38(16.5%)	0.000*
no	162(88.0%)	22(12.0%)	0.000*

BMI (body mass index).
*Statistically significant.

Table 3 : Multivariate analysis of correlates of reduced eGFR (< 60ml/min) among HIV-infected patients.

Variables	Odd ratio	95% confidence limits	P-value
Age (years)			
<50	Referent		
≥50	1.973	2.809 - 18.411	0.000
Gender			
Males	Referent		
Females	0.175	0.342 - 2.062	0.703
Haemoglobin			
≥10.00	Referent		
<10.00	1.310	0.095 - 0.766	0.014
White cell count			
≥3.00	Referent		
<3.00	0.374	0.229 - 2.063	0.504
Platelets count			
≥150	Referent		
<150	0.010	0.306 - 3.329	0.987
CD4 Count			
≥350	Referent		
<350	0.101	0.077 - 1.433	0.139
HIV-1 RNA			
≥100000	Referent		
<100000	0.157	0.312 – 4.392	0.816
AIDS Status			
no	Referent		
yes	0.573	0.701 - 4.486	0.226
BMI			
Normal (18.5-25.0)	Referent		
Abnormal weight	1.239	0.089 - 0.943	0.040

IV. Discussion

Our study examined the pattern of renal impairment and its associated factors among highly active anti retroviral naive HIV infected individuals. The prevalence of renal impairment as defined by an eGFR<60 ml/min/1.73m2 among HIV patients in our cohort was 14.7% similar to previous studies that reported a prevalence rate of 10 to 30% (Weiner *et al.*, 2002; Szczechet al., 2004; Winston *et al.*, 1979). It was however lower than prevalence rate of 53.3% reported in south south (Okafor*et al.*, 2011) and 23.8% determined in north central region (Agbajiet al., 2011), but higher than 7% reported by DART Trial group (Krawczyk*et al.*, 2004), 3% reported in California (Crum-Cianflone*et* al.,2010) and 3.5% in a predominantly Caucasian EuroSIDA cohort. (Mocroft*et al.*,2007).Discordance may be explained by study design, variations in patient population characteristicsincluding demographic characteristics, stage of HIV infection, and access to health care services. Of note, our population was relatively young (mean age 44 years), presented at late stage of the disease.Although somehow expected, this finding of prevalence of 14.7% in our cohort was worrisome for us. We used Cockcroft-Gaultequations to estimate glomerular filtration rate (eGFR), and since these equations can underestimate the actual GFR or creatinine clearance in patients with malnourishment or reduced muscle mass related to advance HIV, it is possible that the true prevalence of CKD in our cohorts is underestimated.

This study demonstrates older age, abnormal weight (under weight or over weight/obesity) and anaemia at presentation to be independent predictors of renal impairment in our cohort. Renal function is known to decline with age. Older age is an established risk factor for a decline in creatinine clearance in the general population (Davies and Shock, 1950). Similarly, older agehas been independently associated with renal function decline among HIV-infected subjects. (Mocroft*et al.*,2007; Cheung *et al.*, 2007).The preponderance of renal impairment in our male cohort may be related to significantly older male than female population.

The mean CD4 count of 222 cells/ul in patients with normal renal function was significantly higher than 182 cells/ul in our cohort with renal impairment. This is consistent with earlier studies that reported an association between impaired renal function in HIV infected patients with significantimmuno suppression, having CD4 cell count less than 200ul/L. Immunological AIDS (CD4 count <200ul/L) is known to be associated with development of opportunistic infections, malignancies and other organ diseases that affects kidney functions. (Winston *et al.*,1999; Szczech *et al.*, 2004; Winston *et al.*,2001; Krawczyk*et al.*,2004). CD4 cell had a protective role in the development of renal

diseases except acute tubular ischaemia (Wang *et al.*, 2005). Previous studies reported that in addition to CD4 cellcount of less than 200cells/ul, high viral load and proteinuria in HIV infected patients were othervariables that are associated with progressive renal impairment (Mulomaet *al.*, 2005; Chaparro *et al.*, 2009). However, Renal failure index (RFI) including HIVassociated nephropathy was recently reported in HIV patients with normal or mildly impaired immunestate with CD4 cell count above 200cells/ul and who usually were asymptomatic (Wang *et al.*, 2005; Ham *et al.*, 2006; Bourgoigni*et al.*, 2005).These information suggest that avoiding the occurrence of low CD4 cell counts, by early HIV diagnosis and treatment, may be important components of preventing future kidney disease among HIV patients; however further studies are needed to establish this preposition.

