response indicating a normal recognition arm of the immune response. It is possible that the cells involved in retaining the memory of the sensitisation are overwhelmed by the antigen load, resulting in a negative response to DNCB in spite of normal afferent and effector arms.

Treatment with busulphan suppresses the efferent arm of the immune response as the responses to recall antigens and PHA are impaired in this stage of the disease.

During remission the responses to all 3 indicators of *in vivo* CMI show that the patients are generally immunocompetent. Several *in vitro* studies in patients of CML have shown that during the phase of remission the immune status is normal. However during the phase of relapse and blast crisis the CMI responses are diminished.

Thus, it seems that during the initial active phase of the disease, the large antigen load makes adequate number of lymphocytes unavailable for retaining the memory of antigenic sensitization; otherwise, the CMI is intact. Therapy with busulphan suppresses the CMI which, however, returns to normal on stopping the drug during remission. Our findings further confirm the impairment in CMI responses in association with advancing disease (relapse and blast crisis). This suggests that rapid reduction in antigen load in the initial phases, stopping the drug during remission and non-specific immunostimulation may be beneficial in prolonging remission and delaying the onset of blast crisis.

REFERENCES

1. Galton DAG. Chemotherapy of chronic myeloid leukaemia. Seminars in Hematology 1969; 6:323-43.
2. Sokal JE, August CW, Grant JT. Immunotherapy in chronic myeloid leukaemia. NY ST J Med 1973; 73: 1180-5.
3. Joshi CG, Gollerkeri MP, Gangal SG. Evaluation of general immune responsiveness in patients of cancer. Indian Med Res 1975; 63: 315-22.
4. Gangal SG, Gathoskar BP, Joshi CS, Advani SH. Demonstration of cellular immunity in chronic myeloid leukaemia using leucocyte migration assay. Br. J Cancer 1976; 33: 267-73.
5. Gangal SG, Joshi CS, Gathoskar BP, Gollerkeri MP, Advani SH. Evaluation of leukaemia specific immunity in chronic myeloid leukaemia. Acta Hematol 1977; 63: 5-11.
6. Gangal SG, Damle NK, Khare AC, Advani SH. Cellular sensitization in chronic myeloid leukaemia patients to leukaemia blast antigens. Br J Cancer 1979; 40: 391-7.
7. Gathoskar BP, D' Silva H, Gharpure H, Advani SH. Leucocyte migration studies in chronic myeloid leukaemia. Eur J Cancer 1976; 12: 815-21.
8. Hester JP, Keneth B, McGredie, Ereinreich EJ. Immunological evaluation in patients with chronic myelogenous leukaemia. Proceedings of AACR and ASCC 1974; 180: 787.
9. Dibella MJ, Brown GC. Immunological dysfunction in myeloproliferative disorders. Cancer 1978; 42: 149-58.
10. Catalona WJ, Tayler PT, Rapson AS, Christias PB. A method for DNCB contact to sensitization — a clinicopathological study. Eng J Med 1972; 286: 399-402.

NITROBLUE TETRAZOLIUM TEST IN THE DIAGNOSIS OF AMOEBIC LIVER ABSCESS

BM Gandhi*, M Irshad**, SK Acharya***, G Choudhuri† BN Tandon‡

SUMMARY

Nitroblue tetrazolium (NBT) test was evaluated in the diagnosis of amoebic liver abscess (ALA). In normal healthy controls and non-suppurative hepatic amoebiasis (NSHA), the percentage of NBT reduced neutrophils was less than 20%, while in ALA, all the patients had an NBT score of over 20 per cent (mean 70 ± 20%). This test is recommended for easy diagnosis of amoebic liver abscess, with an NBT score of more than 20% being diagnostic.

INTRODUCTION

In tropical countries, the liver is frequently infected with viruses, bacteria, parasites and toxins, resulting in tender hepatomegaly. The prompt diagnosis of amoebic liver abscess (ALA) enables the institution of specific therapy with encouraging response. In most peripheral health centres, expensive and complicated diagnostic tests like radioisotope scanning, ultrasonography and amoebic serology are not available. Blind diagnostic aspiration of the liver with its attendant risks is also neither possible nor desirable.

Nitroblue tetrazolium (NBT) test is simple and inexpensive and can be easily performed with a microscope. The present report is the evaluation of the role of this test in resolving the clinical dilemma of tender hepatomegaly due to liver abscess and non-suppurative hepatic amoebiasis (NSHA).

*Senior Biochemist, Department of Gastroenterology **Biochemist, Human Nutrition Unit ***Lecturer, Department of Gastroenterology †Lecturer, Indira Gandhi Institute of Medical Sciences, Patna ‡Prof & Head, Department of Gastroenterology and Human Nutrition, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110 029.

