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The Excretion of Thormählen Positive Melanogens in Melanoma Patients and its Clinical Significance

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Abstract—*Thormählen positive melanogens (TPM) were determined in the urine of 585 primary and/or metastatic cutaneous melanoma patients, in 105 primary and/or metastatic ocular melanoma patients, in 100 patients suffering from other diseases with the exception of melanoma and in 72 healthly persons. On the basis of our results we can recommend the determination of the sum of TPM as sufficient for routine monitoring of patients in the course of malignant melanoma. The determination of TPM urinary excretion cannot substitute histological and clinical examinations in an early diagnosis of malignant melanoma, but it is very important and useful for a differential diagnosis and prognosis of malignant melanoma. TPM urinary excretion can be followed in melanoma patients also for the indication of further treatment and the evaluation of the response on chemotherapy or immunotherapy.*

INTRODUCTION

MELANOGENURIA is one of the indications of the interaction of a malignant pigment cell with the host organism. By this term we mean the excretion of urinary melanogens, i.e., specific compounds which are excreted in an elevated amount in the urine of melanoma patients. Urinary melanogens are phenolic and indolic derivatives and up till now at least 15 of these compounds have been identified in melanoma urine [1]. Urinary melanogens can be divided into two principal groups [2] according to their behaviour during the Thormählen test—Thormählen positive melanogens (TPM) and Thormählen negative melanogens (TNM). An unsubstituted pyrrol ring of the indole nucleus is a condition of a positive Thormählen test.

The group of TPM consists of at least 4 compounds designated as A, B, C and E [3, 4]. The compound A is most probably the monoglucuronide of 5,6-dihydroxyindole or hydroxymethoxyindole, the others are also conjugates of 5,6-dihydroxyindole or hydroxymethoxyindole with glucuronic or sulphuric acid.

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The clinical significance of the determination of the urinary melanogens excretion level depends predominantly on: their clinical specificity; the frequency of their occurrence and, finally, on the relation between the stage and type of disease and the corresponding analytical data.

The aim of this paper is to verify the clinical significance of the evaluation of TPM urinary excretion during the course of malignant melanoma and its significance for patient's monitoring.

MATERIALS AND METHODS

Patients

The study of TPM urinary excretion was carried out with 690 melanoma patients. Of which 585 were patients with melanoma of cutaneous origin and 105 were patients with melanoma of ocular origin. The patients were divided into disease stages according to WHO classification, i.e., stage I—primary tumour or local recidive, stage II—swelling of regional lymph nodes and stage III—metastases and dissemination of the tumour. In all patients the diagnosis was verified by histological examination and the stages of the disease were

also clinically and histologically proved. In order to compare TPM urinary excretion in a group of patients with other diseases and in healthy subjects, we examined TPM urinary excretion in 72 arbitrarily selected healthy subjects and in 100 patients with diseases different from melanoma (10 patients with cutaneous tumours different from melanoma, 20 patients with non-tumorous liver diseases, 70 patients with common non-tumorous diseases).

Determination of the sum of Thormählen positive melanogens

The concentration of TPM was measured in fresh morning urinary samples (no preservatives added) of all patients and control subjects using a quantitative modification of the Thormählen test [5] as follows: 4 ml of urine were mixed with 0.5 ml of 2% sodium nitroferricyanide (*ad hoc* prepared). Two ml of 10% potassium hydroxide were added to this mixture and the contents of the test tube was well mixed. Finally, the solution was acidified by 0.2 ml of concentrated acetic acid and well mixed again (A). Simultaneously the same procedure was made with a sample of urine to which 0.1 ml of standard indole solution (1.7 mmole/l. i.e., 0.2g/l) was added (A_i). The absorbance at 620 nm was measured in 20 min. The results were obtained using formula $[A/(A_i-A)] \times (20/4)$ and expressed in μg of indole equivalents per ml of urine. The quantity of TPM in urine may also be calculated using the standard curve for indole [6].

RESULTS

In the group of 72 control subjects an average value of TPM urinary excretion equal to 3.23 ± 1.02 μg equiv. of indole/ml was found. In this group values exceeding 10 μg equiv. of indole/ml have never been found. In agreement with previous observations [7] this value has been considered as the upper limit of normal values. (Table 1).

In the group of 100 patients with diseases different from malignant melanoma values of TPM urinary excretion higher than 10 μg equiv. of indole/ml were found only in 4 patients with serious liver diseases, i.e. in 4% of all patients (Table 1). From this it follows that an increased TPM urinary excretion is specific for malignant melanoma.