Reports from sub-Saharan Africa, indicated that the prevalence of decreased eGFR is high and varied substantially depending on the estimating method used (Chukwuonye,2007;Van Deventer *et al.*,2008;Eastwood *et al.*,2010). However the use of Cockcroft-Gault equations have been validated for use as it has been shown to predicts renal function in black HIV population (Chukwuonye, 2007). Renal dysfunction is an increasingly recognized non-AIDS–defining comorbidity among HIV-infected persons, with both HIV-associated nephropathy (HIVAN) and HIV-related ESRD disproportionately affecting black population (Choi *et al.*,2007;Lucas *et al.*, 2008). With the recent discovery of a locus on chromosome 22 that is associated with genetic susceptibility to HIVAN and other forms of CKD and ESRD among African-Americans (Kao *et al.*, 2008; Genovese *et al.*, 2010), there is increasing concern about the burden of HIV-related CKD in sub- Saharan Africa (Arendse*et al.*,2010). Available data suggest substantial regional variability in the prevalence of HIV-related CKD. The highest burden has been observed in West Africa, consistent with the predominant ancestry of the genetically susceptibleAfrican- American population (Emem*et al.*, 2008). With expanding access to ART across Africa, including the use of agents with nephrotoxic potential, screening of patients at commencement of ART to identify those with renal impairement is valuable. Also, early initiation of patients on ART in line with the new WHO guideline should be advocated to avoid AIDS related Kidney diseases.

V. Limitations

This study is limited in its retrospective design, with the greater proportion of HIV-infected with AIDS with advanced clinical disease, it implies that prevalence estimates derived from this study may not be generalizable to patients with early stage of HIV infection. In addition, we were limited by the use of a single serum creatinine, hence spurious results were not excluded. Finally, there was no assessment for protenuria; however, this was the standard of care in the centre at the time of this study.

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Toxic Effects of Heavy Metals (Cu, Ni, Fe, Co, Mn, Cr, Zn) to the Haematology of Mastacembelusarmatus Thriving in Harduaganj Reservoir, Aligarh, India

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Keywords : *Reservoir, Haematology, Heavy metals, R.B.C, Mastacembelus armatus.*

GJMR-L Classification : *NLMC Code: QV 290-298, QV 600*

Strictly as per the compliance and regulations of:

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I. Introduction

Aquatic pollution from sources like effluents from industries, power plants untreated domestic and sewage waste etc. have adverse effects on aquatic ecosystem. Due to which the animals thriving in these water bodies exposed to unnaturally high levels of contaminants. Along with other animals fishes are the animals that cannot escape from the detrimental effects of these contaminants. The heavy metals are of particular concern due to their persistence and undegradable nature. The metal contamination in aquatic ecosystem is considered to be unsafe not only for fishes but, also for human beings because they consume fishes whuch are best sources of proteins and essential amino acids . Fish blood is known to exhibit pathological changes before the onset of any external symptoms of toxicity and it truly reflects the physical and chemical changes occurring due to heavy metal accumulation in body of fish. Fish blood is being studied increasingly in toxicological research and environmental monitoring as a possible indicator of physiological and pathological changes in fishery management and disease investigations (Mulcahy 1975; Bansal et al. 1980). It is a pathophysiological indicator of the whole body function and therefore blood parameters are important in diagnosing the structural and functional status of fish exposed to a toxicant. The accumulation of heavy metals in a fish depends mainly on the concentration of the metal in the water and exposure period. Fishes exposed to heavy metal pollutants can induce either increases or decreases in haematological levels. A number of haematological indices such as haemoglobin (Hb), haematocrit (Hct), red blood cells (RBCs), white blood cells (WBCs) and so on, have been used as an indicator of metal pollution in the aquatic environment. Furthermore, it should be noted that haematological indices are of different sensitivity to various environmental factors and chemicals (Vosyliene, 1999b). Previous haematological studies of pollutants brought to the knowledge that erythrocytes are the major and reliable indicators of various sources of stress (O' neal and Weirich, 2001). Hematological abnormalities have also been studied in various toxicants exposed fish: *Channa punctatus* to lead (Hymavathi and Rao 2000); *C. punctatus* to cadmium (Karuppasamy et al. 2005); and *Labeo rohita* to synthetic detergents (Chellan et al.1999).