Received: 24.1.1986
Accepted : 11.7.1986

MATERIAL AND METHODS

Blood samples were obtained from 34 patients with ALA (32 males; aged 17-62 years), 11 patients with NSHA (8 males; aged 22-58 years) and 41 healthy members of the medical and paramedical staff (31 males; aged 17-57 years) of the Department of gastroenterology, All India Institute of Medical Sciences.

The diagnosis of ALA was confirmed as per WHO recommendations[1]. All the patients presented with tender hepatomegaly and showed space occupying lesion(s) on liver scan or ultrasound examination. "Anchovy sauce" pus was aspirated in all the patients from the liver abscess and this was sterile on bacterial culture. Amoebic serology was positive, both with IHA and ELISA. Further, all patients responded to antiamoebic therapy with metronidazole (2.4 g/day for 5 days). The diagnosis of NSHA (11 patients) was based on WHO recommendations[1], which included; (a) presence of tender hepatomegaly without space occupying lesion on ultrasonography/isotope scanning; (b) clinical response to antiamoebic therapy assessed by regression of hepatomegaly and disappearance of tenderness. None of the patients of NSHA had any alteration in liver function tests. Amoebic serology (IHA/ELISA) revealed very low titre antibody in 1 of 11 patients of NSHA, and was negative in the other 10 patients. None amongst the normal healthy controls with negative amoebic serology had any evidence of present or near-past bacterial or parasitic infection.

Histochemical NBT test: The test was performed using heparinized whole blood[2]. Briefly, 0.05 ml of blood was mixed with 0.05 ml 0.05 per cent NBT in phosphate buffer saline (PBS), pH 7.2 and incubated at 37°C for 15 min. Following incubation, 2 May-Grunwald-Giemsa stained slide smears were made and 200 neutrophils in each slide were counted by light microscopy and classified as positive when it contained bluish black deposits of formazan. The percentage of NBT-positive neutrophils was recorded as the NBT score.

RESULTS

In the 41 normal healthy controls, the percentage of NBT-positive neutrophils ranged from 2-20 (mean ± SD 9 ± 5). Based on mean ± 2 SD value, scores above 20 per cent were regarded as positive NBT tests. All the 34 patients with amoebic liver abscess had positive NBT scores (mean ± SD 70 ± 20; range 24-99), whereas none of the 11 patients with NSHA had positive NBT test (10 ± 4; 4-18).

DISCUSSION

Polymorphonuclear leucocytes (PMN) are present in amoebic liver abscesses[3]. Following membrane interaction with *E histolytica* these leucocytes undergo a burst of respiratory activity; as a result of the activation of a non-mitochondrial respiratory chain (NADPH oxidase)[4], oxygen is reduced and superoxide anions and H_2O_2 are produced. The stimulated leucocytes reduce nitroblue tetrazolium.

Hepatomegaly occurs frequently enough in the tropics to be described under the term 'tropical liver'[5]. ALA can be dangerous but easily responds to specific treatment. Hence the differentiation of ALA from NSHA and other benign causes of tropical hepatomegaly is of paramount importance.

Our data demonstrates that the peripheral blood PMN from patients with ALA show markedly elevated NBT-reduction activity compared to those from healthy controls or from subjects with NSHA. Besides being important in the understanding of the pathogenesis of invasive amoebiasis, this finding is helpful in the diagnosis of ALA. The high NBT scores in patients with ALA indicates stimulation of neutrophils to produce toxic substances[6,7] unlike patients with NSHA. In acute viral hepatitis, which is yet another cause of tender hepatomegaly, NBT score has been reported to be very low [2,8]

NBT test is positive with a number of pyogenic abscesses[2]. However, high positivity of NBT test in a sterile pyogenic abscess is of significant diagnostic importance.

NSHA is an ill defined clinical entity with tender hepatomegaly with normal liver function tests and no space occupying lesions on ultrasound and liver scan, and regresses with antiamoebic treatment[1]. The lack of any diagnostic test makes NSHA a vague entity and hence is not accepted by all clinicians. However, NBT test distinctly differentiates ALA from NSHA.

NBT test is easy to perform, rapid, inexpensive and does not need any sophisticated instrument. It is recommended for the diagnosis of amoebic liver abscess particularly at centres where radio-isotopic, sonographic or pathologic diagnostic procedures are not easily available.

ACKNOWLEDGEMENTS

The authors are thankful to the Norwegian Agency for International Development (NORAD), Norway, for providing necessary financial assistance to carry out this study. Prof Claus Ola Solberg, University of Bergen, Norway has assisted in this project as Co-investigator for the NORAD Collaborative Study on Amoebiasis at the All-India Institute of Medical Sciences, New Delhi.

