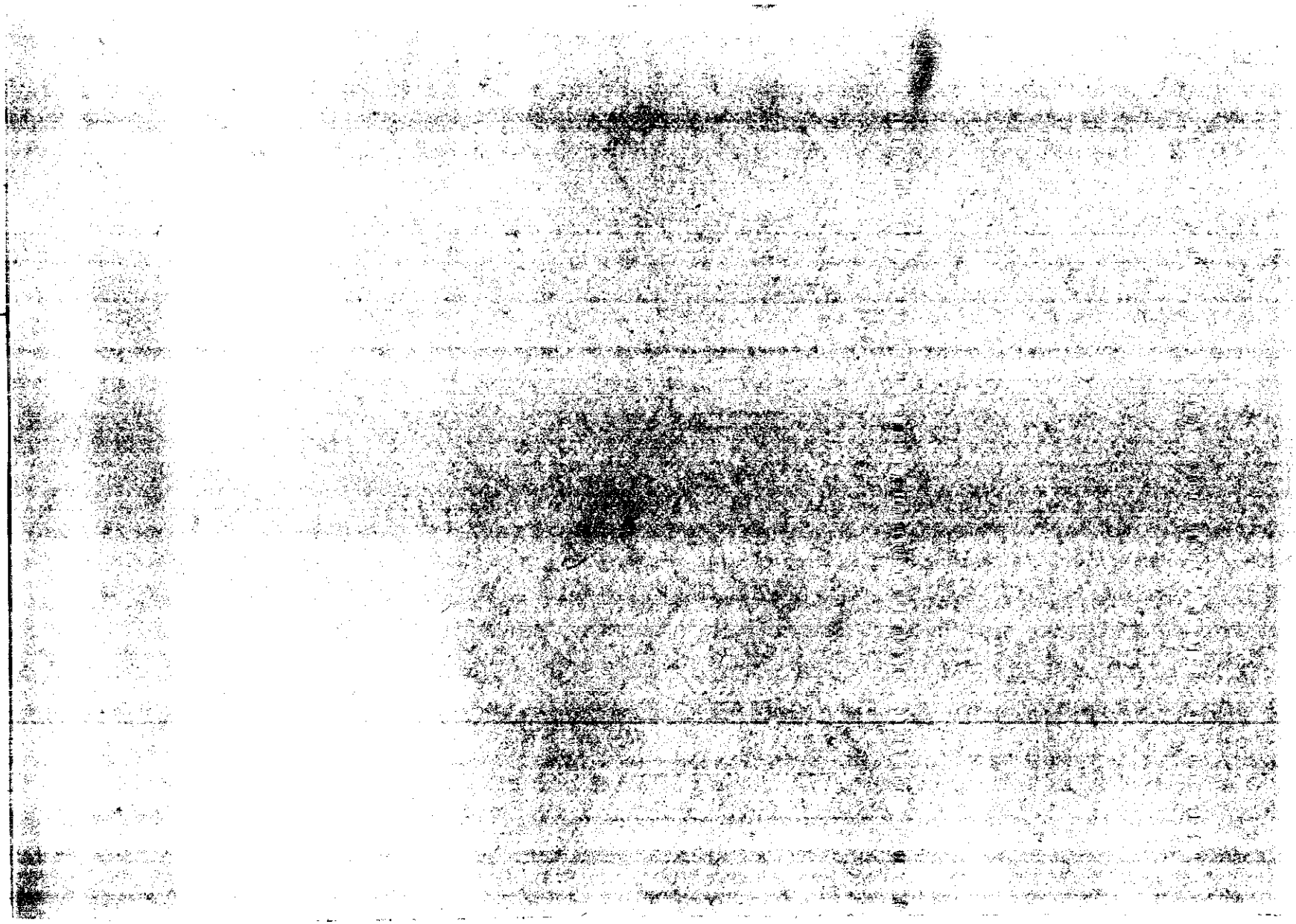
Results



RESULTS

Patients were divided into four groups. Group A included fifty patients, they were of equal sex distribution (table 2). The age ranged from 3 years to 59 years (Mean ± SD "standard deviation": 22.5 ± 10.8) (table 3). They were complaining of different types of warts. The total number of warts was 145 and according to type there were 51 plantar, 33 palmar, 34 common, 20 periungual, 4 filiform warts and 3 cases of plane warts. The duration of disease ranged from 6 months to 20 years (Mean ± SD : 22.2 ± 39.2).

Table (2): Comparison between group A & B as regards sex of patients.

Sex	Group A		Group B		Total		P value	Significance
	No	%	No	%	No	%		
Male	25	50	16	64	41	55	>0.05	Non significant
Female	25	50	9	36	34	45		

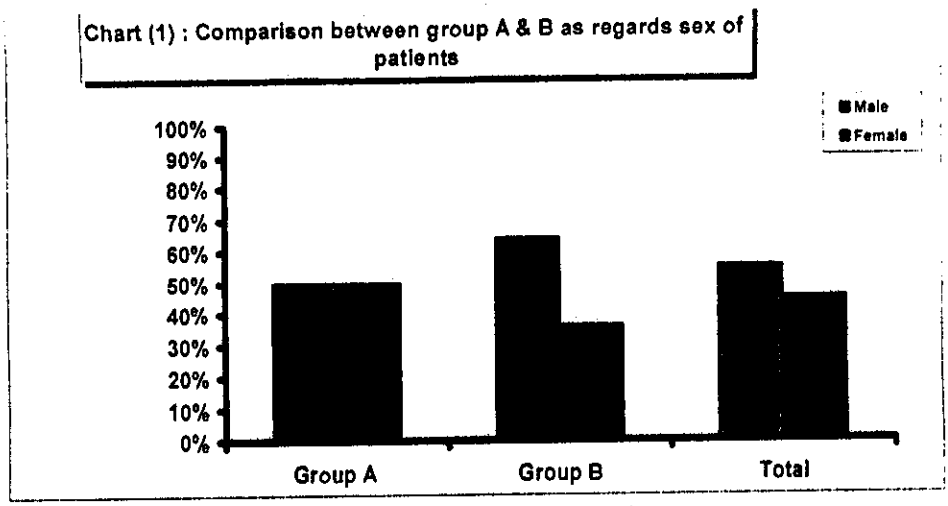


Table (3): Comparison between group A & B as regards age of patients.