Harduaganj Thermal Power station (HTPS) / Kasimpur power station (Fig. 1) is located at Harduaganj (27.218° N and 79.378° E), district Aligarh, India. The waste water containing heavy metals from the power plant reaches the nearby Harduaganj reservoir. This reservoir has water filled area of 13.5 ha. This power plant have a total capacity of power generating of 700 MW, uses sulphur rich bituminous coal as fuel at the rate of 11,65,069 tonnes/ annum. The pollutants,being released in the man made Harduaganj reservoir through condensation process using certain gases, fly ash and traces of heavy metals. Hence, it is obvious that this may also exert tremendous impact on the aquatic ecosystem. Fishes, specially are being the potential indicators of pollution, and clearly indicate the pollution status of the reservoir.

Thus haematological studies are useful not only in assessing the health of fish subjected to changing

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environmental conditions but, also for the deteriorating water quality. Keeping this in view, a study was conducted to assess the changes in fish blood exposed to excess of potentially toxic heavy metals in the wastewater and to provide the fish as a bioindicator.

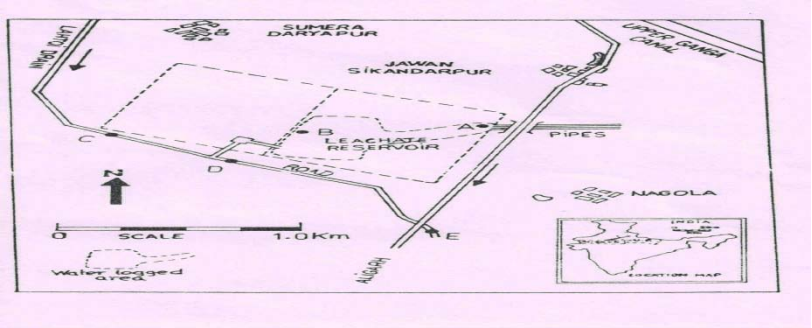

Figure : 1 Map showing the Harduaganj Reservoir, Aligarh, India.

II. Materials and Methods

Water was collected at the time of fish collection into the pre-cleaned and acidified glass bottles. The bottles were immediately brought to the laboratory and acidified with concentrated HNO_3 to pH less than 2.0. On spot fixation of water was done to measure the dissolved oxygen (D.O). Total solids (T.S), total dissolved solids (T.D.S) and total suspended solids (T.S.S) using standard techniques (APHA 2005).The temperature and pH were recorded at the site using laboratory thermometer (Deluxe, 6) and pH strips (S.D Fine chemicals, 0 - 0.1).

The analysis of water for the presence of heavy metals (Cu, Ni, Fe, Co, Mn, Cr and Zn) was performed according to APHA (2005). Blanks were prepared along with each set of the sample. Standard solutions for heavy metals were prepared using standard techniques (APHA 2005).

The water sample was analyzed for heavy metals by Atomic Absorption Spectrometer (Perkin Elmer, AA 800, multiple cathode lamps) with specific cathode lamps for each metal and Nitrous oxide-Acetylene was used as flame. The following analytical conditions of the instrument were used for atomic absorption of these metals Table 1.

Table : 1 Instrumental analytical conditions of heavy metal analyses.

