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PROGNOSTIC SIGNIFICANCE OF LEVEL AND NUMBER OF LYMPH NODES METASTASES IN COLORECTAL CANCER

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Great difference in survival may be solved by Dukes C patients subclassification related to positive nodes number. Chemical lymph nodes clearance is a superior technique to identify the total number and the positive lymph nodes in colorectal cancer surgery. In the present study we investigated the clinico-pathological association of positive lymph node numbers, level, with their number in every level and their prognostic value. Also, the effect of mesentry level ligation on patient's survival was considered.

This study was conducted on 54 patient of Dukes C colorectal adenocarcinoma, who were subjected to colectomy radical operations. Xylene chemical clearance was applied to resected specimens for lymph node dissection. The lymph nodes were identified in three levels, pericoloic within 5 cm. of tumor edge, paracolic within 5-10 cm of tumor edge and those along the main blood vessels. The patients were followed for minimum period of two years.

The mean number of examined nodes per specimen was 21.03 (range, 5-39) while, the mean number of positive nodes was 3.09 (range, 1-14). There was a significant correlation between young age and higher number of positive nodes and perineural invasion. Also, significant correlation was identified between nodes level and higher grade as well as perineural invasion. The 2 years disease free survival (2DFS) was significant with nodes level but not the number. On the other hand, the 2 years disease free time (2DFT) was significant with nodes number, level, and the number within every level.

The tumor grade, positive lymph nodes within every level as detected by chemical clearance should be considered in accurate assessment of prognosis after colectomy.

Key words : *Disease free survival – level and number of lymph nodes – Chemical clearance – Colorectal cancer*

INTRODUCTION

Still Dukes classification of colorectal carcinoma (CRC) is the most valuable guide to prognosis, so proper histopathologic evaluation of the resected specimen and its related lymph nodes (LN) metastases is essential to stage the tumor and predict the outcome[1].

However, a sharp decline in the survival has been found, once there is lymph node involvement (5 DFS for Dukes A, B, C is 98, 78 and 32% respectively). This necessities accurate detection of metastatic lymph nodes which is a difficult problem due to the extensive mesenteric vascular plexus, presence of fatty tissue and the general small size of the lymph nodes [2]. Moreover, in one 1/3 of cases the disease skips so the pathologist must examine as many nodes as possible [3].

The above reasons together with lateral lymph nodes spread in lower 1/3 rectal cancer cases may explain the wide difference in the 5 DFS of Dukes C patients between 23% to 52% [4].

That great difference in the survival may be solved by Dukes C patients subclassification related either to the positive nodes level [5], or to the positive nodes number [6].

Moreover, Yasuda et al., 2001 [7] found the number and level of positive micrometastatic lymph nodes correlated with postoperative recurrence .

Recently, Dukes C stage subclassification is facilitated by the chemical lymph nodes clearance techniques. This help to identify a greater number of lymph nodes, the level of these nodes and their number within every level [8].

Tang et al., 1995 [9] suggested that: Based on the nodal status, stage III patients (TNM classification of colorectal cancer) were separated into three groups; III A (1-3 positive nodes and PN3 –ve), III B (4-9 nodes and PN3-ve) and III C (> 10 positive nodes or PN3 +ve).

In the present study we investigated, the clinicopathologic associations of the positive lymph nodes number, positive nodes level and the number of positive nodes within every level.

Also, the effect of mesentry level ligation on the patients survival was considered.

PATIENTS AND METHODS

This study was conducted on 54 patients of Dukes C colorectal adenocarcinoma with complete surgical resection and rectal cancer cases above the peritoneal reflection.

Following surgical resection the vasculature of the mesentery is labelled as two ties for the main blood vessels and a single tie for the named blood vessels: The technique of chemical clearance involves washing with tap water to clear blood and debris, fixing with 10% formol saline at room temperature for 48 hours, and clearance in xylene for one hour. This renders the tissue translucent with all lymph nodes clearly visible [4].

Macroscopically, the nodes were picked up and under the Japanese general rules at 1983[10]: Firstly, classified into N1 & N2 & N3 levels where N1 involves pericolic nodes within 5 cm of tumor edge, N2 includes paracolic nodes within 5 cm to 10 cm of tumor edge and those nodes along named blood vessels and N3 defines nodes along main blood vessels. Secondly, the total number of nodes per specimen is known. Thirdly, the type of operation is identified where R3 (high ligation) operation is defined when lymph nodes dissection up to N3 level and R2 (low ligation) operation is defined when lymph nodes dissection up to N2 level.

