POLYMORPHONUCLEAR LEUKOCYTIC SIALIC ACID AND SIALIDASE ACTIVITY IN OBESITY

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ABSTRACT

The level of sialic acid in the obese polymorphonuclear leukocytes (PMNL) was found to be significantly reduced as compared to normal. Activity of sialic acid degrading enzyme, sialidase, was found to be increased in the obese state. Restoration in both sialic acid content and sialidase activity was also evidenced in the PMNL of treated obese patients.

KEY WORDS: Polymorphonuclear Leukocytes (PMNL), Sialic acid, Obesity, Sialidase.

INTRODUCTION

Alteration in the contents of sialic acid, as glycoprotein were evidenced earlier in certain pathological conditions (1,2). The content of sialic acid in blast cells (3) and leukocytes (4) was found to be considerably altered in certain types of leukemic states. Variations in the content of sialic acid in certain tissues and in serum of diabetic animals were also reported by earlier workers (5, 6, 7). Recently a decreased level of sialic acid was reported in diabetic erythrocytes (8) and leukocytes (9). It is, however interesting to note that none till date have made any observations on the possible alterations in the content of leukocytic sialic acid in obesity. Therefore it was thought worthwhile to carry out the present study to assay the content of polymorphonuclear leukocytic sialic acid in obesity. The study became more relevant since the hyperinsulinemic condition was found to be closely associated with obesity (10,11) and adversely affect the cell surface characteristics (12) and sialic acid is known to be closely related to such surface characteristics (13,14). It was Warren and Spearing (15) who for the first time reported the sialidase activity in mammary glands, which has now been recognised

to occur in particle bound form in human leukocytes resembling closely to the lysosomal sialidase of the liver (16). During the present study attempts were made to assay the activity of PMNL sialidase in obesity as well, so as to correlate the activity of this enzyme with the content of sialic acid. Treated obese PMNL were also examined for sialic acid content and sialidase activity.

MATERIALS AND METHODS

Chicken egg white ovomucoid was obtained from sigma chemicals, US and Dextran (M.Wt, 150,000-200,000) was obtained from BDH chemicals Ltd, England. All other chemicals and reagents used were of analytical grade.

Experimental

From amongst the obese patients attending clinical O.P.D. of Govt. Medical College and Hospital, Nagpur, fourteen male volunteers between the age group of 30 to 40 years were chosen for the study (Group II). The obesity was confirmed clinically by using B.M.I. (Body Mass Index) scale (17). Ten age and sex matched normal healthy individuals with B.M.I. of range between 19kg/m² to 24 kg/m² (mean 22.5 kg/m²) were considered for normal control group (Group 1). The BMI of obese subjects ranged between 30 kg/m² to 40 kg/m² (mean

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36.5 kg/m²), predominantly with the increased localization of fat in the abdominal region. None of the above subjects had any hepatic or renal pathology or diabetes mellitus.

Seven from the above obese volunteers were treated for obesity specifically following the course of an antiobese agent viz. Ponderax-60, an effective weight reducing drug (18) for a period of 7 weeks, in addition to an intake of low caloric diet and regular exercise (Group III). At the end of the treatment the reversal in obesity was confirmed clinically by using B.M.I. scale.

Heparinized blood samples were drawn from the normal, obese and treated obese subjects.

Isolation of PMN leukocytes

PMN leukocytes were separated by the method of Harlan (19) with slight modifications as follows.

Heparinized blood was allowed to stand for 45 min at 20°C to 25°C, and the plasma was aspirated carefully and centrifuged at 170 x g for 10 mins. Pellets were then suspended in Hank's balanced salt solution (with glucose) and placed on top of a

ficoll-paque mixture and centrifuged at 400 x g for 40 mins. Pellets thus obtained were washed with 0.34M sucrose to remove platelets. A few remaining erythrocytes were disrupted with hypotonic lysis with cold double distilled water for 30 sec. Isotonicity was restored by 3.5% NaCl. PMN leukocytes were finally washed and suspended in 0.34M sucrose. This solution contained 99% PMNL as ascertained by phase contrast microscopy. The entire operation was carried out in cold (0-4°C).

