TESTICULAR ALTERATIONS IN HANSEN'S DISEASE[1]

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ABSTRACT - *Fifty testicular specimens and seminal fluid of 10 normal individuals and 24 patients with Hansen's disease were studied. General and early histological alterations, the frequency of young spermatides in tubular lumen and the measure of the diameters of 1.450 seminiferous tubules were checked. According to the results, it was not possible to establish a qualifying correlation within histological descriptions and/or a quantifying correlation within morphometry from a specific clinical group with Hansen's disease. Thus, the simultany and the variability of the findings seen in a same testis are opposite to the evolving phases described by Grabstald and Swan and to the classification presented by Kumar. The authors suggest that the testicular alterations be a consequence of an auto- aggression to the gland.*

Key words: *Hansen's disease. Testes. Histology. Morphometry. Auto-aggression.*

1. INTRODUCTION

The first comments about testicular injury in Hansen's disease were reported by A. Neisserl [2], V. Gomil &V. Babes[3] and H.C. Lelooir1 in the last century. But only circa 1943 it was established that the testes could be destroyed as a functioning organ[6].

H. Grabstald & L.L. Swan[7] classified the testicular alterations of Hansen's disease In an evolving way: vascular, interstitial and obliterative. However, recently B. Kumar et all 0 contested such an evolving classification since they detected all kinds of alterations simultane- ously In virchowian patients; and they proposed that the histopathological findings of the testes be grouped in a) minimum (10%), b) intermediary (35%) and c) maximum (30%).

Looking over these reports, it was observed that the studies were conducted preferably within virchowian patients and little is know about the alterations seen In the mild presentations of the disease. Thus, aiming at studying testicular injury in the various Hansen's disease clinical groups, this research was made.

2. MATERIAL AND METHODS

2.1. Hansen's group

Twenty-four consecutive patients with Hansen's disease, and from 18 to 64 years of age (x - 41.3 years), registered in the Hansen's Disease Program of São Paulo State Public Health Service (SESSP), were included In this study. Diagnosis and classification of the disease were performed by only one dermatologist, who made use of Madrid's claSsification13. Among the 24 patlehts, 12 were virchowians (V), 6 were dimor-

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phous (D), 4 were tuberculoid (T) and 2 were Indeterminate (I). They had all been undergoing a specific, one-to-seven clinical treatment period (x = 4.6 years). Those with clinical manifestations of uretritis, prostatitis, epldidymorchitis and varicocele were excluded. After being in- formed about and giving written consent for this study, all the 24 patients with Hansen's disease were submitted, on rachianesthesia, to a bilateral testicular biopsy scrotal via. The testicular specimens were immediately fixed In Bouim liquid for 6 hours and, then, parafinized. Following, they were cut 5 micrometers thick and stained by Periodic Accid-Schiff (PAS), Masson Tricromlc (MA), Hematokilin-Eosin (HE) and Ziehl-Neelsen (ZN) techniques, for common optic-microscopy examination. It must be said that the glasses of the study group were coded so as to disallow the investigator to know which Hansen's disease was being analysed. Twenty five seminiferous tubules for each specimen were analysed in relation to histology and morfometry. In this analy- sis, the measuring of the diameter of 1.450 seminiferous tubules and the description of the most differentiated element of the germinative epithelium were outlined. The material destined to seminal analysis was obtained by masturbation after a 5-day sexual abstention, and only the quantity of spermatozoa per mm3 was considered.

2.2. Control group for testicular histology

The testicular fragments used for histo-logical control were taken from 5 individuals who had died of accidental causa mortis, proceeding from the "Autopsy Service Department of São Paulo" (IMLSP). They were from 20 to 30 years old and their death might have occurred for 6 hours at the most. The material obtained was processed and analysed according to the same criteria for the Hansen's group.

2.3. Control group for seminal analysis

The seminal fluid analysed destinated to to the control group was taken from 9 patients from 28 to 35 years old at the "Family Planning Serv- ice", and had been waiting voluntary sterelization procedure (vasectomy).

2.4. Statistical analysis

For statistical analysis of the results, no parametric tests were used: Kruskal-Wallis vari- ance analysis and Dunn test were applied In orderto comparethe results of the diameters and the quantity of tubules with young spermatides, and Wilcoxon test for the investigation of a pos- sible difference between' the measurement re- sults of the right and left testes8,16. This proce- dure was applied solely in the V and D patients due to the small dimension of the other samples. The mean (x), the standard desviation (SD) and the Pearson variance ratio of the seminiferous tubule diameters were also calculated in order to s udy the heterogeneity of the results. It was stated a Alfa <0,05 for all those tests and the statistically significant results were asterisked.