Nextly, the nodes are sectioned, stained with haematoxylin and eosin for histopathologic examination together with the resected specimen and the Duckes C patients are classified. Firstly, into n1+ & n2+ & n3+ levels related to the most distant microscopically positive nodes where, n1+ indicates positive nodes at N1 level, n2+ includes positive nodes at N2 level irrespective to n1 level, and n3+ defines positive node at N3 level [5]. Secondly, classified related to the number of positive nodes either <3, or >4 [6]. Thirdly, classified related to the number within every level.

The patients were followed up for at least 2 years and the follow up data were procured from clinical notes in the outpatient clinics, phone calls and a letter program.

Statistics:-

Survival curves were constructed using the Kaplan-Meier method, and evaluated using the log Rank test. The Chi Square test was used to test for differences in distribution between groups. P-values of less than 0.05 were considered statistically significant. The Cox analysis was used to detect the discriminant factor [11].

RESULTS

54 Dukes C patients were classified according to positive nodes number, positive nodes level and a modified system for both (level + number) (Table I).

The mean number of examined nodes per specimen was 21.03 (range, 5-39) while, the mean number of positive nodes was 3.09 (range, 1-14).

When the patients were subclassified according to the positive nodes number, the higher number was correlated significantly with the younger patients age and positive perineural invasion (Table II-A & Fig.A).

On the other hand, when the patients were subclassified according to the level of positive nodes, the n3+ class of patients was correlated with higher grading and positive perineural invasion (Table II-B), and interestingly n2+ class of patients with skip lesions was correlated with higher grading (Table II-B & Fig.B).

Meanwhile, when the patients were classified according to the number and location of positive nodes, the only significant correlation was between n1+>4 class of patients and positive blood vessels invasion (Table II-C).

As regards to patients survival it has been found neither the total number of lymph nodes nor the number of positive nodes affected the 2DFS, but both the total number of nodes and the number of positive nodes were higher in the recurrent group than in the free group (Table III).

Also, there was no significant difference between the 2 DFT of patients with one to three positive nodes and that of those with four or more positive nodes (Table IV-A).

In contrast, the 2DFS was inversely significantly correlated with the positive nodes level being (61.5%, 40%, 12.5% for n1+, n2+ n3+ respectively) (Table IV.B).

In general, within all levels, the higher the number of positive nodes the lower the 2 DFS and that correlation was statistically significantly in n3+ level (Table IV-C).

Finally, the effect of ligation of the mesenteric artery on the 2DFS of n1+ and n2+ patients was examined and there was no significant effect on the survival although the survival rate in the high ligation group was slightly better than in the low ligation group (Table IV.D).

The Cox analysis resulted in definition of positive central nodes number (n3+ number) as the discriminant factor ,and the B for positive nodes >4 was nearly two folds as those with positive nodes <3.

As regard the disease free time there was a significant difference between the DFT of patients with positive nodes <3 (mean = 22.66) and those with positive nodes >4 (mean = 17.32) (Table V-A, Fig. I).

Similarly, there was inverse correlation of DFT by positive nodes level being (the means were 22.5, 20.8, 11.25 for n1+ & n2+ & n3+ respectively) (Table V-B, Fig. 2). Interestingly, those patients with out skip lesions had better DFT (mean 21.0) than those with skip lesions (mean = 20.56) (Table V-B).

Also, within every level the higher the number of positive nodes the worse the DFT (Table V-C), and moreover, n1+ patients with positive nodes <3 had better DFT than n2+ patients with positive nodes <3 (Fig. 3). Similarly, n1+ patients with positive nodes >4 had better DFT than n2+ patients with positive nodes >4 (Fig. 4), and as expected n2+ patients with positive nodes <3 had better DFT than n2+ patients with positive nodes >4 .

Finally, there was a slight clinically better effect of high ligation procedures on DFT for patients either n1+ & n2+ (Table V-D).

The Cox analysis for DFT revealed the location of positive nodes is the most discriminant factor and B for n3+ = 1.7, for n2+ = 1.1 and for n1+ =1.