Age	Group A n=50	Group B n=25	T	P	Significance
Mean±SD	22.5±10.8	21.8±9.7	0.12	>0.05	Non significant
Range					
minimum	3	20			
maximum	59	40			

Chart (2) : Comparison between group A & B as regards age of patients

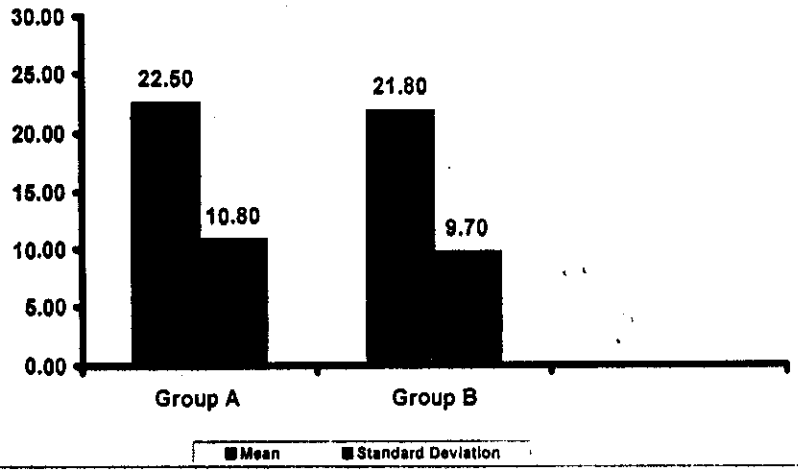
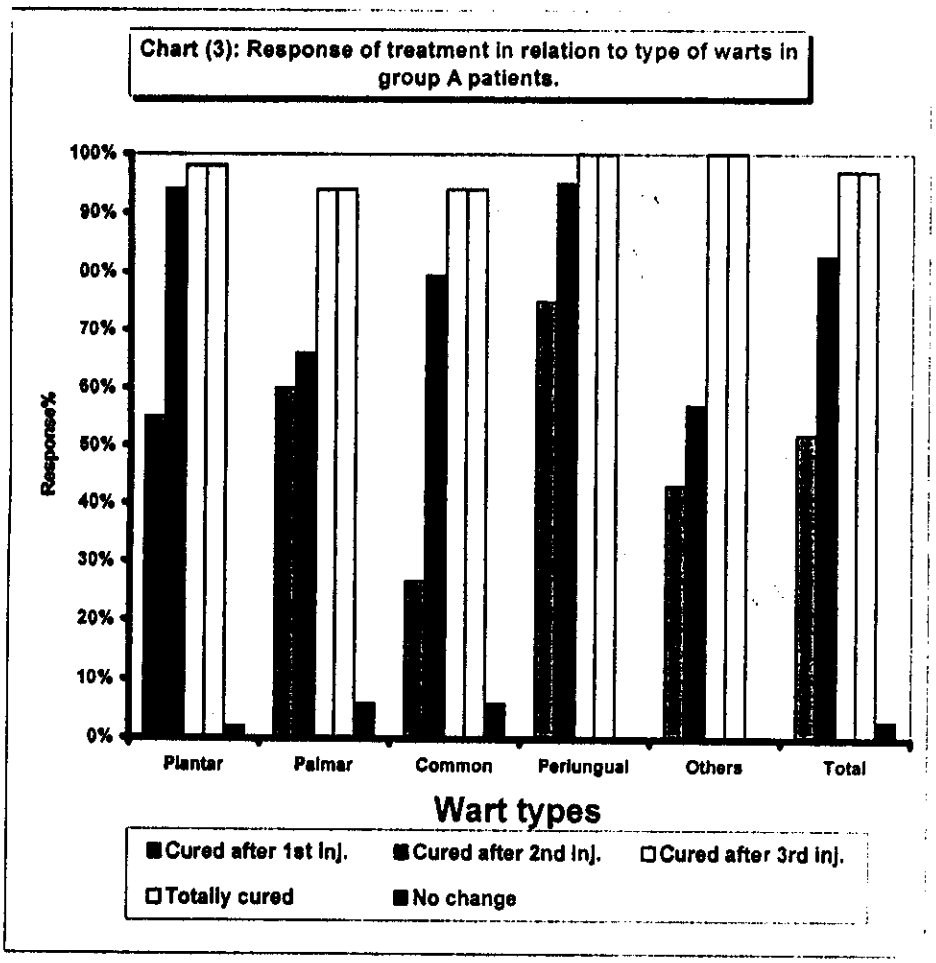


Table (4) shows response to treatment in relation to the type of wart in patients of group A. After 3 sessions of bleomycin injection, plantar, palmar, and common warts showed a total cure rate of 98%, 93%, and 93.5% respectively. On the other hand, periungual, filiform, and plane warts showed a total cure rate of 100%.

Table (4): Response to treatment in relation to the type of wart in group A patients.

Parameter	Plantar (N=51)	Palmar (N=33)	Common (N=34)	Periungual (N=20)	Others (N=7)	Total (N=145)
Cured after 1st injection						
(N)	28	20	9	15	3	75
(%)	55%	60%	26.5%	75%	43%	52%
Cured after 2nd injection						
(N)	20	2	18	4	1	45
(%)	39%	6%	52.5%	20%	14%	31%
Cured after 3rd injection						
(N)	2	9	5	1	3	20
(%)	4%	27%	14.5%	5%	43%	14%
Totally cured						
(N)	50	31	32	20	7	140
(%)	98%	93%	93.5%	100%	100%	97%
No change						
(N)	1	2	2	0	0	5
(%)	2%	7%	6.5%	0%	0%	3%
Worsened						
(N)	0	0	0	0	0	0
(%)	0%	0%	0%	0%	0%	0%



Group B included twenty five patients (table 2). The age ranged from 20 to 40 years (Mean ±SD : 21.8±9.7) (table 3). They were complaining of different types of warts. The duration of disease ranged from 6 months to 10 years (Mean ±SD : 24.1±32.7). None of the 25 control cases (treated by intralesional saline) showed any response after two weeks observation period.

