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COMPARATIVE ERYTHROPOIETIN LEVELS IN URINE OF THE ANEMIC AND MALNOURISHED PATIENTS

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การตรวจวิเคราะห์หาระดับของฮอร์โมนอีริทโธรปอยอีตินซึ่งถูกขับถ่ายออกมาทางปัสสาวะของคนไข้โรคโลหิตจางจำนวน 33 ราย เปรียบเทียบกับของคนไข้โรคขาดสารอาหารจำนวน 8 ราย ที่เพิ่งเข้ามารักษาในโรงพยาบาลนครเชียงใหม่ มหาวิทยาลัยเชียงใหม่ และของคนปกติ จำนวน 18 ราย กระทำโดยวิธี **radiobiological assay** ในหนูขาวที่อดอาหาร จากการศึกษาพบว่าปริมาณของฮอร์โมนอีริทโธรปอยอีตินในปัสสาวะของคนไข้โรคโลหิตจางสูงกว่าของคนไข้โรคขาดสารอาหารประมาณร้อยละ 62 กล่าวคือในปัสสาวะของคนไข้โรคโลหิตจางมีค่าเฉลี่ยของฮอร์โมนชนิดนี้เท่ากับ **297.11 ± 46.95 Co Units/ 24 hr. Urine** เมื่อศึกษาระดับของฮอร์โมนชนิดนี้เปรียบเทียบกันในปัสสาวะของบรรดาคนไข้โรคโลหิตจางนานาชนิดแล้ว ปรากฏว่าในคนไข้โรคโลหิตจางชนิด **aplastic** มีปริมาณของฮอร์โมนอีริทโธรปอยอีตินสูงที่สุด คือมีค่าเฉลี่ยเท่ากับ **725.94±88.31 Co Units/24 hr. Urine.**

ABSTRACT

The 24-hour urinary excretion of erythropoietin was biologically assayed in the 33 anemic patients and the 8 patients with protein-energy malnutrition from Chiang Mai Hospital, Chiang Mai University, Thailand. The erythropoietic activity in the patients' urine was measured by radiobiological technique in starved rats, comparing with that of the 18 healthy subjects living in the same area. This investigation indicates that the urinary erythropoietin level in patients with all types of anemias is approximately 62 per cent higher than in the malnourished patients. The average urinary erythropoietic activity of anemic

patients is 297.11±46.95 Co Units per 24-hour Urine, while that of the patients with protein-energy malnutrition is only 187.66±16.15 Co Units per 24-hour Urine. In the case of anemic patients, the urinary erythropoietin level in aplastic anemia is the highest among the group, in which the average activity is equal to 725.94±88.31 Co Units per 24-hour Urine.

INTRODUCTION

Erythropoietin or erythropiesis stimulating factor (ESF) has been known as a glycoprotein-hormone (MW 50,000-60,000) produced by kidney secreted into circulation and primarily regulated the erythropoiesis in higher organisms (1). The hormone appears in increased quantities in the body fluids of animals subjected to various types of hypoxia, in oxygen deficiency states in man, as well as in the urine and plasma of normal subjects (2). An target site of action of the ESF is the hematopoietic stem cell. The erythropoietin committed cell is stimulated by the hormone to differentiate into the earliest recognizable members of the erythroid cell series namely, the proerythroblasts(3). Moreover, ESF have been demonstrated to enhance effects on erythroblast proliferation, reticulocyte release, nucleic acid and hemoglobin synthesis(5,6).

The excess ESF in serum is relatively excreted into urine. Urine usually contains approximately one third to two thirds of the ESF presenting in an equal volume of corresponding plasma. The amount of the urinary ESF therefore indicates the serum level of the ESF and its production. Since highly purified erythropoietin is not commercially available with a low price in our country. Urine from anemic patients might be a good source for the preparation of the ESF. This report shows the abnormally high amount of urinary ESF in different types of Thai anemic patients.

Anemia is commonly found in protein-energy malnutrition (PEM) children. Its ethiology is still unknown. This is also a preliminary investigation of the urinary level of the ESF in the Thai PEM children.

MATERIALS AND METHODS

1. Animals :

Albino rats in both sexes weighing from 150-250 g were well fed and supplied by the animal-house of the Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand.

2. Chemicals :

Sodium chloride was obtained from City Chemical Corporation, New York, U.S.A. Cobalt chloride was taken from May and Baker, Ltd., London, England. Radioisotope iron (Fe-59) as ferric citrate and/or ferric chloride was directly purchased from the Radiochemical Center, Amersham, U.S.A.

