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Histopathology of Skin Lesions in Chronic Arsenic Toxicity—Grading of Changes and Study of Proliferative Markers

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ABSTRACT

Chronic arsenic toxicity (CAT) manifests predominantly as cutaneous lesions in the form of melanosis, keratosis and neoplastic changes. We have studied skin biopsies from 42 patients of CAT. Histological study of H/E stained sections showed - hyperkeratosis in 13, parakeratosis in 13, acanthosis in 12, papillomatosis in 24, elongation of reteridges in 21, increased basal pigmentation in 27 and dysplastic changes in 8 cases. Squamous cell carcinoma was present in 2, basisquamous in 1 and basal cell carcinoma in 1 case. Changes of skin lesions after drug DMSA and DMPS therapy compared to placebo were studied. The result was inconclusive. Proliferative activity of skin lesions in CAT were studied by AgNOR stain to assess the biological behaviour of the lesions. AgNOR score showed - normal control 1.08, benign changes (e.g. Hyperkeratosis, parakeratosis, acanthosis, papillomatosis etc.) without dysplasia - 1.35, mild to moderate dysplasia - 1.735, severe dysplasia - 3.0 and carcinoma - 3.56. Thus, AgNOR score gives some idea on the biological behaviour of CAT lesions. It is suggested that AgNOR staining should be done regularly along with H&E staining for proper assessment of the cases.

Key words : Chronic arsenic toxicity, Melanosis, Keratosis, Neoplasia, AgNOR, Proliferatiave marker.

INTRODUCTION

Drinking of ground water having arsenic concentration above the maximum permissible limit of 0.05 mg/l has been reported from 8 districts of West Bengal[1]. Similar reports are also available from other countries e.g. Bangladesh, Taiwan, Argentina, Chile, Brazil etc. Prolonged exposure to such water results in various clinico-pathological conditions affecting skin and internal organs.

Arsenic is a naturally occuring element, usually in combination with other elements in earth's crust. Inorganic form of arsenic is more toxic over its organic form and present in ground-water. After systemic absorption, it is eliminated mainly through urine. But on overdose, arsenic accumulates in different body tissues e.g. liver, skin, hair, nail etc. and presents predominantly with cuta-neous features[2] which include:

1. Palmo-plantar/body/mucous membrane melanosis. May be spotted or diffuse.
2. Palmo-plantar keratosis diffuse or nodular.
3. Carcinomatous changes e.g. squamous cell carcinoma, basal cell carcinoma etc.

These changes may progress or regress either spontaneously or on therapeutic interventions. An effective therapeutic agent is yet to be found.

Simple mentioning of presence or

absence of a particular skin change does not specify the extent of damage occured. Therefore, it was felt that there must be some system of histological grading of the skin changes following fixed criteria. To assess the biological behaviour of any lesion particularly in regard to malignant potential, various methods[3] eg. mitotic count, PCNA and ki-67 immunostain, DNA flow-cytometry, AgNOR score etc. are being utilised. Of these methods, AgNOR is of special interest particularly in our country as the method is simple, cheap and reproducible.

MATERIAL AND METHODS

42 patients of chronic arsenic toxicity were selected for study by following criteria :

1. Characteristic skin lesions.
2. History of taking arsenic contaminated water for prolonged period.
3. High arsenic content of urine/hair and nail.
4. No history of chronic alcoholism or taking hepatotoxic drugs.

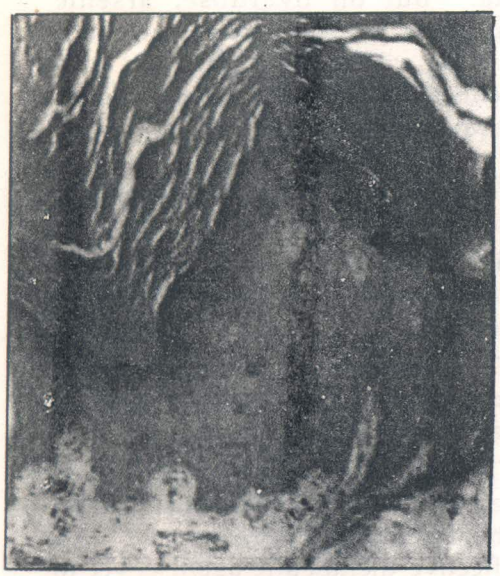

Fig.1 Photomicrograph showing marked hyperkeratosis and acanthosis (H&E Stain, X 40).

Laboratory examination of blood for sugar and urea, urine for R/E, LFT, USG of liver and chest X-ray were done. Estimation of arsenic content of drinking water, urine (24 hours), hair and nail of patients were done.