Table (5) shows comparison of disease duration and cure rate in patients of group A. Twenty four patients with disease duration less than 1 year showed total cure rate of 95.8%. Twenty six patients with disease duration of 1 year or more showed total cure rate of 96.1%. There was no statistically significant difference between the two groups of patients as regards cure rate (P value >0.05).

Table (5): Comparison between disease duration and cure rate in group A.

Disease Duration	< 12 months	> 12 months	P value	Significance
No of patients	24	26	>0.05%	Non significant
Cure rate	95.8%	96.1%		

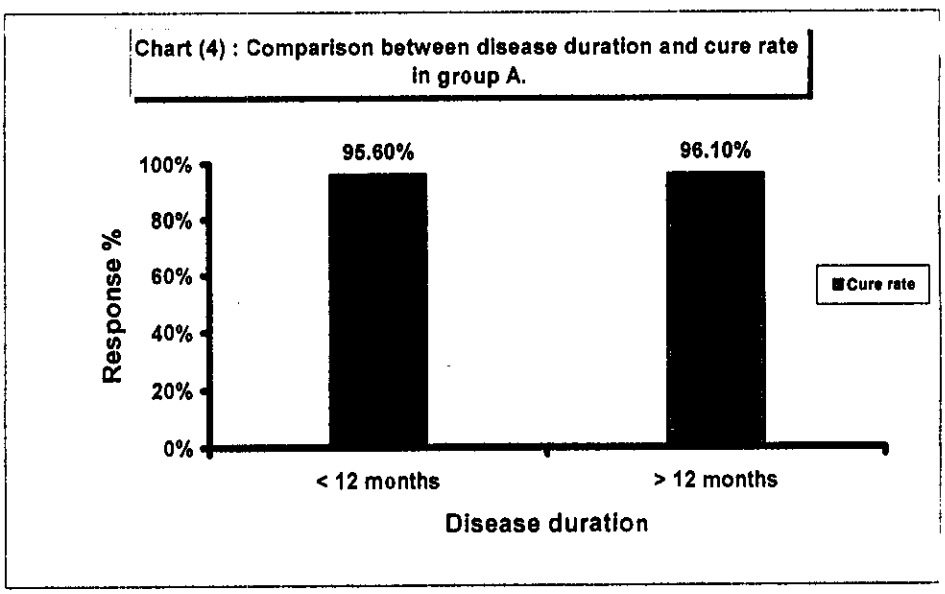
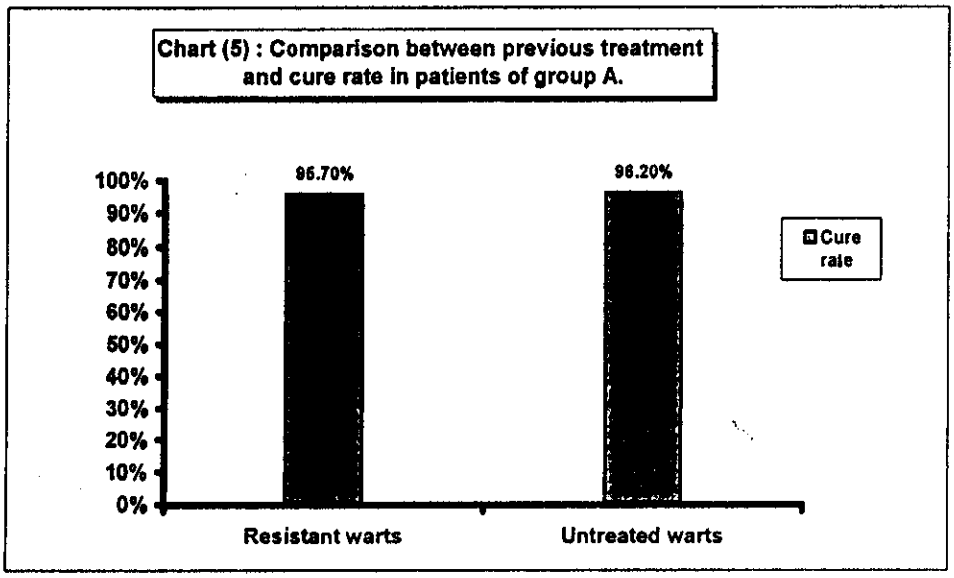


Table (6) shows comparison between past history of treatment and cure rate in group A. Twenty three patients had resistant warts and they showed cure rate of 95.7%. On the other hand, the number of patients who did not receive treatment before was 27 and they showed a cure rate of 96.2%. There was no statistically significant difference as regards the cure rate between the two groups of patients (P value >0.05).

Table (6): Comparison between previous treatment and cure rate in group A.