Increased TPM urinary excretion (higher than 10 μg equiv. of indole/ml) has been found in 216 patients, from the total number of 690 melanoma patients tested, i.e. in 31.3% of all melanoma patients. Practically the same results have been achieved when the patients were divided according to the origin of the tumour. In 105 patients with primary ocular melanoma we found an increased TPM urinary excretion in 32 patients, i.e., in 30.5% and in 585 patients with primary cutaneous melanoma, we found an increased TPM urinary excretion in 184 patients, i.e., in 31.45% . Similar results have been obtained, when the patients were divided according to sex. (Tables 2 and 3).

When the patients were divided according to the stage of the disease, we found in patients with primary ocular melanoma in stage I increased TPM urinary excretion only in 2 of 67 patients, i.e., 2.95%, in stage II in 3 of 10 patients, i.e., 30% and in stage III in 27 of 28 patients, i.e., 96.4%. The only patient in stage III whose TPM urinary excretion was lower than 10 μg equiv. of indole/ml suffered from amelanotic melanoma, the widespread metastases of which were also apigmented. In some patients in stage III the TPM urinary excretion achieved extreme values of up to 800 μg equiv. of indols/ml, i.e., more than 5000 μmole equiv. of indole/24 hr

Table 1. *The urinary excretion of TPM* (μg. equiv. *indole*/ml) *in healthy persons and in patients suffering from other diseases with the exception of malignant melanoma*

	No. of cases	Mean	S.D.	R	Range
Healthy subject	72	3.23	1.02	5.80	1.30–7.10
Skin tumours except m.m.	10	3.50	0.80	2.60	1.90–4.50
Liver non-tumorous diseases	20	8.80	4.50	13.80	3.20–17.00
Common non-tumorous diseases	70	4.37	2.56	7.25	1.85–9.10

Table 2. The frequency of an increased TPM urinary excretion in patients with ocular malignant melanomas

Stage	No. of patients			TPM > 10 μg equiv. indole/ml		
	Male	Female	Total	Male	Female	Total
I	31	36	67	1	1	2
				3.2%	2.7%	2.95%
II	6	4	10	2	1	3
				33.3%	25.0%	30.0%
III	16	12	28	15	12	27
				93.7%	100%	96.4%
Total	53	52	105	18	14	32
				33.9%	26.9%	30.5%

Table 3. The frequency of an increased TPM urinary excretion in patients with cutaneous malignant melanomas

Stage	No. of patients			TPM > 10 μg equiv. indole/ml		
	Male	Female	Total	Male	Female	Total
I	171	228	399	40	34	74
				23.4%	14.9%	18.5%
II	76	61	137	42	28	70
				55.2%	45.9%	51.1%
III	32	17	49	26	14	40
				81.2%	82.3%	81.6%
Total	279	306	585	108	76	184
				38.7%	24.8%	31.45%

(Table 2). In patients with primary cutaneous melanoma in stage I we found increased TPM urinary excretion in 74 patients out of 399, i.e., 18.5%, in stage II in 70 patients out of 137, i.e., 51.1% and in stage III in 40 patients out of 49, i.e., 81.6%. In some of these patients in stage III the TPM urinary excretion was extremely high and reached values of up to 500 μg equiv. of indole/ml, i.e., more than 3000 μmole equiv. of indole/24 hr (Table 3, Fig. 1).

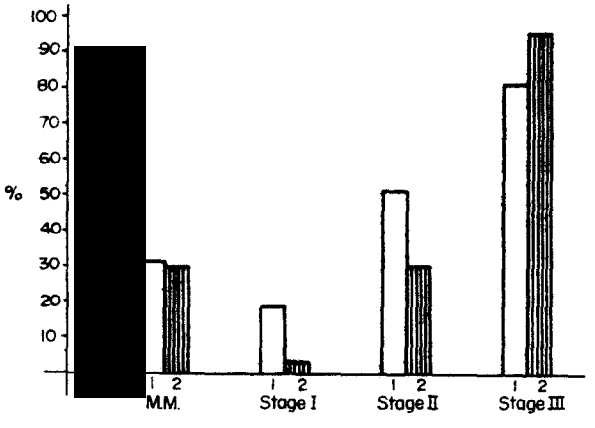

Fig. 1. Frequency of an increased TPM urinary excretion in patients with malignant melanomas of different origin. (1 = cutaneous malignant melanoma, 2 = ocular malignant melanoma, MM = all melanoma patients).