Heavy Metals	Wavelength (nm)	Slitwidth (nm)	Optimm working range(μgml^{-1})	Sensitivity (μgml^{-1})	Lamp current (mA)
Cu	327.4	0.2	2.5- 10	0.050	3.0
Ni	341.5	0.2	6-25	0.12	3.5
Fe	372.0	0.2	20-80	0.45	7.0
Co	346.6	0.2	90-450	2.3	6.0
Mn	403.1	0.2	7-27	0.15	5.0
Cr	425.4	0.2	7-40	0.17	6.0
Zn	213.9	0.2	0.4-1.5	66	5.0

Fish *Mastacembelus armatus* is a predominant species of the reservoir, (stock 5, n= 5, length 14.20±1.5 cm, weight 35±0.42g,), were collected from the fishermen working beside the Reservoir. Procured fishes were washed with double distilled water and wiped dry with clean muslin cloth. Blood was collected from heart puncture of fish by heparinised syringe in vials containing heparin anticoagulant, which was used to estimate the haematological parameters. The vials and fishes were kept in separate ice boxes and brought to the laboratory for further analysis. Control fishes (Stock 5, n=5, length=14.99±1.43cm, weight= 36.12±0.98g) were procured from market and reared in laboratory. These fishes were kept in chlorine free tap

water which was changed every morning and maintaining ideal water quality conditions.

The RBC counts were made by Neubaur Haemocytometer. Blood was diluted 1:200 with Hayem's solution. Counting was done under the binocular microscope in the five smaller squares i.e. in the 1st, 5th, 13th, 21st and 25th. The RBC's on the lower and right sides of a square were added in the total, while those on the upper and left sides were rejected. Total numbers were reported as10^6 mm^{-3} (Wintrobe, 1967).

The WBC counts were made by Neubauer Haemocytometer. Blood was diluted 1:20 with Turk's diluting fluid and placed in haemocytometer. Four large (1 sq mm) corner squares of the haemocytometer were counted under the binocular microscope. The total number of WBC was calculated in mm^3 x 10^3 (Wintrobe, 1967).

Haemoglobin (Hb) was determined with haemoglobin test kit (DIAGNOVA, Ranbaxy, India) using the cyanmethaemoglobin method.

Experiments were conducted in triplicates. Values are presented as Means ± SEM. Values of RBC, WBC and Hb of fish blood were compared statistically with control by using student's t-test (2- tailed) with the help of SPSS 17. The level of significance was established at $P < 0.01$.

Table : 2 Water quality parameters of Harduaganj Reservoir water.

Water quality parameters	Values
Temperature	27.6±0..36°C
pH	6.9±0.29
Dissolved oxygen (D.O)	6.9±0.0 mgL^{-1}
Total solids (TS)	652±0.70 mgL^{-1}
Total Dissolved solids (TDS)	407±0.06 mgL^{-1}
Total Suspended solids (TSS)	245±0.50 mgL^{-1}
Cu	0.86± 0.06 mgL^{-1}
Ni	0.12± 0.01mgL^{-1}
Fe	8.71± 1.66 mgL^{-1}
Co	0.11± 0.01 mgL^{-1}
Mn	0.21± 0.05 mgL^{-1}
Cr	0.10± 0.01 mgL^{-1}
Zn	0.30± 0.02 mgL^{-1}

Values are Mean ± SEM, (n= 3).

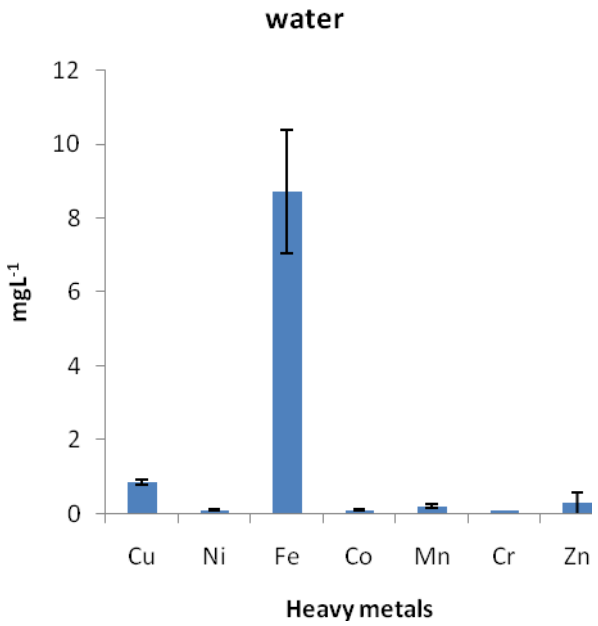

Figure : 2 Heavy metal concentration in water of Harduaganj Reservoir.