Table I: ***Patients classification***

Serial	*Classification*	*No.*	*%*
A	Positive nodes number		
	<3	29	53.6
	≥4	25	46.4
B	***Positive nodes level***		
	n1+	26	48
	n2+	15	27
	n2+ with skip	5	9
	n3+	8	25
C	***Positive nodes (level + number)***		
	n1+ <3	16	62
	≥4	10	38
	n2+ <3	8	60
	≥4	7	40
	n3+ <3	6	75
	≥4	2	25

Table II (A,B,C): ***Significant clinicopathologic association***

Serial	*Variable*	*Character*			*P-value*
	Positive nodes number	Patients age			
A	<3	<45	≥ 45		
	≥4	13 (44.8)	16 (55.2)		
		18 (72.0)	7 (28.0)		0.04*
		Perineural invasion			
	<3	+ve	-ve		
	≥4	1 (3.8)	28 (96.2)		
		6 (24.0)	19 (76.0)		0.02*
B	Positive nodes level	Grade			
		I	II	III	
	(n2+with skip)	0 (0)	1 (20)	4 (80)	0.008*
	N3+	1 (12.5)	1 (12.5)	6 (7.5)	0.001**
		Lymph vessel invasion			
		+ve	-ve		
					0.001**
	N3+	8 (10)	0 (0)		
C	Modified system	Blood vessel invasion			
		+ve	-ve		
	n1+ <3	2 (12.5)	14 (87.5)		
	n1+ ≥4	6 (60)	4 (40)		0.01*

Table III: ***The correlation of the lymph nodes number by 2 year disease free time (2DFT)***

Serial	*Lymph nodes number*	*Free group*	*Recurrent group*	*P-value*
A	***Total***			
	Mean	21.03	22.4	0.37
	Range	5 – 37	13 – 39	
B	***Positive***			
	Mean	2.68	3.44	0.13
	Range	1 – 7	1 – 9	

Table IV (A,B,C,D): ***Significant predictors of 2 year disease free survival (2 DFS)***

Serial	*Variable*	*Free*	*Recurrent*	*P-value*
A	Positive nodes No.	No. %	No. %	
	<3	16 (55.2)	13 (44.5)	
	≥4	9 (36)	16 (64)	0.15
B	Positive nodes level			
	n1+	16 (61.5)	10 (38.5)	
	n2+	8 (40)	12 (60)	0.04*
	n3+	1 (12.5)	7 (87.5)	
C	Modified system			
	n1+ <3	11 (68.8)	5 (31.5)	
	≥4	5 (50)	5 (50)	0.33
	n2+ <3	5 (38.5)	8 (61.5)	
	≥4	3 (42.9)	3 (57.5)	0.83
	n3+ <3	1 (16.7)	5 (83.3)	
	≥4	0 (0)	2 (100)	0.04*
D	Level of mesenteric artery ligation			
	n1+ low	4 (50)	4 (50)	
	n1+ high	12 (66.6)	6 (33.3)	0.42
	n2+ low	4 (36.4)	7 (63.6)	
	n2+ high	4 (44.4)	5 (55.6)	0.71
	n1+ & n2+ low	8 (42.1)	11 (57.9)	
	n1 & n2+ high	16 (59.3)	11 (40.7)	0.25

Table V (A,B,C,D): ***Significant predictors of disease free time (DFT)***

Serial	*Variable*	*Mean*	*S.E*	*P-value*
A	***Positive nodes number***			
	<3	22.66	0.39	
	≥4	17.32	1.20	0.01*
B	***Positive nodes level***			
	n1+	22.46	0.47	***
	n2+	20.80	0.84	0.000
	n3+	11.25	1.78	
	n2+ with skip	20.56	1.33	
	n2+ no skip	21.00	1.88	0.65
C	***Modified system***			
	n1+ <3	23.31	0.3	
	≥4	21.10	0.98	
	n2+ <3	21.85	0.74	0.05*
	≥4	18.86	1.77	
	n3+	11.25	1.78	
D	Level of mesenteric artery ligation			
	n1+ low	21.25	1.10	
	n1+ high	23.0	0.42	
	n2+ low	19.36	1.33	0.17
	n2+ high	22.56	0.50	