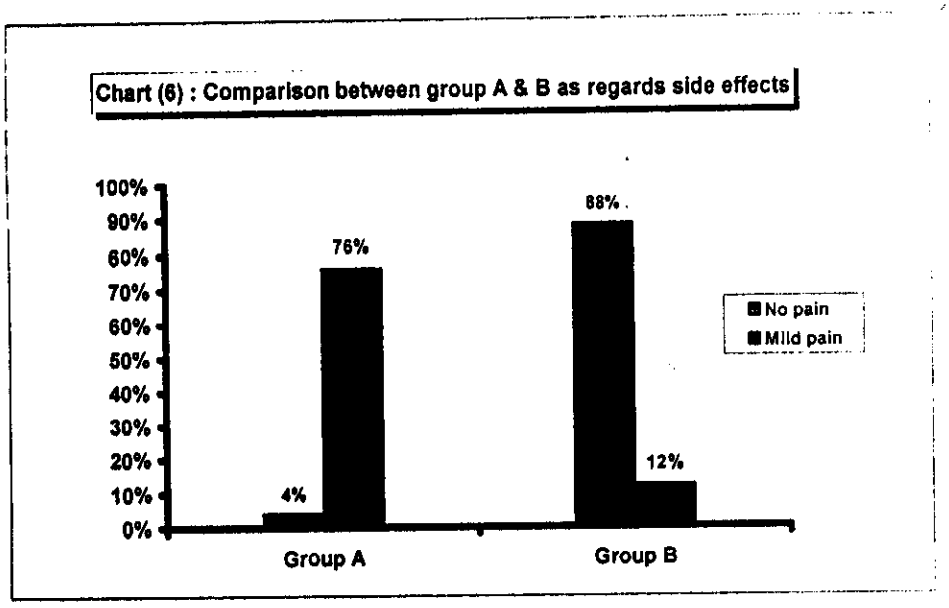
Past history of treatment	**Resistant (treated before)**	**Untreated**	**P value**	**Significance**
Number	23	27	>0.05	Non significant
Cure rate	95.7%	96.2%		



All patients of group A & B perceived pain during injection. This pain lasted for few seconds in group B (during saline injection) but, persisted for 15 minutes in group A (during bleomycin injection). Pain perceived by patients continued for a period of time. After injection, there was no pain in 2 cases (4%) of group A and in 22 cases (88%) of group B. Mild to moderate pain was perceived after bleomycin injection in 48 cases (96%) while in group B mild pain was perceived by 3 cases only (12%). Table (7) showed highly significant difference between the two groups as regards pain.

Table (7): Comparison between group A & B as regards side effects.

	Group A		**Group B**		**P value**	**Significance**
	No	**%**	**No**	**%**		
Pain during injection	50	100	25	100		
Pain after injection						
- No pain	2	4	22	88	<0.001	Highly
- Mild pain	38	76	3	12	<0.001	significant
- Moderate pain	10	20	0	0		
- Severe pain	0	0	0	0		
Hemorrhagic bulla	1	2	0	0		

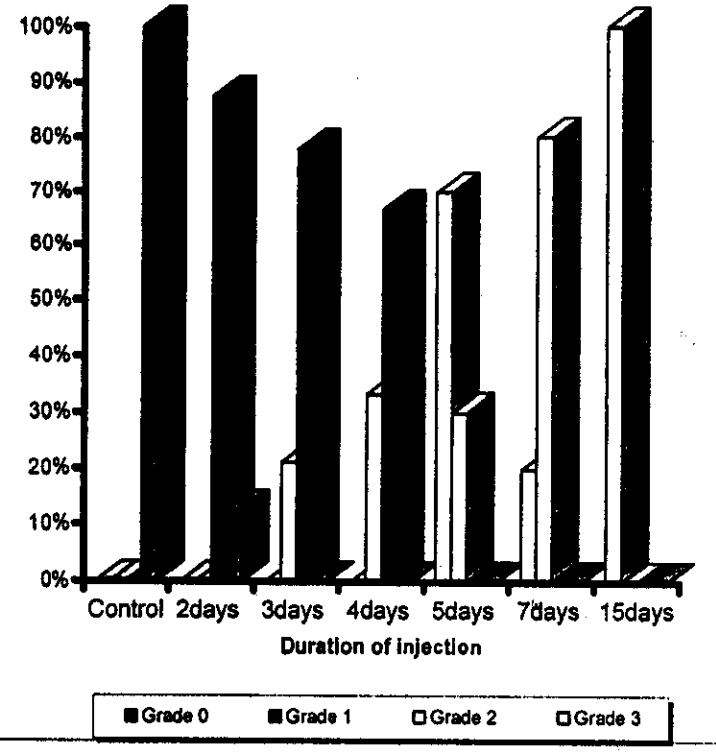


Patients complaining of common or plantar warts (not treated before) were included in group C. Bleomycin was injected intralesionally then these warts were biopsied. Wart biopsies were stained by hematoxylin and eosin stain (H&E). Seven warts not injected by bleomycin were examined as internal control of this group. Keratinocyte necrosis, hemorrhage, and neutrophilic infiltrate in dermis and epidermis were noticed in wart biopsies after bleomycin injection. The histopathological changes that occurred in wart biopsies after bleomycin injection were shown in table (8).

Table (8): Characters of wart biopsies as regards the grades of keratinocyte changes, hemorrhage and neutrophil infiltration by H&E staining in patients of group C.

	keratinocyte necrosis, hemorrhage, and neutrophil infiltration			
	Grade 0	Grade 1	Grade 2	Grade 3
Control N=7 %	7 100	0 0%	0 0%	0 0%
2days after injection N=8 %	1 12.5	7 87.5	0 0%	0 0%
3days after injection N=9 %	0 0%	7 77.8	2 21.2	0 0%
4days after injection N=9 %	0 0%	6 66.7	3 33.3	0 0%
5days after injection N=10 %	0 0%	3 30	7 70	0 0%
7days after injection N=10 %	0 0%	0 0%	8 80	2 20
15days after injection N=7 %	0 0%	0 0%	0 0%	7 100

