N-ACETYL-NEURAMINIC ACID (NANA) IN PATIENTS WITH CARCINOMA CERVIX UTERI@

by

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Considerable interest has been manifested in the serum carbohydrate-bound proteins (glycoproteins) during the last few years, centred mainly around the demonstration of elevated levels of the glycoproteins in a wide variety of human diseased states. N-acetyl neuraminic acid (commonly known as sialic acid) is important in biological chemistry as the parent compound of number of acylated derivatives and is widely distributed throughout the animal kingdom. It is generally considered that human serum does not contain free "NANA" and that 90% of the NANA is bound to the alpha and beta globulins (Barker *et al*, 1959). In certain diseases like tuberculosis, rheumatic fever, nephrosis, leukaemia and cirrhosis of liver, quantity of NANA in serum increases in comparison to the normals in coincident with the increase in relative proportion of globulins. From our country little work has been published on serum sialic acid levels in normal healthy adults, but there is no publication on cancer patients. In the present work NANA has been estimated in the serum of healthy normals and in the serum of patients suffering from cancer of cervix uteri.

Material and Methods

The present study consisted of 35 healthy fefmales. Each individual was regarded as healthy only when thorough history-taking, clinical examination and necessary investigations ruled out the presence of any significant illness which she might have had either at the time of present study or in immediate past. Fifty-one individuals with advanced histopathologically and clinically diagnosed cancer of cervix in various stages were studied (Table I). Maximum care was taken to exclude all those conditions which are known to alter the values of NANA, such as tuberculosis, rheumatic fever, leukaemia and hepatic diseases etc. Venous blood samples were drawn from the subjects and serum was separated. The haemolysed samples were discarded. NANA was estimated in serum by diphe-

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TABLE I
Distribution of Cases

		No. of cases
(A) NORMAL		35
(B) CANCEROUS PATIENTS:		51
—Stage I	8	
—Stage II	22	
—Stage III	15	
—Stage IV	6	

nylamine reaction, according to the procedure followed by Winzler (1955) which is a slight modification of that developed by Ayala et al (1951) using 50 mg/100 ml solution of crystalline acetylneuraminic acids (supplied by Sigma Chemicals, USA) for standardization. All other chemicals used in the preparation of reagents were of analytical grade of guaranteed purity. Readings were taken on EEL photoelectric-colorimeter and the results are expressed in milligrams of NANA per 100 ml of serum. Some of the serum samples were analysed in duplicate also and the results with this procedure have been reproducible.

Results

The concentration of NANA in the serum of abnormal individuals with advanced cancer of cervix uteri is shown in Table II.

Discussion

It could be seen from the data presented in the tables that NANA levels in healthy females are 58.0 ± 4.5 mg/100 ml. of serum which is almost the same as that reported by Winzler (1958) and Macbeth and Bekesi (1962). Elevated NANA levels are seen in all cases of histopathologically confirmed cancer patiens wih a mean value of 149.7 ± 42.2 mg/100 ml of serum in this study. Winzler (1955) has also reported a value of 142 ± 13.1 mg/100 ml of serum in 15 patients suffering from advanced cancer. Turumi and Dawes (1958) reported that the level of sialic acid in serum is increased in mice carrying some transplanted mammary carcinoma. In some cases of human breast, colon and stomach carcinomas, Barker *et al* (1959) found 160 to 290 mg/100 ml of NANA which was distinctly more than the adjacent normal tissue, surrounding the malignant tissue. Masamune and Kawasaki (1958) reported that glycoproteins from the cancerous tissue had more sialic acid. Thus, variation in the serum NANA concentration is of considerable interest. Macbeth and Bekesi (1962) have concluded that tumour growth stimulates the liver (probably by some humoral mechanism) to synthesize glycoprotein, which subsequently makes its appearance in the circulation.

TABLE II
Estimation of Sialic Acid in Controls and the Study Group

Subjects	No. of Cases	NANA in Serum (mgm/100 ml.)	± S.D.
(A) NORMAL	35	58.00	± 4.5
(B) CANCEROUS PATIENTS	51		
Stage I	8	142.10	± 32.8
Stage II	22	149.30	± 42.2
Stage III	15	155.20	± 13.4
Stage IV	6	162.10	± 35.6

While it seems unlikely, determination of serum NANA fraction will prove to be useful diagnostic procedure in cases of cervical cancer, it is possible that the estimation of specific carbohydrate in NANA may eventually be of some assistance in this regard. This facet of the subject will be approached by means of serial plasma tissue NANA and mannose determinations following the malignant tumour production in experimental animals.

Summary

N-acetyl-neuraminic acid was estimated in serum by diphenylamine reaction, according to the procedure followed by Winzler (1955) which is a slight modification of that developed by Ayala (1951) in healthy normals and patients suffering from cancer of cervix uteri. A statistically significant rise of NANA was noticed in cancer patients. The increased serum level of NANA is explicable on the basis of incrased production of mucoproteins containing NANA.

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THE SIGNIFICANCE OF CORRELATION IN DETERMINING B-GLUCORONIDASE ACTIVITY IN CONJUNCTION WITH CYTOLOGY FOR THE EARLY DIAGNOSIS OF CANCER OF THE CERVIX UTERI

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Material and Methods

Eighty cases chosen from the total number of women examined in the Obstetrical and Gynaecological Department of the General Hospital of Nikeas-Piraeus, whose cytologic examination presented Class II or Class III according to Papanicolaou, comprised the material of the present investigation.