Fig.(1) Survival functions according to postive lymph nodes numbers

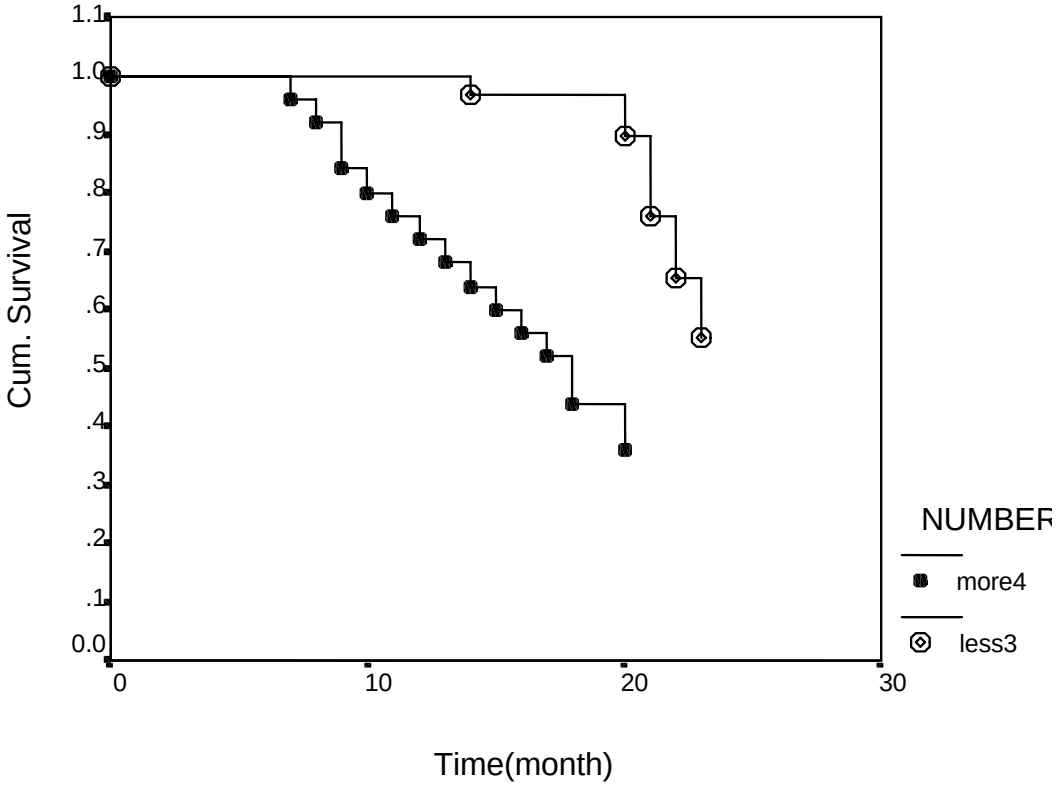


Fig.(2) Survival functions according to the level of postive lymph nodes

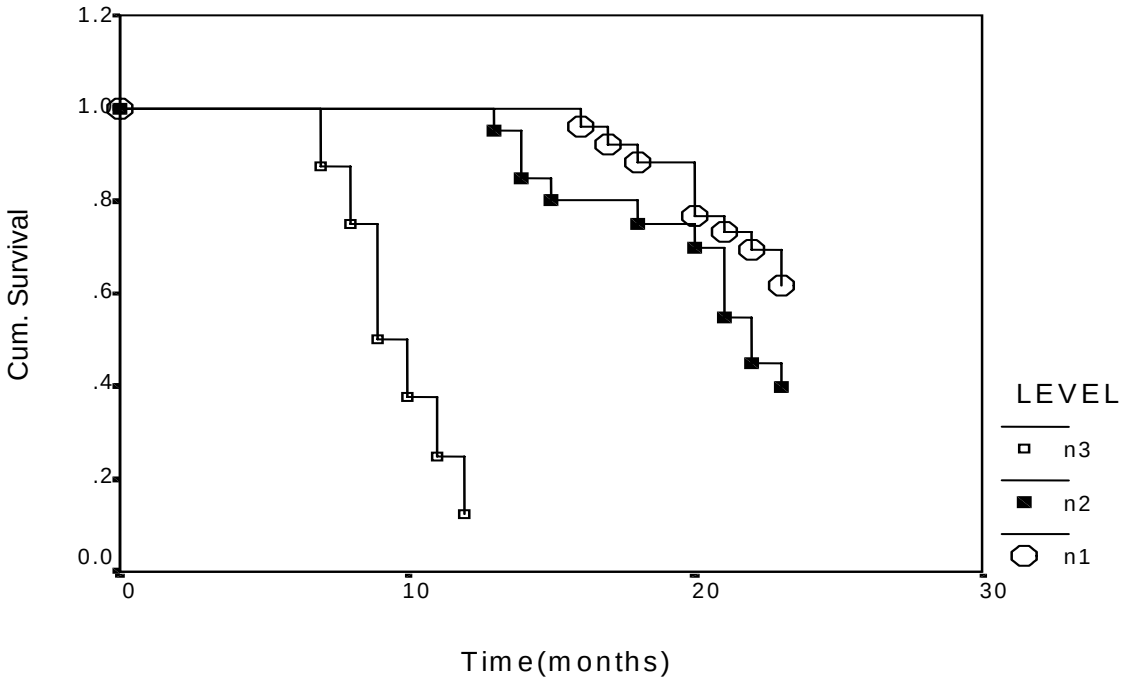