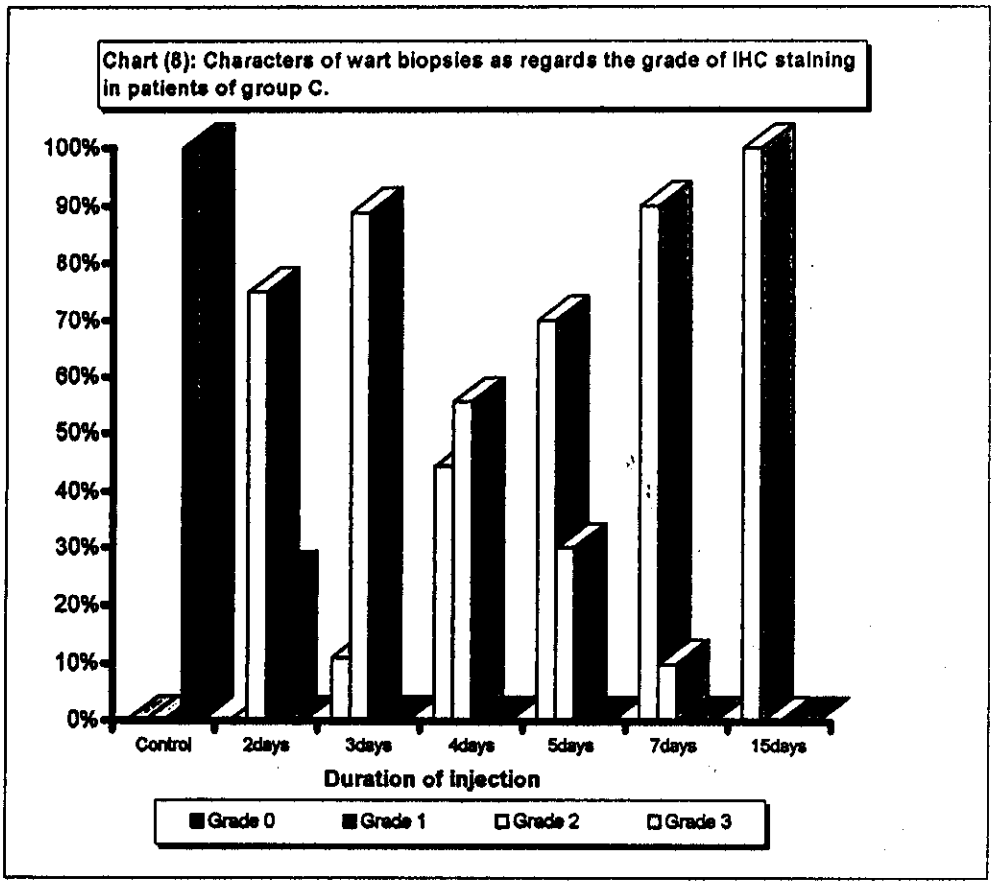
Chart (7): Characters of wart biopsies as regards the grades of keratinocyte changes, hemorrhage and neutrophil infiltration by H&E staining in patients of group C.



All the biopsies including the control (group C) were subjected to immunohistochemical (IHC) staining to visualize the adhesion molecule CD44. The stain was exclusively membranous. The control biopsies showed complete staining of full thickness of the epidermis. Examination of wart biopsies after bleomycin injection revealed gradual loss of IHC stain by different degrees according to bleomycin duration. The pattern of IHC stain expression in wart biopsies before and after bleomycin injection was shown in table (9).

Table (9): Characters of wart biopsies as regards the type of wart and grade of IHC staining in group C.

	Total	Grades of IHC staining			
		Grade 0	Grade I	Grade II	Grade III
Control Number Percentage	7 100%	7 100%	0 0%	0 0%	0 0%
2days after injection Number Percentage	8 100%	0 0%	2 25%	6 75%	0 0%
3days after injection Number Percentage	9 100%	0 0%	0 0%	8 88.9%	1 11.1%
4days after injection Number Percentage	9 100%	0 0%	0 0%	5 55.6%	4 44.4%
5days after injection Number Percentage	10 100%	0 0%	0 0%	3 30%	7 70%
7days after injection Number Percentage	10 100%	0 0%	0 0%	1 10%	9 90%
15days after injection Number Percentage	7 100%	0 0%	0 0%	0 0%	7 100%



Completely normal six volunteers were included in group D. A normal skin biopsy was taken from volar surface of the forearm before injection of bleomycin and was examined by H&E and IHC stain.

Other skin biopsies were taken 3 days after intradermal injection of bleomycin. These biopsies revealed (grade 1) keratinocytes necrosis, hemorrhage and neutrophilic infiltrate after H&E stain.

All skin biopsies, after intradermal bleomycin injection, were also stained by IHC stain to visualize CD44. The stain was

exclusively membranous. There was complete expression of IHC stain in full epidermis in normal skin biopsies. On the other hand, there was loss of stain by different degrees in skin biopsies after bleomycin injection. Most skin biopsies showed grade 1 (3 cases), two cases showed grade 2 and one case showed grade 3.

Fig. (2): Common wart before bleomycin injection.

Fig. (3): Common wart after bleomycin injection showing blackening due to local thrombosis and hemorrhage.

Fig. (4): Palmar wart before bleomycin injection.

Fig. (5): Palmar wart after 2 weeks of bleomycin injection (totally separated).

Fig. (6): Multiple plantar warts before bleomycin injection.

Fig. (7): Multiple plantar warts after 2 weeks of bleomycin injection.

Fig. (8): Multiple common and periungual warts before bleomycin injection.

Fig. (9): The previous case after bleomycin injection.

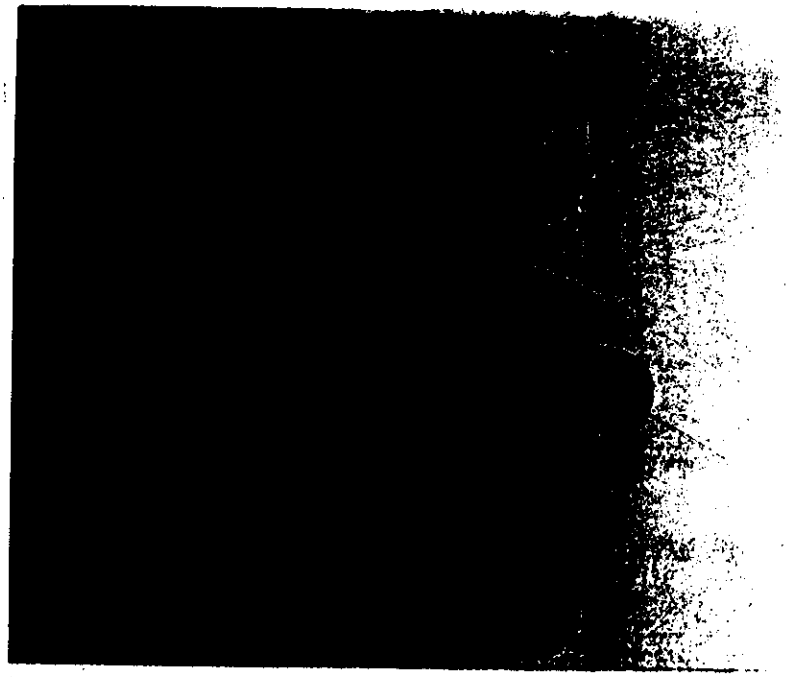


Fig. (10): Large periungual wart before bleomycin injection.