3. RESULTS

3.1. Testicular histology

The histological study of the 10 testes fragments of the control group has not shown up alterations (Figure 1).

However, the Hansen's group showed bilateral testicular alterations in all cases studied. The histological results of the coded glasses were re-organized according to the classifica- tion of the disease for its analysis. Thus, in the dimorphous group (Figure 2) the histologyshowed enlarged interstice caused by an accumulation of amorphous eosinophilic material, a slight short- ening in the mean diameter of the seminiferous tubules, loss of cellular stratification, loss in the height of the seminiferous epithelium, and the presence of young cells in the lumen. In the virchowian patients (Figure 3) the testicular In- jury was most marked. There were focal areas of destruction with sclerosed tubules so severely damaged that they could only be detected when stained by MA technique. A thickening in the

blood vessel walls and in the tubule basal membrane was observed. In the remaining tubules there was an intensive reduction in the median diameter and the epithelium ceased maturarion. In some specimens, the interstice showed a peritubular lynphocytic infiltrate and an increase in the quantity of Leydig cells (Figure 4). The patients of tuberculoid group showed a histology similar to that of D and V groups, although with less intensity in the focus areas. Tuberculoid granuloma was not observed (Figure 5). Like- wise, the patients of indeterminate group showed to be similar to the other groups, with focus areas of intense alterations in the interstice and in the seminiferous tubules, and sites with histological aspect close to normal (Figure 6). The examina- tion of the material of all cases did not detect bacilli.

3.2. Testicular morfometry

The mean diameter (d) of the seminiferous tubules of each specimen are shown in Table 1. After statistical analysis, it was concluded that the D in the V, T and I was smaller and statistically significant, when compared to the D of the healthy subjects of control group. The D in the D patients was smaller but not significant. There was no difference between aggression to right and left testes. The result of the counting of the seminiferous tubules with young spermatides in the lumen is shown in Table 2. The mean obtained from the tubules examined in every Hansen's group was superior to the one obtained from the control group, but not significant.

3.3: Seminal analysis

The result of the seminal fluid analysis is shown in Table 3. The mean of the count of sperm per mm3 of the seminal fluid in the Hansen's patients was smallerthan that in the control group, though statistically significant was only the difference from that of the V group.

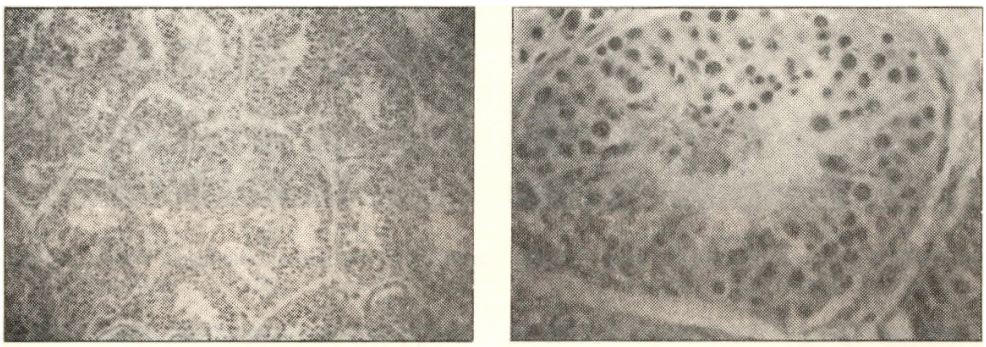

FIGURE 1 - Photomicrograph of a control patient's testis. Hematoxilin-Eosin stain; x 125 and x 500.

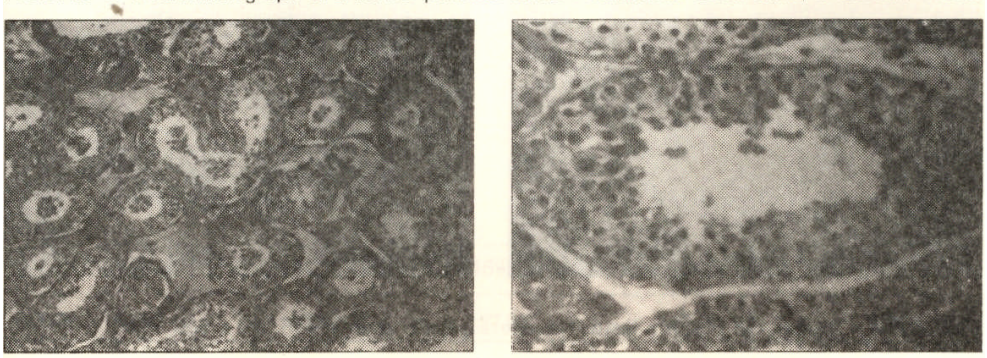

FIGURE 2 - Dimorphous patient's testis. Hematoxilin-Eosin stain; x 125 and x 500.