Table : 3 Summary of water quality guidelines and standards by International Organization or country.

Parameter↓	WHO (guidelines) mgL^{-1}	E U (standards) mgL^{-1}	Canada (guidelines) mgL^{-1}	Australia (guidelines) mgL^{-1}	N Z (guidelines) mgL^{-1}	Japan (standards) mgL^{-1}	USA (standards) mgL^{-1}	Present Study mgL^{-1}
Copper	2	2	2	1	2	1	1.3	0.86
Nickel	0.02	0.02	0.02	0.02	0.02			0.12*
Iron		0.2	0.2	0.3	0.01	0.3	0.3	8.71*
Cobalt								0.11
Manga-nese	0.5	0.05	0.05	0.5	0.5	0.05	0.05	0.21
Chrom-ium	0.05	0.05	0.05		0.05	0.05	0.1	0.1
Zinc	3			3		1	5	0.3

Adapted for Water Quality for Ecosystem and Human Health, 2006 (prepared and published by the United Nations Environment Programme.
Global Environment Monitoring System (GEMS)/ Water Programme).
Blank cells indicate that no, citable information was available.
*Indicates these values are far exceeding the recommended limit.

Table : 4 Total count of RBC's, WBC's, and Haemoglobin in the control and exposed *Mastacembelus armatus.*

Variable	Control (Mean±SEM)	Exposed (Mean±SEM)
No. of RBC (10^6 mm^{-3})	4.31±0.34*	2.16±0.08*
No. of WBC (10^3 mm^{-3})	2.55±0.16*	3.84±0.18*
Haemoglobin (g/dL)	9.70±0.23	9.48±0.52

Values are given as Mean ± SEM, (n = 5), *Significantly different at ($P < 0.01$

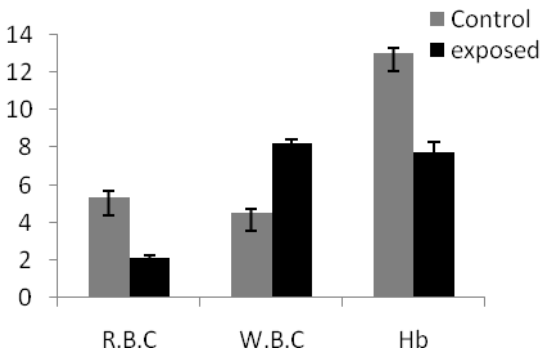

Figure : 3 Graphical representation of total Count of RBCs, WBCs and Hb in the Control and exposed *Mastacembelus armatus.*

III. Results and Discussion

Table 2 and figure 2 shows the mean values of physicochemical parameters and concentration of heavy metals (mg L^{-1}) in water. The temperature, pH, dissolved oxygen and total, suspended and dissolved solids are upto the ideal water quality guidelines (Vijay kumar, 1999). Therefore these are no more responsible for stress which the *Mastacembelus armatus* was undergoing. The heavy metal content in reservoir water were in the order of Fe (8.71) > Cu (0.86) > Zn (0.30) > Mn (0.21) > Ni (0.12) > Co (0.11) > Cr (0.10). The heavy metal content in reservoir water was compared with the quality guidelines and standards of United Nations Environment Programme Global Environment Monitoring System (UNEPGEMS, 2006) (Table 3). The Fe and Ni were found to be exceeding than the recommended limits. The results of hematological studies of *Mastacembelus armatus* are summarized in Table 4 and Figure 3.