Fig. (11): The previous case after the 1st injection (smaller in size).

Fig. (12): The previous case after 2nd injection (completely cured).

Fig. (13): Multiple plantar warts before bleomycin injection.

Fig. (14): The previous case after bleomycin injection (completely cured).

Fig. (15): Multiple plane warts before bleomycin injection.

Fig. (16): The previous case after two injection sessions (completely cured).

Fig. (17): Another view of the previous case.

Fig. (18): Multiple common warts before bleomycin injection.

Fig. (19): The previous case showing hemorrhagic bullae in two warts and blackening of the other warts after bleomycin injection.

Fig. (20): Common wart before bleomycin injection (control) showing hyperkeratosis, parakeratosis, acanthosis, hypergranulosis with koilocytes (H&E ; X40).

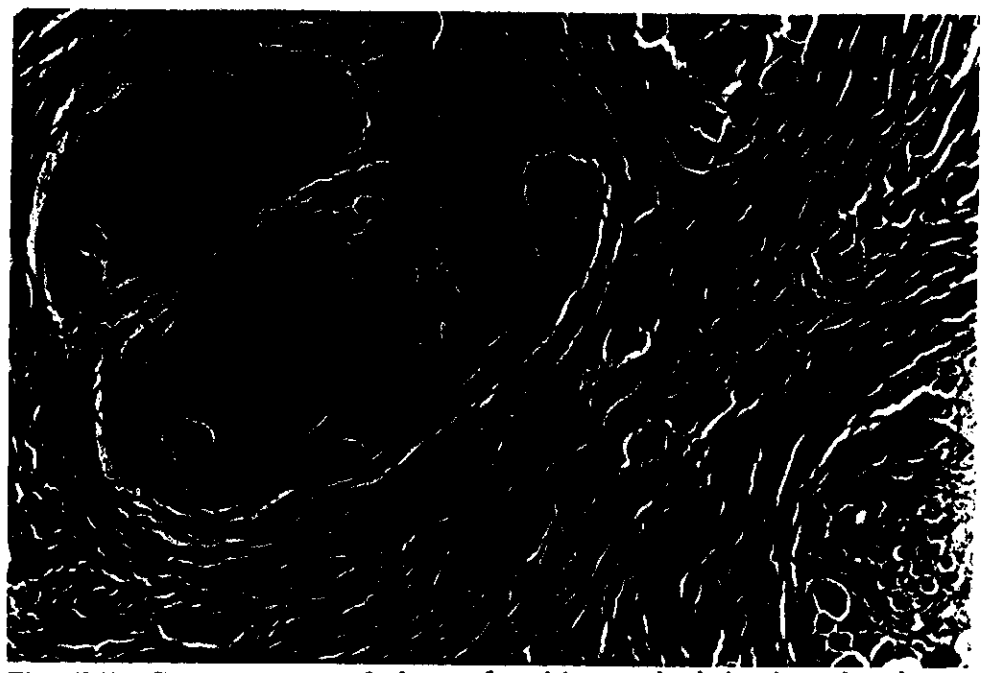


Fig. (21): Common wart 3 days after bleomycin injection showing moderate keratinocyte necrosis, hemorrhage and neutrophilic infiltrate (H&E ; X100).

Fig. (22): Common wart 3 days after bleomycin injection showing moderate keratinocyte necrosis, hemorrhage and neutrophilic infiltrate (H&E ; X40).

Fig. (23): Common wart 5 days after bleomycin injection showing moderate keratinocyte necrosis and prominent neutrophilic infiltrate (H&E ; X100).

Fig. (24): Common wart 7 days after bleomycin injection showing severe keratinocyte necrosis, hemorrhage and neutrophilic infiltrate (H&E ; X40).

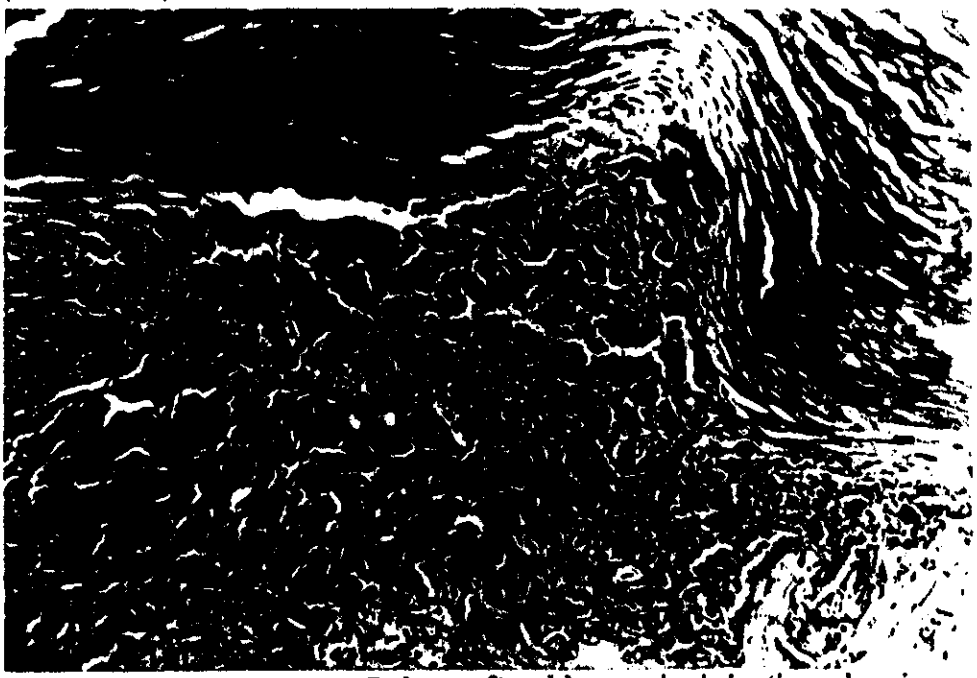


Fig. (25): Common wart 7 days after bleomycin injection showing severe keratinocyte necrosis, hemorrhage and neutrophilic infiltrate (H&E ; X100).