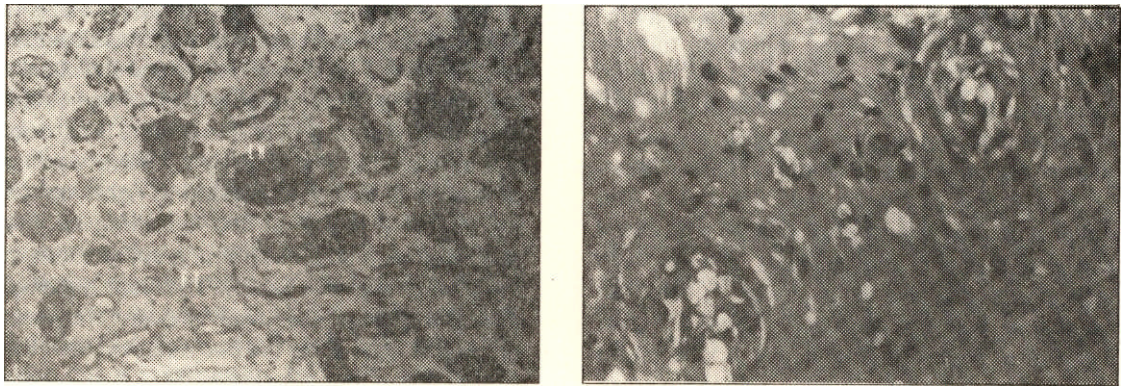

FIGURE 3 - Virchowian patient's testis. Area with fibrous tissue (FT.) and Leydig cells hiperplasia (L.H.). Hematoxilin-Eosin stain; x 125 and x 500.

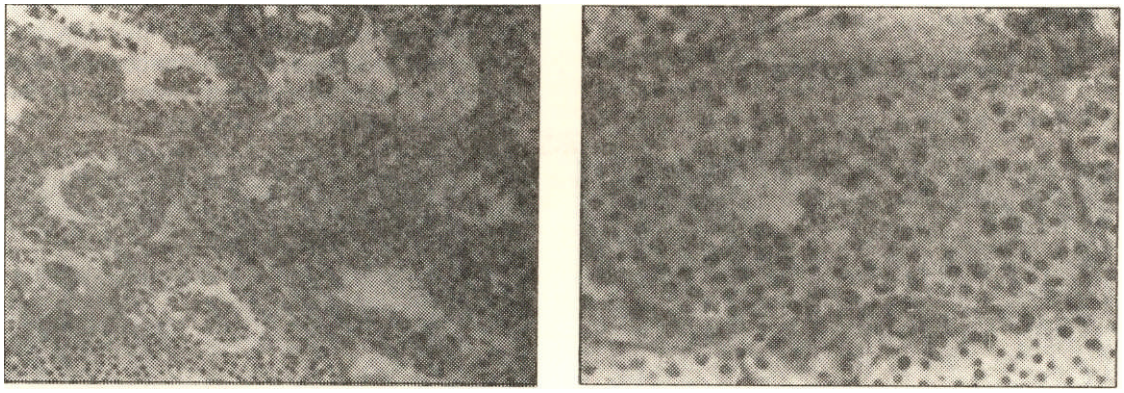

FIGURE 4 - Virchowian patient's testis. Relatively preserved area.Hematoxilin-Eosin stain x 125 and x500.