Water quality parameters are one of the major factors responsible for individual variation in fish hematology. Since hematological parameters are necessary for clinical diagnosis of a disease and pathological conditions in human, these criteria should receive enough attention in assessing the health of the fish with regard to aquatic pollution and has been accepted by many workers such as McCarthy et al. (1973) and Christensen et al. (1978). While evaluating the total effect of heavy metals on the hematological indices of *Mastacembelus armatus*, a synergetic effect of these metals was found on the erythrocyte count, concentration of hemoglobin, and the present leucocytes. Blood forms a unique compartment between external and internal environments and any agent including toxic substances that causes stress and can alter blood composition either directly or indirectly by altering osmotic and ion regulation.

In the present study, the erythrocyte count of healthy controls showed a mean value of 4.31 x 10^6 mm^{-3} and Hb 9.70 gdL^{-1}. The fish *Mastacembelus armatus* procured from Harduaganj Reservoir showed mean values of RBC 2.16 x 10^6 mm^{-3} and Hb 9.48 gdL^{-1}. The values mentioned above showed a significant ($P < 0.01$) decrease when compared to the control. The reported value of Hb is insignificant. This study on hematological changes in fish serves as an effective tool in the diagnosis of the extent of environmental pollution and also the abiotic fish diseases. Hypoxia, anemia, and hyperthermia are related stresses causing an osmotic imbalance and decreased capacity of the RBC to carry sufficient oxygen unless otherwise compensated by erythropoiesis or suitable physiological adjustments. Lowering of TEC count coupled with low Hb content here may be due to destructive action of pollutants on erythrocytes and as a result of which the viability of the cells may be affected as was also reported by Karuppasamy (2000). This supports the findings of present study that is lowering in RBC count and Hb content. Multiple form of hemoglobin allows fish to adjust more efficiently to physiological stress such as varying water temperature and oxygen concentration (Hochachka and Somero 1973). Hemolysis occurs in response to toxicity that leads to alteration in the selective permeability of the membrane (Das et al. 1987). All these reports are in agreement with the present study of reduction in TEC count and Hb content of fish from polluted lakes due to the inhibition of aerobic glycolysis curtailing synthesis of iron and hemoglobin via the lowered energy status in fish (Joshi et al. 2002). It has been suggested that heavy metal exposure decreases the TEC count, Hb content due to impaired intestinal absorption of iron.

The results of the total count of white blood cells revealed that the blood of the control fish showed a mean value of 2.55 x 10^3 mm^3. Exposed Mastacembelus armatus showed the mean value of WBC as 3.84 x 10^3 mm^3. The values mentioned above showed a significant increase when compared to the control ($P < 0.01$).

Increase in TLC in the present study was a result of direct stimulation for its defense from diseases due to the presence of polluted substances. Progressive increased levels of TLC have been reported in *C. punctatus* exposed to lead (Hymavathi and Rao 2000) and *Clarias batrachus* exposed to mercuric chloride (Joshi et al. 2002). Leukocytosis is directly proportional to severity of stress condition in maturing fish and is a result of direct stimulation of immunological defense due to the presence of pollutants (heavy metals) in this reservoir. The observations made in present study for WBC are also in good agreement with those of Karuppasamy et al. (2005) and Hardikar and Gokhale (2000).

Hence, the present investigation results confirm that stress due to various heavy metals present in the reservoir does create hematological disturbances, erythrocyte destruction (hemolysis), and leukocytosis in fish population, affecting the immune system and making the fish vulnerable to diseases.

IV. Acknowledgements

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References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

INDEX

A

Absorption · 90, 94
Acquaint · 5
Adhesive · 10
Aminian · 66, 67
Anastomosis ls · 55
Anastomotic · 55, 58
Anthraquinones · 73, 75
Antigen · 8, 10, 11, 12, 14, 16, 17
Antigenaemia · 8, 11, 12, 13, 14, 16, 17
Asymmetry · 66
Autonomic · 6

B

Bancroftian · 8, 10, 12, 14, 16, 17, 18
Benzene · 71, 74, 75
Biomérieux · 22
Bourgeois · 35, 38
Bourgoigni · 86

C

Cadmium · 89, 95, 96
Carbamazepine · 19, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 40, 51
Centrifuge · 23, 24
Chukwuonye, · 83, 86
Cimolin · 60, 65, 67, 68
Cirrhosis · 71, 73, 75, 77, 79, 81
Cynthia · 12, 13

