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THE INTRACELLULAR OXIDATIVE METABOLIC ACTIVITY OF GRANULOCYTES IN INFLAMMATORY EXUDATES OF CHILDREN WITH PCM STUDIED BY THE NBT-REBUCK SKIN WINDOW TECHNIQUE.

By

Prapon Thubdimphun, B.Sc. (Med. Tech.) *

Pramote Teowsiri, B.Sc. (Med. Tech.) **

Panja Kulapongs, M. D., Dip. Am. Bd. of Ped. *

ABSTRACT:

The intracellular oxidative metabolic activity of polymorphonuclear leukocytes in the inflammatory exudates of 6 children with severe protein calorie malnutrition (PCM) and 6 well-nourished healthy control children were evaluated by the new technique, NBT-Rebuck skin window. The results obtained indicated that the oxidative activity of leukocytes especially the polymorphonuclear neutrophils in circulation and in the inflammatory exudates (including monocytes and fixed tissue macrophage) of children with severe PCM is not impaired when compared with well-nourished healthy children of the same age.

* Hematology Lab., Dept. of Pediatrics, Faculty of Medicine.

** Dept. of Clinical Microscopy, Faculty of Associated Medical Sciences Project, CHIANG MAI UNIVERSITY.

INTRODUCTION:

It is well accepted that the malnourished child is more susceptible to infection and that infection is a major factor in the high morbidity and mortality associated with protein calorie malnutrition or PCM [1]. Clinical observations suggest that the malnourished individual's body defense system may respond to infection in a way which is different from that of the well nourished one. An organism which may be relatively harmless in the well-nourished child may give rise to a severe or were fatal in the malnourished child. Our recent experience as well as the others indicated clearly that children with PCM tend to develop gram negative septicemia [2,3]. In addition, when localized infection spreads in these patients it done so with the development of gangrene but not with suppuration [4]. Since phagocytes especially polymorphonuclear neutrophils play a major role in protection and limitation of infection through their phagocytosis and intracellular bactericidal activities against the invading organisms both in the circulation and tissues, the observations mentioned above necessitated the systematic studies of the functional integrity of phagocytes in malnourished individuals. Selvaraj et al (5) and Seth et al (6) found that in children with PCM granulocyte function is compromised and that with nutritional repair there is an improvement in both phagocytosis and killing function. Vithayasai et al (7) found that some but not all of the children with PCM has the defect as those observed earlier. The in vivo study, using standard Rebuck skin window technique, indicated that the mobilization of polymorphonuclear leukocytes into the inflammatory exudates in children with PCM is

not impaired (8). Therefore it is important to study the functional integrity of the migrating phagocytes in the inflammatory exudates in these children.

The new techniques, called NBT-Rebuck skin window technique, was designed specifically for the study of the phagocytosis and killing function of leukocytes in the inflammatory exudate. The oxidative activity of leukocytes which is essential for their intracellular bactericidal activity is evaluated by the capacity of leukocyte to reduce the colorless NBT particles to dark-colored formazan precipitates.

MATERIALS AND METHOD:

Six children, 1-5 year of age, with severe degree of protein calorie malnutrition (9, 10) was admitted to the Pediatric Ward for study and treatment. Six control children of the same age were the well-nourished patient admitted for elective surgery and children recovered from malnutrition.

NBT-Rebuck skin window technique. The skin abrasions were made on the volar surface with a sterile scalpel according to the standard technique (11, 12). Care was taken to avoid bleeding. Sterile glass coverslips were placed over each of the skin abrasion lesions. After 4 hour the coverslips were removed. One drop each of either 1% NBT (Sigma Chemical Co., St. Louis, Mo., USA.) in phosphate buffered saline with or without 5% latex were applied on the lesions. New coverslips were then placed over the lesions for an additional 60 minutes. The coverslip preparations were then stained with Wright's stain and differential count was done on each samples. The granulocytes with large chunks of dark-stained intracellular

formazan precipitates were counted as NBT-positive cells. (13).

The in vitro NBT test using heparinized capillary blood (13) was carried out in each patient at the same time of the in vivo test.