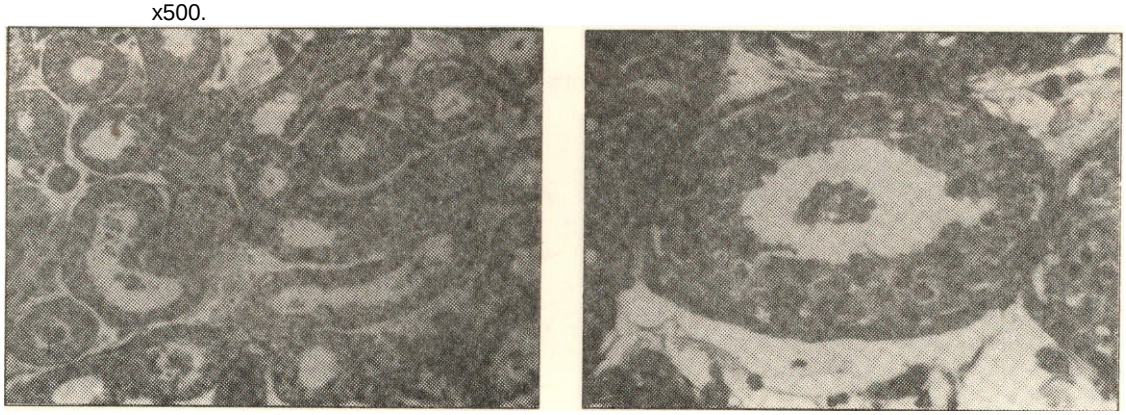

FIGURE 5 - Tuberculoid patient's testis. Hematoxilin-Eosin stain; x 125 and x 500.

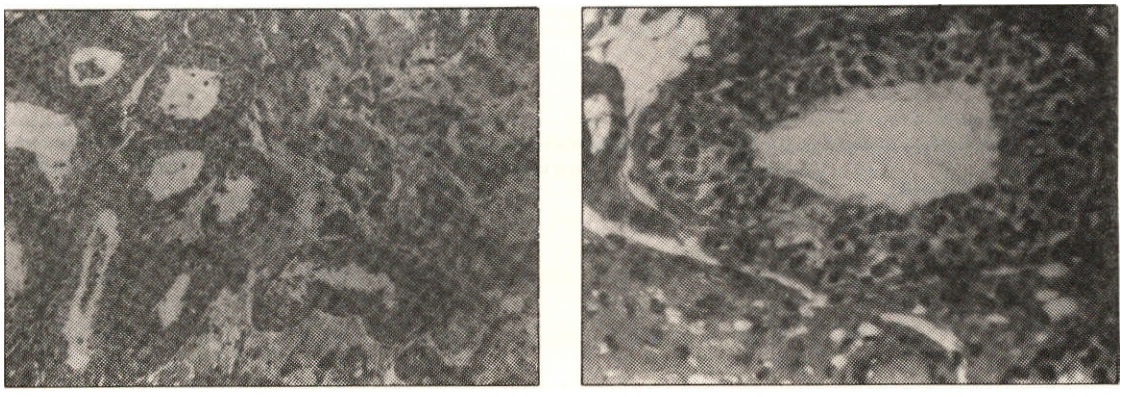

FIGURE 6 - Indeterminate patient's testis. Intense aggression on the right side and a relatively preserved area on the left side. Hematoxilin-Eosin; x 125 and x 500.

TABLE 1 - Range of mean values of 25 seminiferous tubules in micrometers, of each testis from Control Group and Hansen's Group.

n	Controls	Virchowian		Tuberculoid		Dimorphous		Indeterminate	
		R	L	R	L	R	L	R	L
1	207,4(L)	165,2	0	180,4	156,2	163,2	155,6	139,0	122,4
2	206,8(R)	171,6	142,4	180,0	198,6	202,6	178,4	154,2	100,8
3	270,0(L)	117,0	128,4	145,0	121,8	181,8	198,6		
4	242,6(R)	0	0	144,6	150,8	182,8	178,4		
5	240,4(L)	154,2	174,0			167,8	182,2		
6	227,6(R)	180,4	166,2			162,0	152,4		
7	204,0(L)	87,8	58,4						
8	228,2(R)	86,0	87,1						
9	197,2(L)	159,6	155,0						
10	221,8(R)	160,6	166,6						
11		150,8	143,0						
12		14,4	11,4						
MEAN	**224,60**	**117,67**		**159,68**		**175,48**		**129,10**	
SCORE	**53,20**	**18,52**		**27,94**		**37,46**		**15,58**	

R = right, **L** = left, **H** = 35,37*, Critical Value (0,05) = 9,49

TABLE 2 - Moan of seminiferous tubules with young spermatides In 25 tubular lumen of each Control and Study Patients.

n	Controls	Virchowian		Tuberculoid		Dimorphous		Indeterminate	
		R	L	R	L	R	L	R	L
1	**0**	0	0	**4**	4	5	3	4	3
2	0	2	6	9	3	0	1	1	1
3	0	6	6	1	1	3	2		
4	2	!	!	1	0	1	1		
5	0	4	4			2	0		
6	1	2	2			0	1		
7	1	0	0						
8	1	0	0						
9	0	2	0						
10	1	1	1						
11		1	1						
12		!	!						
T	**600**	**200**		**300**		**100**			
MEAN	**2,4**	**7,6**		**11,5**		**6,3**		**9,0**	

R right, **L** left, **T** total tubules measured and **n** number of patients studied. ! =sclerosed tubules.