Skin biopsies were taken from unexposed part of the body as well as from affected part in cases with suspicion of malignancy by punch biopsy. Skin biopsies were also taken from 25 apparently normal persons (with history of taking safe water) for control. Patients were divided in two groups. In 1st group of 21 patients, 11 were given DMSA (Dimercapto succinic acid) and 10 patients were given placebo. In 2nd group of 7 patients, 4 were given DMPS (Dimercaptopropane sulphonate) and 3 were given placebo. Skin changes after therapy with these chelating agents were assessed by taking follow-up biopsy and a comparative study was carried out. All the biopsy specimens were routinely processed using formalin fixative and paraffin embedding.

5μm thick sections were prepared and stained with H&E stain. Different epidermal and dermal chanages were noted. Epidermal changes were graded[4] according to the following criteria. The minimum value for these gradings were taken from the findings of the control cases.

1. Hyperkeratosis: measured by ocular micrometer.
   Absent (-) : <20μm Mild (+) : 20-40μm
   Moderate (++): 41-75μm Marked (+++): >75μm
2. Parakeratosis: graded by visual assessment.
   Absent (-), Mild (+), Moderate (++) and Severe(+++).
3. Acanthosis: graded by ocular micrometer.

Absent : <140μm Mild : 140-195μm
Moderate : 196-350μm
Severe : >350μm

4. Papillomatosis: graded by visual assessment.
Absent (-), Mild (+), Moderate (++) and Severe(+++).

Basal pigmentation : could not be graded because of dark skinned patients.

Carcinomatous changes : Severe dysplasia without break of basement membrane was described as carcinoma-in-situ while those breaking basement membrane and invading deeper tissues as invasive carcinomas. Type of carcinoma and degree of differentiation were noted.

The representative sections from all the biopsy specimens were stained by AgNOR technique[5]. Here, 2% gelatin in distilled water with 1% formic acid mixed with double its volume of 50% silver nitrate solution in distilled water, freshly prepared, covered the deparaffinised sections. The sections were incubated in the dark for 45 minutes washed in distilled water, dehydrated and mounted. AgNOR dots, brown-black or black, were counted[6] in 200 consecutive cells under oil-immersion lens. Average number of dots per cell were calculated for each case.

Table . 1 Arsenic contents

Sample	Arsenic Conc.	Normal Values
1. Drinking water	0.06 - 1.43mg/l	<0.05mg/l
2. Urine	0.064 - 0.892mg/l	<0.025mg/l
3. Hair	0.46 - 8.50mg/Kg	<1mg/Kg
4. Nail	0.48 - 11.04mg/Kg	<1mg/Kg

Changes achieved after treatment with drug in respect to their pre-therapy status in comparison to post-placebo changes were noted.

OBSERVATION

In the present study, skin biopsies from 42 patients were examined. Ages of the patients varied from 10-65 years. Male patients were 33 and female were 9. Exposure to arsenic contaminated water varied from 5-25 years. Arsenic content of drinking water, urine, hair and nail of patients were much above normal acceptable limit (Shown in Table-1).

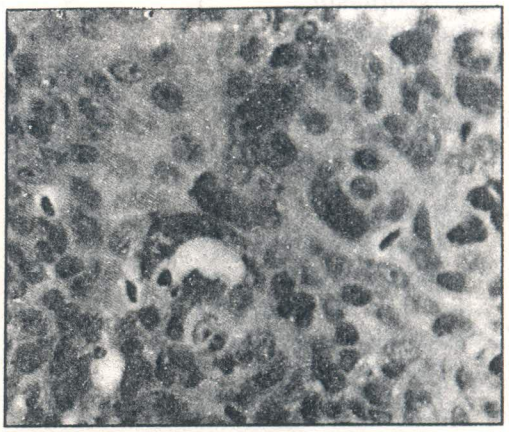

Fig. 2 Photomicrograph showing sq.cell carcinoma (H&E stain, X 200).

Out of 42 cases studied, 1 case had atrophied epidermis. The epidermal changes of remaining 41 cases are shown in Table-2. As the epidermal changes in 4 neoplastic cases and 2 severely dysplastic cases were haphazard instead of usual pattern of hyperkeratosis, parakeratosis, acanthosis etc. they were separated from non-neoplastic cases on grading. In our study, though all the patients, clinically, had hyper pigmention; histologically, increased pigmentation was noted in 64% of cases. Hyperkeratosis was present in 31% parakeratosis in 31%, acanthosis in 28.5% papillomatosis in 57% and elongation of rete-ridges was present in 50% cases.

Table2. Histopathological changes in CAT cases Total case-42, atrophied epidermis-1, Non-neoplastic-35, Neoplastic-6.