D

Diazepam · 23, 24, 26, 52, 53
Dysfunction · 86

E

Ecosystem · 88, 89, 96
Endemicity · 16, 17
Enrollment · 6
Epilepsia · 35, 36, 37, 38
Erythrocyte · 93, 95
Estimated · 13, 83
Estimation · 75
Ethnopharmacol · 80, 81
Enzymatically · 79, 80
Enzyme · 6, 33, 34, 46, 60, 62, 64, 69, 70, 74, 75, 77, 79
Erythrosine · 42, 44, 47

F

Febiger · 96
Fibrosis · 71, 79
Frequency · 39

G

Glycolysis · 94
Guangxi · 1, 2, 3, 7
Gunawardena · 17

H

Haematological · 88, 89, 90, 96
Haematological · 96
Hardikar · 95, 96
Harduaganj · 88, 89, 90, 91, 92, 93, 94, 95
Hemiplegia · 60, 65, 67, 68, 69
Hepatic · 22, 33, 37, 71, 75, 77, 79
Hepatitic · 84
Hepatocellular · 32, 33
Hepatoprotective · 73, 75, 76, 80
Hepatotoxic · 76, 80

I

Illiteracy · 31
Immunochromatographic · 8, 16
Immunological · 95
Intraoperative · 55, 57

K

Karuppasamy · 89, 94, 95, 96
Keruly · 87
Kimura · 17
Kinematics · 60, 61, 62, 63, 64, 65, 66, 67
Knotts · 11, 12, 13, 16
Krawczyket · 85
Kurroa · 73, 81

L

Lammie · 16
Leucocytes · 55, 93
Lujan · 57
Lymphatic · 16, 17
Lymphoma · 96

M

Magnitude · 72
Majority · 2, 5, 6, 12
Mastacembelus · 88, 90, 91, 92, 93, 94, 95
Mehjbeen · 88, 95
Microfilaraemic · 8, 11
Microfilaria · 8, 11, 12, 14
Miranda · 80
Mukorossi · 71, 73, 74, 75, 76, 77, 79, 81
Mulcahy · 88, 96

N

Natiopns · 96
Necrosis · 75, 77, 79, 96
Neighbouring · 13, 82
Neurologic · 29

O

Okaforet · 85

P

Palsy · 60, 61, 65, 66, 67, 68, 69
Paulo · 12, 37
Permeability · 94
Permitted · 1
Pharmacokinetics. · 36
Picrorhiza · 73, 81
Plasma · 19, 30, 34, 83
Protenuria · 86

Q

Questionnaire · 1, 4, 55
Questionnaire · 1, 6

R

Randomized · 31, 37
Recommended · 92, 93
Relevant · 5, 8
Retrospective · 19, 21, 24, 25, 26, 27, 28, 29, 30, 32, 33, 34, 35, 39, 40, 42, 44, 46, 47, 48, 82, 86

S

Sapindus · 71, 73, 75, 77, 79, 81
Schuppan · 79
Seizure · 19, 24, 29, 30, 31, 35, 38, 39
Serum · 19, 22, 23, 25, 26, 27, 38, 40, 42, 44, 45, 46, 47, 77
Shlipak · 86
Significantimmuno · 85
Simulation · 1, 3, 5, 6
Stpierre · 80

T

Testini · 55, 58
Therapeutic · 19, 21, 23, 25, 27, 29, 30, 31, 33, 35, 36, 37, 39, 40, 42, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54
Topiramate · 19, 21, 24, 27, 28, 29, 30, 32, 34, 36
Toxicity · 31, 74, 81, 88, 94, 95

U

UNEPGEMS · 88, 93, 96
Urbanization · 12

V

Valproic · 19, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 34, 35, 36, 37, 40, 42, 52, 53
Vosyliene · 88, 96
Vulnerable · 35, 95

W

Wavelength · 23
Weerasooriya · 17
Wintrobe · 91, 96
Wuchereria · 8

X

Xinjiang · 7

Z

Zamora · 80
Zoologica · 96

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