The summary of the results is shown in Table I below. There is no statistical significant difference between the results obtained from children with PCM and healthy control children regarding the percent of NBT positive granulocytes (especially polymorphonuclear neutrophils) in the inflammatory exudates with or without stimulation with latex particles. This, in turn, indicated that the intracellular oxidative metabolic activity of granulocytes (particularly of polymorphonuclear neutrophils) in the inflammatory exudates of children with PCM is not impaired. In addition, the increased in vitro NBT positivity of the granulocytes in peripheral blood of children with PCM compared to normal control children further substantiate the in vivo findings that the overall oxidative activity of the granulocytes is remained intact or at least on apparent impairment in these patients.

TABLE 1:
RESULTS OF NBT-REBUCK SKIN WINDOW AND IN VITRO NBT TEST *

TESTS	PCM	CONTROL
NBT-Rebuck skin window		
-NBT alone	2.92 ± 2.49	3.16 ± 1.40
-NBT and latex	7.33 ± 2.89	5.10 ± 3.27
In vitro NBT test	33.40 ± 25.00	4.75 ± 4.49

* Expressed as mean ± 1 S.D. of NBT-positive cells.

COMMENTS :

The results of elevated NBT positivity of circulating granulocytes in our PCM children confirms those observed earlier (14, 15) which indicated that the oxidative activity of leukocytes in these patients remain intact and the increased NBT positive cells can be explained on the basis of increased "resting" activity and frequent intercurrent and concurrent infections observed in these patients on admission (16). The results obtained from the in vivo study indicated that the granulocytes and monocytes which migrated into the inflammatory exudates were functionally normal. The lower NBT positivity values obtained from the inflammatory exudate are not statistically significant different from those obtained from circulating granulocytes in normal healthy children. This is apparently not due to the cell death since almost all granulocytes and majority of monocytes/macrophages in the inflammatory exudates ingested latex particles heavily.

Our results are supported by observation of Kumate et al (17) which indicated that the phagocytosis and intracellular killing function of leukocytes was not impaired in children with PCM. Smith et al (18) made similar observation in malnourished pigs. Various possible explanations for the difference of the results of the in vitro studies obtained by investigators are the different poputation studied, different criteria, ratio of bacteria to leukocytes used, presence or absence of amino acids supplementation, complement inactivation etc. (19). These factors were largely eliminated in our in vivo system. In addition to granulocytes, it is noticed that the degrees of NBT positivity of monocytes/macrophages in the inflammatory

exudates of normal controls and children with PCM were also comparable.

SUMMARY:

The intracellular oxidative activity of polymorphonuclear neutrophils and other granulocytes in the inflammatory exudates with and without stimulation (with latex particles) were studied in 6 children with severe PCM.. The results obtained indicated that granulocytes in the inflammatory exudates as well as in the circulation of children with PCM remain functionally unimpaired.

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ย่อเรื่อง

คณะ ผู้ ทด สอบ ได้ ศึก ษา เกี่ยว กับ intracellular oxidative metabolic activity ของเม็ดเลือดขาวชนิด polymorphonuclear ใน inflammatory exudates จาก เด็ก ที่ ป่วย ด้วย Protein calorie malnutrition (PCM) อย่างรุนแรงจำนวน 6 คน เปรียบเทียบกับเด็กที่ได้รับอาหารอย่างดี และสุขภาพสมบูรณ์ จำนวน 6 คน เป็น normal control โดยใช้วิธี NBT-Rebuck skin window

ผลที่ได้จากการทดสอบ ชี้ให้เห็นว่า oxidative activity ของเม็ดเลือดขาวโดยเฉพาะ พวก polymorphonuclear neutrophils ใน circulation และใน inflammatory exudates (ในที่นี้รวมทั้ง monocytes และ fixed tissue macrophage ด้วย) ของเด็กที่ป่วยด้วย PCM ไม่ได้ลดลงแต่ประการใด เมื่อเทียบกับของเด็กที่ทำเป็น normal control ในขนาดอายุเท่า ๆ กัน