TABLE 3 - Count of spermatozoa per mm3 and motile sperm during the first hour In the Control and Study Patients.

n	Controls		Virchowlan		Tuberculoid		Dimorphous		Indeterminate	
	C	M	C	M	C	M	C	M	C	M
1	84,0	39	0,48	15	15,75	35	48,50	50	28,75	10
2	20,5	5	10,5	30	0,04	5	88,5	5	37,50	20
3	151,0	57	3,55	10	56,0	60	52,0	90		
4	124,0	0	0	0	102,5	80	74,0	30		
5	30,0	0	46,0	86			41,50	60		
6	62,0	29	14,0	60			32,0	25		
7	96,0	34	0	0						
8	340,0	5	0,03	0						
9	150,0	30	52,5	70						
10			63,5	75						
11			31,0	20						
12			0,15	0						
MEAN	**117,38**	**22,1**	**17,31**	**36,6**	**43,57**	**45**	**56,08**	**43,3**	**33,13**	**15**

n=. number of patients studied, **C-** count x 106 and **M =** percentage of

4. DISCUSSION

In this investigation, the observation of testicular alterations in the various groups of Hansen's disease and with a short period of disease, supplied relevant histological findings so far only referred to vIrchowian patients. It must be said that these observations are opposed to the evolving phases described by H. Grabstald & LL. Swan7, and to the quantitative alterations standards presented by B. Kumar et al.[10], due to the intense variability and simultaneity of the findings, which are characterized by the testes. Besides the disparity of those results, the altera- tions referred to as minimum, and found in 10% of B. Kumar et all[10], were detected in 89% of the investigated testes from the hansenians. In these areas, in which the tubular archtteture was relatively preserved, a decrease In the height of the semintferous epithelium, a cease in maturation generally In the phase of young spermatides and the presence of an accented and premature exfoliation of these cells to the lumen were detected. These alterations In the seminiferous tubules generally translate a recent aggression to the gonad1,2 and can suggest that the compartment of Sertoly cells has been injured, once they are responsible for the maturation and liberation of the mature spermatides in thetubular lumen[4,18.] Thus, those findings could reflect a rupture of the hemato-testicular barrier and an autoegression to the tubule by the patient's Immuno-

logical defence, Reenforcing this hypothesis, in this study It was not possible to detect the pres- ence of bacilli, despite the use of ZN technique, making us think that, perhaps, bacilli play only a supporting role in the physlopathology of the lesions17.18, yet at its early onset phase.

As to the results in the seminal fluid analy- sis, they demonstrated a direct correlation to histological aspects, and they also differ from what Is found in literature, where as the percent- age of azoospermia among thevirchowians range from 61% to 97% according to several au- thors5,9,10,15, this study observed a percent- age of only 16,6% (Table 3). In relation to other researches, this discrepancy presented may be explained by the small number of samples and by the disease's short period of injury In the patients studied.

Thus, significant alterations were detected In the germ epithelium through testicular histology and morphometry, in all Hansen's disease's patients studied. However, it was not possible to establish a specific qualitative or quantitative correlation of a determinate group. Those facts, besides being contrary to the results in medical literature, point out that testicular aggression is precocious and Insidious, far anteceding the irreversible lesions presently known.

RESUMO - Estudaram-se 58 fragmentos testiculares e o espermograma de 10 indivíduos normais e 24 hansenianos. Verificaram-se as alterações gerais e precoces na histologia, a freqüência de espermátides jovens no lúmen tubular e a medida dos diâmetros de 1.450 túbulos seminíferosos. De acordo com os resultados obtidos, não foi possível estabelecer uma correlação qualitative entre as descrições histológica e ou quantitativa entre a morfometria, e um grupo clínico específico de hansenianos. Assim, também, a simultaneidade e a variabilidade dos achados observados em um mesmo testículo, se opõe as fases evolutivas, descritas por H. Grabstald & LL Swan e à classificação proposta por B. Kumar of aL Os autores sugerem que as alterações testiculares sejam conseqüência de uma auto-agressão à glândula.

Palavras chave: Hanseníase. Testículos. Histologia. Morfometria. Auto-agressão.

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