Fig. (30): Common wart 15 days after bleomycin injection showing severe keratinocyte liquefactive necrosis, hemorrhage and neutrophilic infiltrate (H&E ; X40).

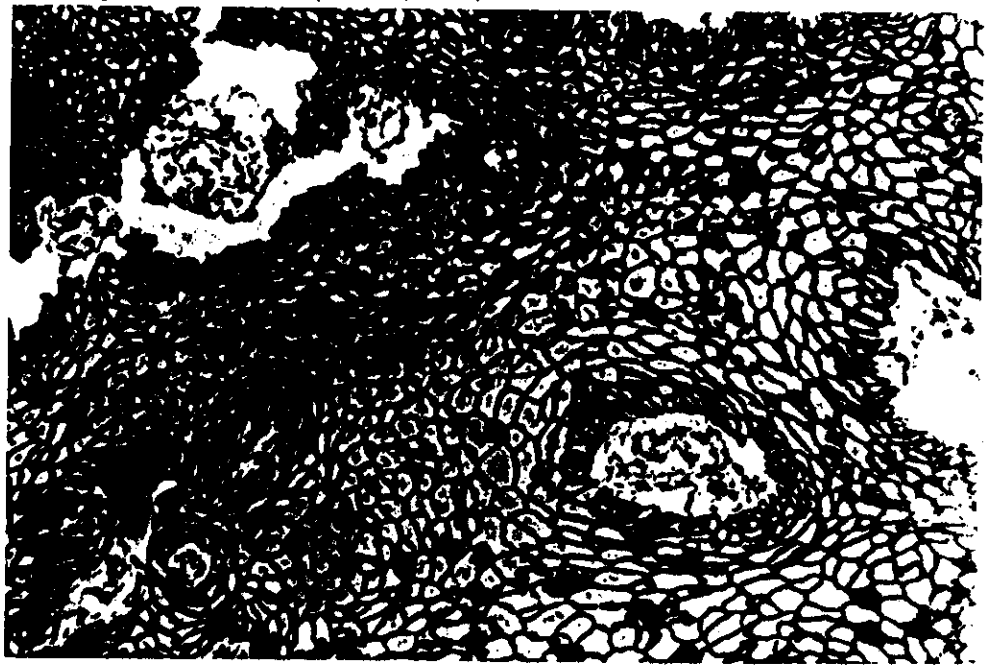


Fig. (31): Common wart before bleomycin injection (control) showing membranous staining of full epidermal thickness (grade 0) (IHC ; X100).

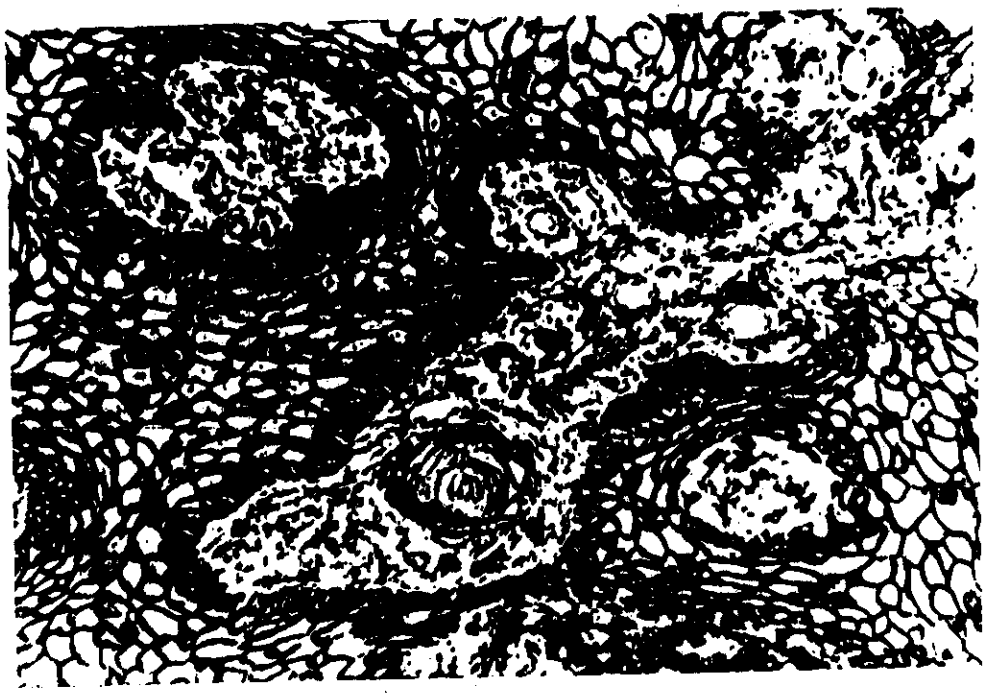


Fig. (32): Common wart before bleomycin injection (control) showing membranous staining of full epidermal thickness (grade 0) (IHC ; X100).

Fig. (33): Common wart 2 days after bleomycin injection showing loss of IHC stain from upper third of epidermal thickness (grade 1) (IHC ; X100).

Fig. (34): Common wart 3 days after bleomycin injection showing loss of IHC stain from upper two thirds of epidermal thickness (grade 2) (IHC ; X40).