When the patients were divided according to the stage of the disease and sex, no significant difference in the frequency of increased TPM urinary excretion in dependence on the sex was found.

Interesting results have been obtained when following TPM urinary excretion in the course of the disease as follows from Table 4 and Fig. 2. The frequency of increased TPM urinary excretion increases in the course of the malignant melanoma, mostly in stage II. According to our experience, this fact is not favourable from the viewpoint of the disease prognosis. Increased TPM urinary excretion is often a first sign of the progress of malignant melanoma.

The absolute daily quantity of TPM urinary excretion has been compared in a group of 20 patients in stage III and it has been found, that its value depends on the origin of the tumour, degree of pigmentation (melanotic or amelanotic) and the metastases localization. High TPM urinary excretion has always been found in melanotic types of melanomas, where metastases were also highly pigmented, achieving extreme values in the case of liver metastases. (Table 5).

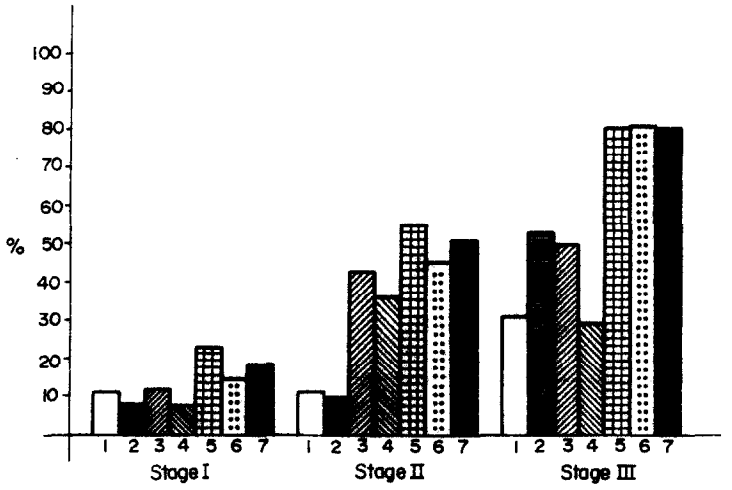

Fig. 2. Frequency of an increased TPM urinary excretion in patients with cutaneous malignant melanomas. (1 = male, 2 = female—% of elevated level of TPM urinary excretion at the beginning of the individual stage of melanoma, 3 = male, 4 = female—% of elevated level of TPM urinary excretion in the course of the individual stage of melanoma, 5 = total male, 6 = total female, 7 = total male and female).

Table 4. The frequency of an increased TPM urinary excretion in patients with cutaneous malignant melanomas at the beginning of the individual melanoma stage and in the course of the disease

				TPM > 10 μg equiv. indole/ml					
	No. of patients			At the beginning of the disease			In the course of m.m.		
Stage	Male	Female	Total	Male	Female	Total	Male	Female	Total
I	171	228	399	19	18	37	21	16	37
				11.0%	7.9%	9.2%	12.2%	7.0%	9.2%
II	73	61	134	9	6	15	33	22	55
				11.8%	9.8%	10.9%	43.4%	36.1%	40.1%
III	32	17	49	10	9	19	16	5	21
				31.3%	53.0%	38.8%	50.0%	29.4%	42.8%
Total	279	306	585	38	33	71	70	43	113
				13.6%	10.7%	12.1%	25.1%	14.1%	19.3%

Table 5. The average of daily TPM urinary excretions in stage III of melanoma patients

		Amount of TPM	
	No. of patients	Mean mg equiv. indole/24 hr	Mean μmole equiv. indole/24 hr
Melanotic ocular melanoma widespread metastases including liver—pigmented	6	431.3	3666.05
Amelanotic ocular melanoma widespread metastases including liver—apigmented	1	1.57	13.3
Melanotic cutaneous melanoma widespread metastases including liver—pigmented	6	345.5	2936.75
Melanotic cutaneous melanoma widespread metastases without liver—pigmented	4	3.01	25.6
Amelanotic cutaneous melanoma widespread metastases including liver—apigmented	3	1.85	15.72

DISCUSSION

In evaluating TPM urinary excretion, we reached agreement with previous results of other authors [6–9], who found increased TPM urinary excretion in an average of 25% of all melanoma patients studied. Our results are as much as 5% higher, however, we assume that this increase is due to the biological variability and thus cannot be considered as absolute. On the other hand some other authors [10] have found a lower frequency of increased TPM urinary excretion in melanoma patients. According to our opinion, the lower frequency can be caused by the fact that usually an acid is used as a preservative during urine collection and it is not generally well known that TPM are very unstable, especially in an acid medium.