Changes	Absent	Mild	Moderate	Severe	Total affected	% of total affected
1. Hyperkeratosis						
Non-neoplastic	27	3	3	2	13	
Neoplastic	1	1	0	4		31.0
2. Parakeratosis						
Non-neoplastic	25	9	1	0	13	
Neoplastic	3	2	1	0		31.0
3. Acanthosis						
Non-neoplastic	28	3	3	1	12	28.5
Neoplastic	1	3	2	0		
4. Papillomatosis						
Non-neoplastic	17	7	11	0		
Neoplastic	0	1	5	0	24	57.0
5. Elong of rete-ridges						
Non-neoplastic	11	4	11	1	21	50.0
Neoplastic	1	2	0	3		
6. Dysplasia						
Non-neoplastic	29	5	1	0	8	19.0
Neoplastic	6	0	0	2		
	Present					
7. Basal pigmentation						
Non-neoplastic	9	26			27	
Neoplastic	5	1				64.0

Table 3. AgNOR Score in CAT Cases.

Skin Changes	Range	Mean	Std deviation	*P-value
1. Normal	1.06±0.14	1.08	0.102	<0.05
2. Benign changes (e.g. Hyperkeratosis, acanthosis etc. without dysplasia)	1.51±0.49	1.35	0.235	
3. Mild to Moderate dysplasia	1.85±0.50	1.73	0.361	<0.01
4. Severe dysplasia (In-situ Ca)	3.0±0.1	3.0	0.02	>0.1
5. Carcinoma	3.52±1.77	3.56	1.486	

* Pooled t test (unpaired)
p value <0.05 indicates significant
& >0.1 indicates insignificant

Tabel. 4 Comparison of changes achieved in post DMSA & post placebo cases

Skin Changes	No. Change	Upgrading	Down grading	*P-value
Hyperkeratosis :				
Post - DMSA - 10	5	1	4	NS**
Post - placebo - 7	6	0	1	
Parakeratosis				
Post - DMSA - 10	5	.2	3	
Post - placebo - 7	7	0	0	NS
Acanthosis :				
Post - DMSA	5	1	4	
Post - placebo	5	1	1	NS
Papillomatosis				
Post - DMSA	2	6	2	
Post - placebo	5	1	1	NS
Elong of rete-redges				
Post - DMSA	5	2	3	NS
Post - placebo	5	1	1	
Dyplasia				
Post - DMSA	7	1	2	
Post - placebo	6	0	1	NS
Basal Pigmentation				
Post - DMSA	4	2	4	
Post - placebo	4	3	0	NS

* Student t test, ** Not significant

Tabel 5. Comparison of changes achieved in post - DMPS and post placebo cases. DMPS given to 4 patients and placebo to 3 patients.

Skin Changes	No. Change	Upgrading	down grading	p-value
1. Hyperkeratosis :				
Post - DMPS	4	0	0	
Post - placebo	2	1	0	NS
2. Parakeratosis				
Post - DMPS	1	1	2	
Post - placebo	1	1	1	NS
3. Acanthosis :				
Post - DMPS	4	0	0	NS
Post - placebo	3	0	0	
4. Papillomatosis				
Post - DMPS	1	2	1	NS
Post - placebo	2	0	1	
5. Elong of rete-redges				
Post - DMPS	4	0	0	
Post - placebo	3	0	0	NS
6. Dysplasia				
Post - DMPS	4	0	0	NS
Post - placebo	3	0	0	
7. Basal Pigmentation				
Post - DMPS	4	0	0	
Post - placebo	2	1	0	NS

In our study, 19% cases had dysplastic changes varying from mild to severe. We found four cases of cancer - 2 cases of squamous cell carcinoma, 1 case of basisquamous and 1 case of basal cell carcinoma. All the 4 carcinoma cases were above 50 years age.

Dermal changes eg. Collagen fragmentation, oedema, increased vascularity and infiltration by chronic inflammatory cells were found in most cases.

AgNOR score of skin lesions in CAT (shown in Table-3) showed that normal control cases had a mean of 1.08 while skin changes without dysplasia scored 1.35, carcinoma had mean score of 3.56 while those with severe dysplasia had 3.0 and mild to moderate dysplasia had 1.73.