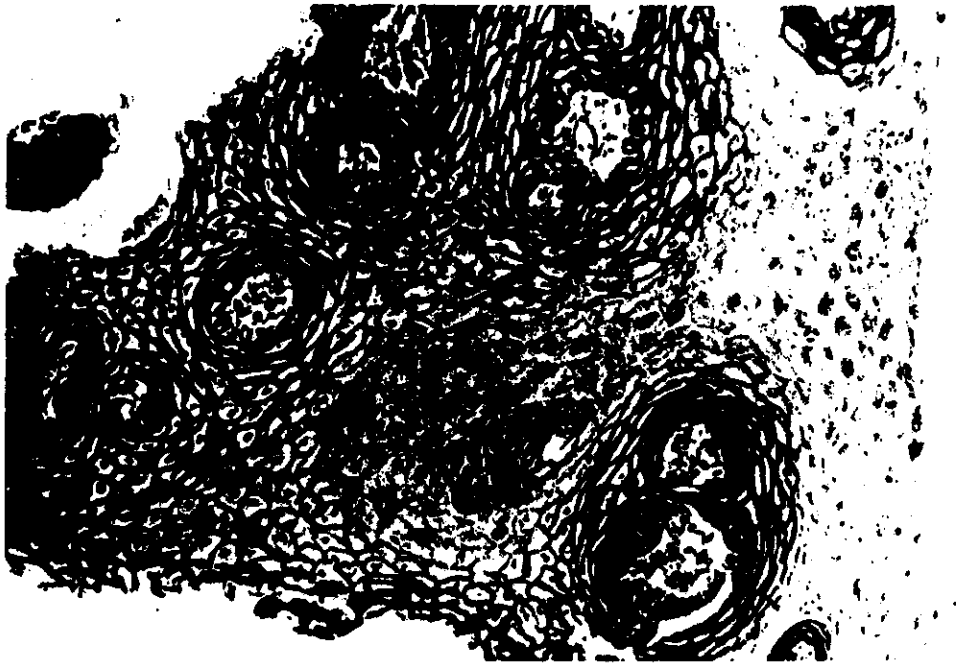


Fig. (35): Common wart 5 days after bleomycin injection showing severe loss of IHC stain (grade 3) (IHC ; X100).

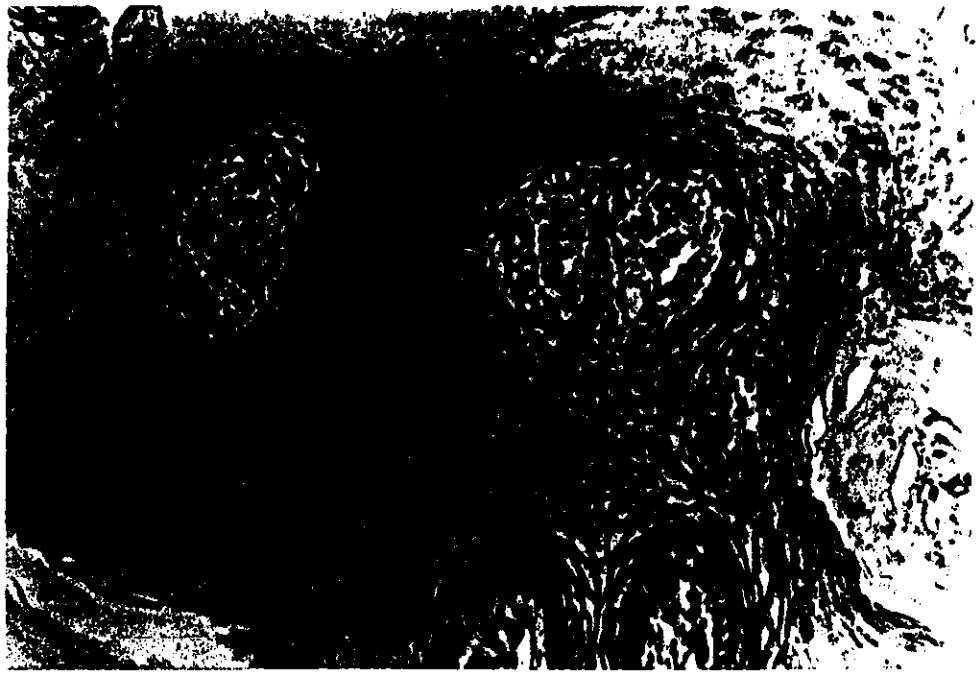


Fig. (36): Common wart 7 days after bleomycin injection showing near to complete loss of IHC stain (grade 3) (IHC ; X100).

Fig. (37): Common wart 15 days after bleomycin injection showing microthrombi and complete loss of IHC stain (grade 3) (IHC ; X100).

Fig. (38): Normal skin 3 days after bleomycin injection showing keratinocyte necrosis, hemorrhage, and neutrophilic infiltrate (grade 1) (H&E ; X40).

Fig. (39): Normal skin 3 days after bleomycin injection showing keratinocyte necrosis, hemorrhage, and neutrophilic infiltrate (grade 1) (H&E ; X100).

Fig. (40): Normal skin 3 days after bleomycin injection showing keratinocyte necrosis, hemorrhage, and neutrophilic infiltrate (grade 1) (H&E ; X100).

membranous expression of IHC stain in full epidermal thickness, endothelial cells lining small blood vessels and epithelium of hair follicles (grade 0) (IHC ; X40).

Fig. (42): Normal skin before bleomycin injection (control) showing membranous expression of IHC stain in full epidermal thickness and endothelial cells lining small blood vessels (high power of previous

Fig. (43): Normal skin 3 days after bleomycin injection showing loss of IHC stain in upper third of epidermal thickness (grade 1) (IHC ; X100).

Fig. (44): Normal skin 3 days after bleomycin injection showing loss of IHC stain in upper two thirds of epidermal thickness (grade 2) (IHC ; X40).

Fig. (45): Normal skin 3 days after bleomycin injection showing near to complete loss of IHC stain from epidermis and hair follicle epithelium (grade 3) (IHC ; X100).

Fig. (46): Normal skin 3 days after bleomycin injection showing near to complete loss of IHC stain from epidermal thickness (grade 3) (IHC ; X100)