The evidence of increased TPM urinary excretion has no great significance in an early diagnosis of malignant melanoma, since it cannot substitute histological techniques and clinical examinations. A different situation arises when the patients are divided according to the melanoma origin (ocular or cutaneous) and the stage of the disease. This question until now has not been considered. We have found increased TPM urinary excretion in patients with ocular melanoma in stage III in all melanotic (pigmented) forms in contrast from stage III with cutaneous melanoma, where increased TPM urinary excretion was found in more than 80% of the patients. This fact, together with the differences in the absolute quantity of daily TPM urinary excretion indicates, that metabolic differences could exist between the individual types of malignant melanomas, just as there exist differences in their biological behaviour. We assume, on the basis of the comparison of the TPM urinary excretion in selected patients in stage III, that these metabolic variations could depend on the melanoma type and/or the degree of pigmentation (melanotic or amelanotic). TPM can only be formed from dopaquinone originating from tyrosine by means of the catalytic activity of tyrosinase (E.C.1.14.18.1) [4]. Thus the differences could also be an indirect evidence of different tyrosinase activity in amelanotic and melanotic melanomas.

From our observations it explicitly follows, that the following of TPM urinary excretion is very important for the disease prognosis. A continuously increasing TPM urinary excretion in the course of malignant melanoma is always an unfavourable sign for the patient, no matter what the absolute TPM urinary excretion level is. An increased TPM urinary excretion can in many cases precede the clinical signs of the progress of the disease, as it appears from Table 4. Repeatedly, we have also observed in agreement with Crawhall *et al.* [8] a decrease of TPM urinary excretion after immuno- or chemotherapy.

We assume that the variation of TPM urinary excretion during the course of the melanoma in dependence on its type and stage may also express an interaction of probably existing different biochemical types of malignant melanomas with different biochemically or immunologically conditioned responses of the host organism. This is in agreement with the findings of Fairley [11] who has found a decrease of melanoma specific antibodies in advanced stages of malignant melanomas, which is in exact reciprocity of the frequency of increased TPM urinary excretion.

Although from our observations it is obvious that an increased TPM urinary excretion is highly specific for malignant melanoma, we must not, when evaluating, omit the fact, that for a correct evaluation of TPM urinary excretion it is necessary to evaluate the dynamics of TPM urinary excretion, i.e., to follow TPM urinary excretion repeatedly, best in regular intervals.

If we compare the frequency of positive results of TPM urinary excretion in melanoma patients with frequency of positive results of other melanogens given by other authors, we may see that they are very similar [1, 2]. Only with 3,4-dihydroxyphenylalanine (DOPA) is the frequency of positive results higher, however the specificity of proof is lower.

In 1975 Agrup *et al.* [12] described increased 5-*S*-cysteinyldopa urinary excretion in melanoma patients and recommended the determination of this melanogen as the most specific test for monitoring melanoma patients. Comparing our results with those of Agrup *et al.*, it can be seen that while increased TPM urinary excretion in stage I of cutaneous and ocular melanoma was found in 16.3% of all our melanoma patients, 5-*S*-cysteinyldopa in stage I has been found in concentrations corresponding to healthy persons. Stage II has not been evaluated by Agrup *et al.* In stage III Agrup *et al.* found increased 5-*S*-cysteinyldopa excretion in 66% of the patients in contrast with 87% of increased TPM urinary in all our melanoma patients. This indicates that the technically

very complex fluorimetric method of 5-*S*-cysteinyldopa determination did not exhibit better results in melanogenuria evaluation in melanoma patients than the technically simple, feasible and time-saving determination of the sum of Thormählen positive melanogens.

On the basis of our results we can summarize that for routine observation of melanogenuria in melanoma patients it is sufficient to determine the sum of Thormählen positive melanogens. The determination of TPM urinary excretion cannot substitute histological and clinical examinations, but it is very important and useful for a differential diagnosis and prognosis of malignant melanoma and it can be used for the indication of further treatment and an evaluation of the response on chemotherapy or immunotherapy.

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