To assess the effect of chelating agents[8], the comparison of follow-up cases after giving DMSA and DMPS along with placebo to separate groups of patients were made. The result is shown in Table-4 and 5 respectively. DMSA was given to II patients, 1 case was not available on follow-up, hence 10 cases were compared. Placebo was given on 10 cases, 1 pre-placebo case

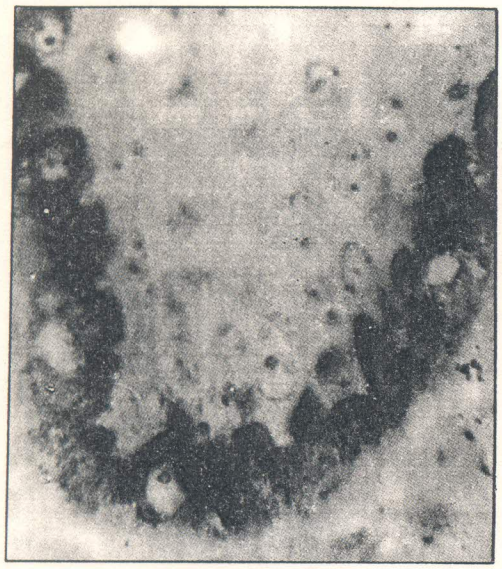

Fig.3 Photomicrograph showing AgNOR dots (score 1.35) in acanthosis (AgNOR Stain, X 500.

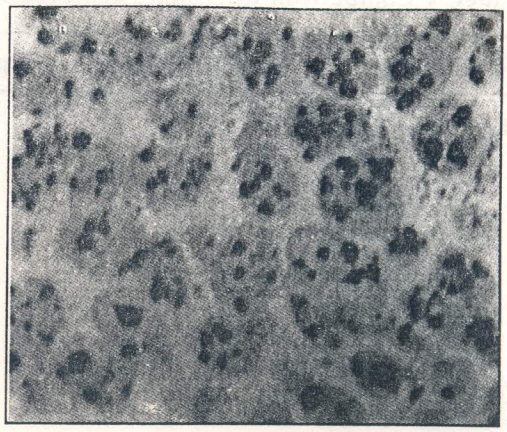

Fig. 4 Photomicrograph showing AgNOR dots (score 5.3) in sq. cell carcinoma (AgNOR stain, X 500).

had atrophied epidermis while 2 cases were not available on follow-up. Hence 7 out of 10 cases were compared. In both the drugs, the changes were not statistically significant.

DISCUSSION

Exposure to high arsenic containing drinking water is widespread because of easy availability of tubewell water which made people to switch over from drinking surface water. Shortest incubation period[9] for development of chronic arsenicosis was found to be 4 years. Involvement of skin is early and draws the attention of patients though internal organs are also frequently involved.

Cutaneous pigmentation is the early and most frequent feature occuring rain-drop like in the skin of body and limbs. Keratosis is another important feature of CAT involving palms and soles. Histologically, keratotic lesions show hyperkeratosis, parakeratosis, acanthosis and elongation of rete-ridges.

Arsenic is a known carcinogen. Chronic exposure to arsenic has a strong relationship with skin cancers. Various skin cancers were reported by different workers[10]. In our study all grades of dysplastic changes were noted. We found 4 carcinoma cases in our study.

The skin changes observed in our patients of chronic arsenicosis are non-specific. Additional information eg. high arsenic containing drinking water, high urinary arsenic excretion, high arsenic content of hairs/nails etc. are supportive of arsenical lesions and help to differentiate from other causes of skin lesions.

To quantify a particular epidermal change and to assess the changes which occurred in follow-up cases, we graded the lesions into mild, moderate and severe by using ocular micrometer for hyperkeratosis, acanthosis and elongation of rete-ridges while parakeratosis, papillomatosis and dysplasia were graded visually.

The traditional histopathologic study can diagnose a lesion but fails to predict its biological behaviours. Study of proliferative activity can help to assess the biological behaviour of such lesions. There are various methods eg. Mitotic count, DNA flow cytometry, Ki-67 scoring, PCNA immunostain, AgNOR scoring etc. which can assess proliferative activity of a lesion. Of these methods, AgNOR technique has been of great interest as this method is simple, relatively cheap, reproducible and can be applied on paraffin sections. Our study revealed that P-value of AgNOR scores in different lesions were significant. On the basis of statistically significant observation particularly in carcinoma cases, high AgNOR score can be utilized as a diagnostic marker for cancer cases. The mean score for each has wide gap from one another and definitely of great diagnostic help.

Till now, no effective drug therapy is available for reversing the lesions of CAT.

Aromatic retinoids[11] have been used elsewhere and shown to have some therapeutic effect. Chelating agents eg. DMSA and DMPS were utilized in arsenic clinic to assess their theraputic effect at SSKM Hospital, Calcutta. Follow up study showed that though some cases improved, other cases did not improve or even deteriorated. The placebo cases on follow-up showed the similar picture indicating that DMSA/ DMPS had no useful effect in therapy for CAT cases, so far as skin histology is concerned.

CONCLUSION

Arsenic is a major health hazard in different districts of West Bengal producing various skin lesions including carcinomas. AgNOR can be used as a good predictor for lesions with higher proliferative potential and can be a substitute for other methods of study for cell proliferation. Till an effective therapeutic agent is available, availability of safe water is the only remedy.

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