3. Collection and preparation of urine samples for biological assay of erythropoietin :

The 24-hour urine samples were collected from anemic and protein-energy malnutrition patients in the wards of Chiang Mai Hospital, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand. A few drops of toluene were used as preservative. The urine samples were kept with frozen condition until use.

One hundred millilitres of each urine sample were divided for determination of the erythropoietic activity. Each sample was dialyzed at 4 ℃ for 48 hours against two litres of deionized-distilled water with at least 4 changes, lyophilized and used as a "testing material" for the radiobiological assay of erythropoietin.

4. Radiobiological Assay of the Erythropoietic Activity :

The measurement of erythropoietic activity in the "testing material" from each urine specimen was modified from the methods of Graham(7), and of Wognum (8). The assay required at least 4 rats per group of each sample and with two remaining group for normal saline solution (NSS) and cobalt chloride solution for negative and positive controls respectively. The procedure has to be completed within 5 days for each experiment as the following:

Day 1. The experimental rats were weighed out, approximately 150-250 g, and starved. Only deionized-distilled water was given throughout the assay.

Day 2. About 24 hours after complete starvation, two millilitres of "testing material" were intraperitoneally injection into the rats.

Day 3. About 24 hours after the first injection, two more millilitres of "testing material" were intraperitoneally injected in addition into the same rats.

Day 4. About 24 hours after the second injection, one microcurie of radioisotope iron (Fe-59) in NSS solution was injected into rats via tail vein, 0.5 ml. per rat.

Day 5. About 18 hours after the injection of radioisotope iron. The rats were placed under light ether anaesthesia to facilitate decapitation. The rats were decapitated and gradually bled. One millilitre of blood was collected with a few drops of Acid Citrate Dextrose (ACD) solution as anti-coagulant. The blood was washed out at least 3 times with an adequate volume of NSS solution. The packed red blood cells were occurred by centri-fugation at the speed of 3,500 rpm for 10 minutes.

The Fe-59 incorporation in the red cells was counted by a Packard Scintillation Gamma Counter, Model 3320. The unit of erythropoietic activity is expressed in term of Cobalt Units per 24-hour Urine.

RESULTS

The erythropoietic activity in 24-hour urine obtained from the 18 normal subjects with both sexes living in Chiang Mai province is shown in Table 1. Their hemoglobin concentrations were in normal ranges which are approximately 12 g% for female and above 13 g% for male. The erythropoietin level is in the range of 3.78 upto 15.63 Co Units per 24-hr. Urine. The average hormonal activity is 10.67±1.35 Co Units per 24-hr. Urine.

Table 2. represents the erythropoietin level in 24-hr. Urine specimens obtained from the 33 anemic patients. All of the patients were just admitted into the hospital and were diagnosed as anemias caused by aplasia, hemolysis, iron deficiency, malaria, hookworm and thalassemia. The hormonal activity varied in a wide range of about 73.27 upto 1,214.34 Co Units per 24-hr. Urine. From this finding, it has been also found that the urinary erythropoietin content in aplastic anemia is the highest among the group. However, the average ESF activity is 297.11±46.95 Co Units per 24-hr. Urine.

Table 1. Erythropoietic activity in 24-hr. Urine from normal subjects.

Number of Subjects	Sex	Age (years)	Hb (g %)	ESF Activity (Co Units/24-hr.Urine)
1	F	27	12.6	4.40
2	M	25	13.0	8.07
3	M	20	13.7	14.53
4	F	18	12.8	9.20
5	M	23	16.4	3.78
6	M	24	16.7	8.03
7	M	23	16.2	7.04
8	M	19	16.6	3.82
9	M	21	16.2	7.43
10	M	20	14.7	5.29
11	F	18	12.1	5.29
12	M	17	15.6	15.63
13	M	19	16.0	7.82
14	M	20	13.7	12.90
15	M	23	16.2	8.18
16	M	22	16.4	14.13
17	M	24	16.5	5.00
18	M	20	16.2	10.65
				$\bar{X}$ + SEM = 10.67 ± 1.35

Notice : *F = Female*
M = Male
One cobalt unit is equal to the activity by which 5 micromoles of $CoCl_2$ solution as a total dose injected into starved rats. From the experiments, the average per cent Fe-59 incorporation caused by 5 micromoles of $CoCl_2$ is 7.76.

Table 2. Erythropoietic activity in 24-hr. Urine from various types of anemic patients.