REFERENCES

1. WHO Tech Rep Ser 1969; 421: 1-2. Amoebiasis.
2. Hellum KB. Nitroblue tetrazolium test in bacterial and viral infections. Scand J Infect Dis 1977; 9: 269-76.
3. Tandon BN, Tandon HD, Ravi VV, Gandhi PC. Diffuse liver injury in amoebic abscess of the liver: an electron microscopic study. Exp Mol Pathol 1975; 23: 155-63.
4. Rossi F, Patriarca P, Romeo D, Zabuccki G. In: Reichard SM, Escobar MR, Friedman H, Eds. The reticuloendothelial system in health and disease. New York: Plenum Press. 1976: 205-33.
5. Cook GC. Tropical liver. In: Tropical Gastroenterology, Ist ed. Oxford, New York: Oxford University Press. 1980. 59-66.

6. Borregaard N. Bactericidal mechanism of human neutrophil. Scand J Haematol 1984; 32: 225-30.
7. Holmes B, Page AR, Good AR. Studies on the metabolic activity of leucocyte from patients with a genetic abnormality of phagocyte function. Clin Invest 1967; 46: 1422-32.
8. Gandhi BM, Acharya SK, Irshad M, Gupta H, Chawla TC, Tandon BN. Elevated NBT reduction activity in polymorphs during amoebic liver abscess. Trans Soc Trop Med Hyg (in Press).

ECHOCARDIOGRAPHIC EVALUATION OF LEFT VENTRICULAR DIMENSIONS AND FUNCTION BEFORE AND AFTER HAEMODIALYSIS

MF El-Hassadi*, K Nimkhedkar**, M Behera**, ME Mousa*, A Bendardaft

SUMMARY

Left ventricular (LV) functions of twenty patients on regular haemodialysis were studied by M-mode echocardiography immediately before and after dialysis. Body weight, blood pressure, heart rate, cardiac output, stroke volume, systolic and diastolic dimensions and volume of LV, ejection fraction, shortening fraction and velocity of circumferential fibre shortening (VCF) were measured.

Haemodialysis resulted in significant decrease in blood pressure, cardiac output and stroke volume, with increase in heart rate. LV function as assessed by VCF showed improvement but it was more so in the group of patients who had depressed LV function before dialysis. M-mode echocardiography provides a simple means for evaluating LV function in patients on regular haemodialysis.

INTRODUCTION

Cardiovascular complications are very common in uraemic patients[1,2]. These are attributed to metabolic disturbances, anaemia, and pressure and volume overload[3]. Five to 10% of patients on regular haemodialysis die each year because of these complications[1]. Abnormalities of left ventricular (LV) size and function in uraemic patients as assessed by non-invasive techniques such as systolic time intervals and echocardiography have been reported in several studies[4-8]. The effect of dialysis on LV function has been variable in some of the studies[9-12]. M-mode echocardiography is a simple means for evaluating LV function in patients on regular haemodialysis. The present study was undertaken to study the effect of haemodialysis on LV function as determined by M-mode echocardiography.

MATERIAL AND METHODS

Twenty patients (10 men, 10 women; aged 15 to 62 years, mean 38 ± 11) on regular haemodialysis were studied by M-mode echocardiography within half an hour before and after haemodialysis. All patients were dialysed for four to six hours each on Gambro AK 10 dialysis machine using acetate containing dialysate. Echocardiograms were obtained using an Ekoline 21 strip Chart Recorder with the patient in the semi-recumbent and the left lateral position. The transducer was placed parasternally in the 3rd, 4th and 5th left intercostal spaces. Only records showing good continuous septal and posterior endocardial echoes were accepted for measurement. Left ventricular dimensions were measured according to standard convention. LV functions were assessed by fractional shortening (FS) and velocity of circumferential shortening fibre (VCF).

Blood pressure (mean arterial pressure = MAP), body weight, heart rate, cardiac output, stroke volume, systolic (Ds) and diastolic (Dd) diameters and volumes of the left ventricle ejection fraction, FS and VCF were measured. MAP was calculated as systolic ± 2 = diastolic pressure) ÷ 3 mmHg. Heart rate was obtained by an electrocardiogram recorded simultaneously with the echocardiograms. Cardiac output was calculated by multiplying heart rate with the stroke volume while stroke volume was calculated by substracting the end systolic volume from the end diastolic volume. The ejection fraction was calculated by dividing as (Dd — Ds) ÷ Dd × 100%. VCF was calculated as FS — LVET (LV ejection time). A value of VCF > 1.0 circ/sec was considered as abnormal. All results were expressed as mean ± SD. Student's *t* test was used for statistical evaluation.

RESULTS

The results are summarised in the Table. Haemodialysis resulted in significant decrease in MAP, diastolic dimensions of LV, cardiac output and stroke volume, and increase in heart rate and VCF.

There was no significant alteration in the body weight, systolic dimension of LV, ejection fraction and FS.

*Assistant Professor **Lecturer †Consultant Nephrologist Department of Medicine, 7th April Hospital, Arab Medical University, Benghazi, Libya.

Received: 10.3.1986
Accepted: 16.6.1986