Fig.(3)Survival function according to the level of postive nodes when nodes number less than 3 in n1&n2 level

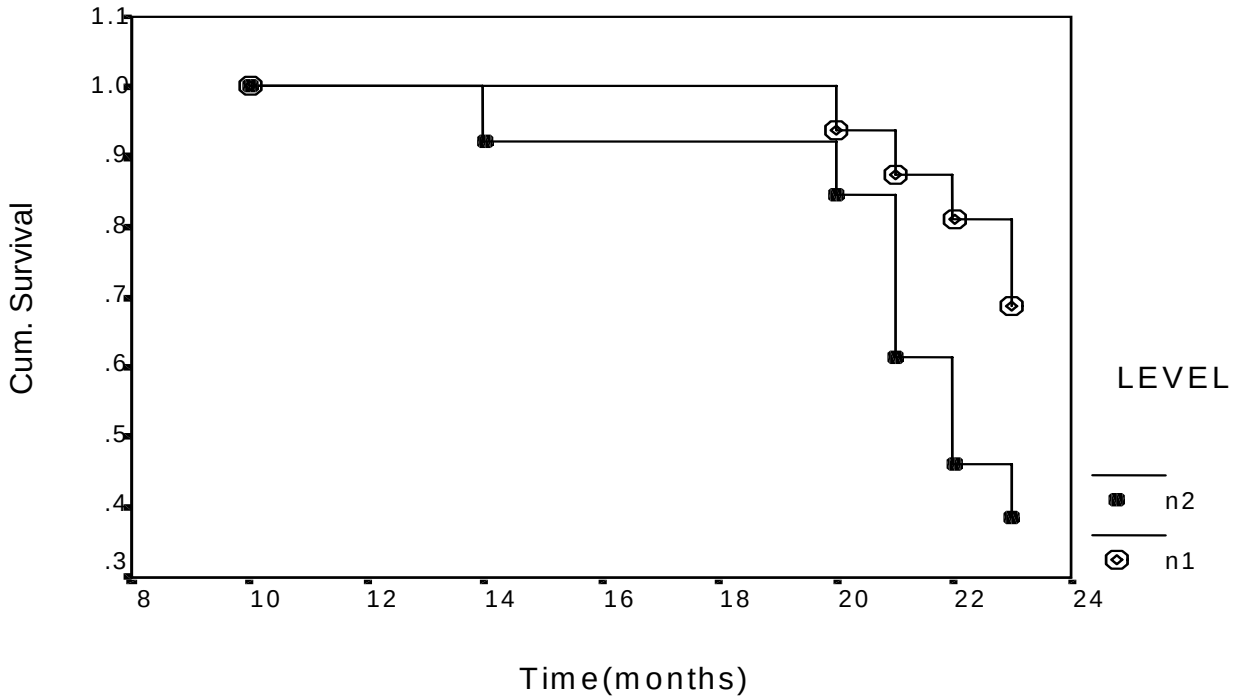
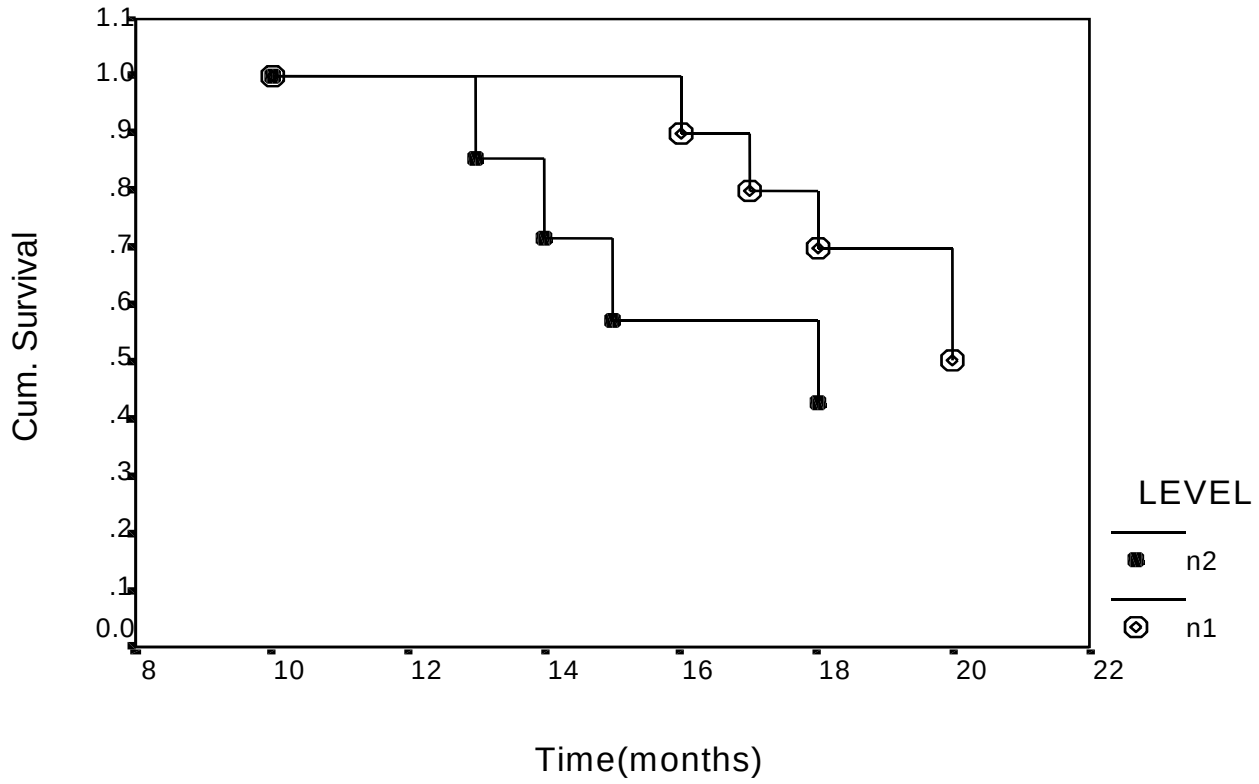