Number of Patients	Sex	Age (years)	Hb (g %)	ESF Activity (Co Units/24-hr. Urine)
1	M	40	3.2	764.74
2	F	19	6.0	553.50
3	M	35	6.0	632.72
4	M	16	5.8	772.20
5	M	18	5.5	1,214.34
6	M	35	6.2	588.60
7	M	26	6.5	555.42
8	M	36	3.4	442.86
9	M	18	5.7	340.03
10	M	24	5.3	378.56
11	M	38	5.8	105.22
12	M	35	5.7	145.49
13	F	43	5.0	166.35
14	F	32	6.9	73.27
15	F	29	6.1	93.20
16	F	24	6.8	114.24
17	F	25	7.3	139.25
18	F	30	6.1	104.59
19	F	40	5.4	165.79
20	F	26	6.4	100.79
21	M	34	7.4	159.10
22	M	38	6.3	109.74
23	M	41	6.1	100.71
24	M	20	7.7	115.19
25	M	22	8.0	99.09
26	M	28	6.7	116.24
27	M	32	6.8	107.60
28	M	36	7.3	128.89
29	M	38	5.8	121.88
30	M	42	6.5	373.66
31	M	35	6.0	377.41
32	M	46	7.5	434.25
33	F	25	5.5	109.57
				$\bar{X}$ + SEM = 297.11 ± 46.95

Notice : *Patient No. 1-7 : Aplastic Anemia*
Patient No. 8-10 : Hemolytic Anemia
Patient No. 11-29 : Iron Deficiency Anemia
Patient No. 30-31 : Malaria-Caused Anemia
Patient No. 32 : Hookworm-Caused Anemia
Patient No. 33 : Thalassemia

One cobalt unit is equal to the activity by which 5 micromoles of $CoCl_2$ solution as a total dose injected into starved rats. From the experiments, the average per cent Fe-59 incorporation caused by 5 micromoles of $CoCl_2$ is 7.76.

The erythropoietin level in the urine specimens from the 8 children with protein-energy malnutrition was also observed and the result occurred in **Table 3.** The hemoglobin concentration and plasma protein content are parameters to exhibit the malnourished condition of these patients. The average erythropoietic activity is 184.66±15.16 Co Units per 24-hr. Urine, or within the range of 108.04 upto 275-36 Co Units per 24-hr. Urine.

Table 3. Erythropoictic activity in 24-hr. Urine from the patients with protein - energy malnutrition.

Number of patients	Sex	Age (years)	Hb (g %)	ESF Activity (Co Units/24-hr. Urine)
1	M	4.2	8.6	198.79
2	M	8.6	9.8	136.48
3	M	9.3	8.5	189.46
4	F	5.6	7.6	215.32
5	M	6.2	9.8	242.69
6	M	7.5	10.0	108.04
7	F	8.1	6.8	275.36
8	M	10.5	9.5	111.12
				X + SEM = 184.66 + 15.16

Notice : *The patients' plasma protein level is in the range of 3.5-8.8 g%.*

Discussion

The trace amount of urinary erythropoiethin could be estimated in the healthy persons studied here. It seems that there is not much difference of the ESF activity in 24-hour urine samples between male and female normal subjects. The various types of anemic patients apparently indicate a very high excretion of urinary ESF, in some case which is upto 120-fold higher than the normal average. The anemic patients caused by hemolysis significantly produced in urine as well as the malaria- and hookworm-caused anemia patients.

Since hypoxia has been normally found to associate with the cases of anemic group, it is well established as the fundamental erythropoiesis stimulus, inducing the kidney to enhance erythropoietin production (9,10,11). A concept on biosynthesis and release of ESF proposed that is the product of biochemical interaction between any specific protein as substrate from the liver and the renal erythropoiesis factor (REF) (12,13). Production of both such the substrate and REF was consequently increased by the hypoxic stimulation. As in the case of anemias, the remarkable excretion of erythropoietin would be therefore dependent upon the severity of disease associated with hypoxia either or the low level of hemoglobin concentration.

It is of special interest to find that the protein-energy malnutrition children readily showed increases in urinary erythropoietin level comparing to healthy persons, and much lower than the patients with anemias. Our findings in this communication seem to be consistent with that of previous reports (14,15). The alterations in erythropoietin excretion by protein-energy malnutrition patients are still obscure. A further investigation in order to achieve the concrete answer how the malnourished conditions could directly either or indirectly affect the erythropoiesis system in children is actually required.

The isotopic starved rat assay was the only method employed in this study due to its simplicity and limited facilities produced a low sensitivity. However, the more reliable procedure, for instance, the radioimmunoassay (16) or even other specific methodology might be recommended to obtain better results for the future observation.

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