Furthermore, these women were subjected, in addition to vaginal smear study, to b-glucoronidase measurement of the vaginal fluid as well as to a final histologic examination.

The detection of b-glucoronidase was made by the chromatometric method using glycuronic phenolphthalein as reagent; b-glucoronidase breaks down the reagent and frees phenolphthalein which stains red. This stain is compared with the predetermined formed solutions.

Results

The present investigation was based on a chosen material which corresponded histologically to the following classes:

(a) Class II Papanicolaou with a clinical picture of cervicitis (20 cases); (b) Class II Papanicolaou with a clinical picture advocating a more developed condition (30 cases); (c) cases of class III of undifferentiated clinical picture (30 cases).

Vaginal fluid was taken in all of the above cases for the assessment of b-glucoronidase. A value of over 400 units of the above enzyme was considered as a possibility of existing cancer (Odell and Burt, 1950; Lorinez *et al*, 1951; Fishman *et al*, 1959; Lawson *et al*, 1966). Histological examination was carried out in 10 cases of group A and in all the remaining groups (Table I).

As shown in the Table, in 50 cases in which the cytologic examination revealed class II, only in 1 patient was cancer in situ discovered by histology. In these cases the b-glucoronidase values ranged within the normally considered limits, except for 3 cases in which this value exceeded the established crucial value of 400 units, including the cytologically negative case as well. The two additional cases corresponded to those of severe changes of dysplasia.

Of the 30 cases with class III Papanicolaou a histologically discovered cancer of differentiating development was found in 10.

In these cases the b-glucoronidase values of the vaginal fluid ranged within

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TABLE I

	No. of cases	Class according to Papanikolaou	Clinical picture	b-glucoronidase	Histological examination
A	10	II	cervicites	107, 89, 116, 266, 205, 102, 180, 398, 138, 280.	cervicites
	10	II	cervicites	192, 375, 267, 370, 310, 215, 307, 380, 376, 181.	—
B	30	II	cervicites	358, 384, 216, 154, 318, 280, 307, 298, 381, 206, 212, 187, 286, 148, 212, 296, 230, 397, 376, 104, 300, 222, 454, 317, 497, 306, 324, 320, 577.	
Ca	10	III	cervicites	332, 328, 487, 219, 300, 394, 275.	Severe changes of cervicites
				605, 720.	Ca in situ (2)
				1004.	Ca invasive (1)
Cb	10	III	—	407, 206, 387, 192, 392, 427.	Severe changes of cervicites
				540, 976, 604, 488.	Ca-commencing (4)
Cc	10	III	Ca	254, 314, 225, 756.	Severe changes of cervicites
				399, 310, 325, 712, 712, 310.	Dysplasia Ca-commencing (2)
				898	Ca-invasive (1).

the expected limits, except for 5 cases in which there was no agreement of value-changes.

The crucial value of 400 units of enzyme activity, as also results from the literature cited by the author, seems to be the most correct and is also established by the histological examination (Table I). This Table shows that b-glucoronidase almost always continues to increase, in parallel to the aggravation of the cytologic as well as the histologic picture.

Discussion

The above mentioned findings lead, in the author's opinion, to the conclusion that whenever the cytologic examination is not able to account for the nature of cervical change the exact diagnosis should be sought by some other easy and bloodless supplemental investigation, such as the measurement of b-glucoronidase activity. Of course, during this investigation, the patient's age should be taken into consideration as well as possible vaginal inflamation, because the above states influence b-glucoronidase value of 400 units which seems to cover the above disadvantages (Mobius *et al*, 1961; Lawson and Watkins, 1966).

Of the bloody methods, simple biopsy performed even after the Schiller test does not absolutely ensure that the suspicious change is in the tissue which was taken. Wedge resection, usually performed in such cases, may even constitute sufficient treatment; it may however make

the local application of radium pre-operatively difficult or may postpone a supplementary extended surgical procedure for a period of at least 4 to 6 weeks. This is so because every pelvic operation attempted in a shorter period of time engenders great danger for septic complications.

However, by combining the study of vaginal smears and the activity of b-glucoronidase it is possible to limit the pseudo negative cases and to clear up the suspicious ones which very often have to be given meticulous care and early treatment.

Summary

Class III, according to Papanicolaou, often marks a situation of greater vigilance for the clinician and doubt for the cytologist. The value of b-glucoronidase activity of vaginal fluid offers great help in these cases in order to establish the final diagnosis, so that possible erroneous conclusions which might be drawn by using these two methods independently are diminished to the least.

It is common experience with all those occupied with the primary diagnosis of cancer of the cervix uteri that the cytologic classification of the vaginal smear in class III may be liable to question. The cytological changes which are found, initially attributed to Ca in situ are now considered by most auhors as corresponding mainly to histologically dysplastic changes (pre-cancerous or border-line), and less to a more developing condition such as in situ cancer (Papanicolaou, 1958; Kaufman and Ober, 1959; Koss, L. G., 1963).

In order to assess these cases further, the possibility of determination of b-glucoronidase activity in the vaginal fluid (Gross, 1964) was attempted, and the results of this enzymatic method were appraised with the histologic examination in relation with and parallel to the study of the vaginal smears (Odell and Burt, 1950; Lorincz *et al*, 1951; Hadzimichael, 1962).

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