Assay of sialic acid and sialidase in PMNL

Total sialic acid of PMNL membrane was estimated by the method of Warren (20) and the activity of sialidase was assayed by the method of Mahadevan et al (21) with slight modification as follows: The reaction mixture consisted of 1 mg of chicken egg white ovomucoid as substrate, 200 mM tris-malate buffer (pH 6.0), in a total volume of 0.4 ml, incubated for 2 hrs at 37°C. The enzyme activity was inhibited by the addition of 0.2 ml of 0.2M sodium metaperiodate and the liberated sialic acid was measured by the method of Warren (20). Protein was estimated by the method of Lowry et al (22), using bovine serum albumin as standard.

Table 1. Sialic acid content and sialidase activity of PMNL in obesity.

Group	Subjects	Body Wt. (Kg)	BMI (Kg/m ²)	Total sialic (μ moles/g protein)	Sialidase* activity
I	Normal healthy individuals (10)	65±7.1	22.5±3.2	41.79±5.21	12.35±2.32
II	Untreated obese individuals (07)	98±8.3	36.5±5.6	23.45±3.84 p<0.001	23.15±3.51 p<0.001
III	Treated obese individuals (07)	92±7.6	32.8±4.1	30.18±5.92 p<0.05	17.53±2.04 p<0.01

Values are mean±SD

Figures in parenthesis are number of subjects.

*Micromoles of sialic acid liberated per gram of protein in 2 hrs at 37°C.

P value calculated by using students 't' test.

RESULTS

Table 1 reveals that the content of sialic acid was found to be significantly reduced in the PMNL of obese subjects, as compared to that of normal subjects ($P < 0.001$).

Sialidase activity of PMNL however was found to be increased in obesity. Although sialidase increase was quite significant ($P < 0.001$) in obesity, its catalytic activity appeared to be restored with antiobese treatment.

DISCUSSION

It is well known that at physiological pH, human leukocytes carry a net negative charge (23) and the surface charge is considered to be one of the factors responsible for the balance between attractive and repulsive forces that operate between the cell periphery and other surface of potential contact (24). Sialic acid is well known to play a significant role in cell physiology (25,26). In the leukocytes the surface charge density which is mostly due to sialic acid (27) may be important in determining the surface characteristics and may affect the migration of cells to inflammatory foci, deformability of the cell periphery, circulatory pattern of cells, antigenic nature of the cell surface and movement of ions across the cell surface (4). Hence alterations caused, if any, in the content of leukocytic sialic acid under certain pathological conditions or metabolic states might have deep implication in assessing the movement of ions across the cell surface and the antigenic nature of the cell surface. However it is peculiar to know that no evidence has yet been forwarded in examining the sialic acid content of PMNL during obesity.

The significant reduction in the content of total sialic acid in the obese PMNL observed during the present study, is thus a new information in obesity. Attempts were not made to assay the content of free sialic acid, since earlier reports indicated that the leukocytic sialic acid is mostly present in the bound form (4). Besides no work has been done till date to evidence the sialic acid synthesizing system in

leukocytes. Thus our results indicate a possible aberration in the membrane integrity of the PMNL in obesity.

The above results might be accounted for either expecting a decreased synthesis or an increased degradation of sialic acid. Since, none have so far reported a synthetic system of sialic acid in leukocytes and reports being available on particulate sialidase activity in PMNL (16), the present study was undertaken to assess the sialidase activity in obese subjects as one of the responsible factors for the observed decrease in sialic acid. Despite the data available related to abnormal alteration of ionic transport across the erythrocytic (28,29) and leukocytic (30), cell membrane in obesity, it is peculiar that none have attempted so far to examine the leukocytic sialic acid in this disease.

Our results clearly demonstrate that there was an increased specific activity of sialidase in the obese PMNL, thus accounting satisfactorily for the reduced content of sialic acid. Although the mechanism related to increase in sialidase activity in this disease is not clear at the present juncture, it may be pointed out that several workers earlier have shown increased activity of tissue lysosomal enzyme in serum of diabetes mellitus (31), lymphocytes of chronic lymphocytic leukemia (32) and pancreatic islets of obese hyperglycemic conditions (33,34). Thus it may be contended that the increased sialidase activity observed during the present study might derive its hyperactivity from PMNL lysosomes in obesity. It may be noted that similar explanation was offered for the derangement in PMNL sialic acid in patients suffering from diabetes mellitus (9) and essential hypertension (35).

The treated obese PMNL were found to show restoration in the content of sialic acid and sialidase activity to a significant manner. The restoration of the sialic acid content in treated obese PMNL clearly indicates that antiobese agents were considerably effective in restoring the turnover rate of sialic acid.

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