Fig.(4)Survival function according to the level of postive nodes when nodes number more than 4 in n1and n2 level



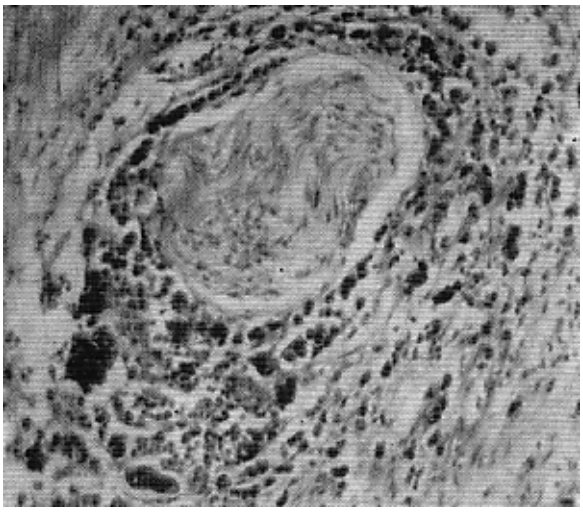


Fig. A

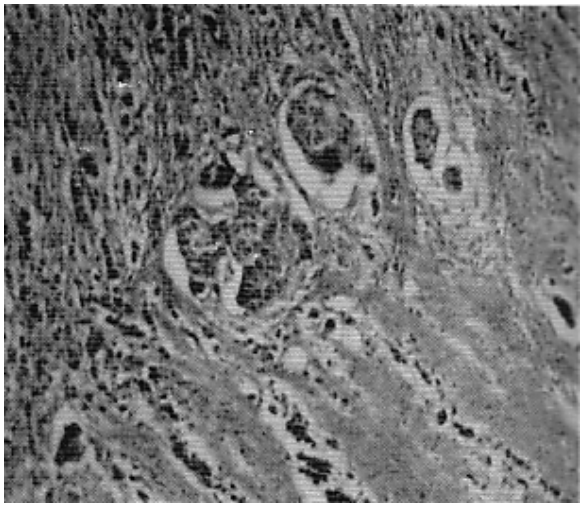


Fig. B

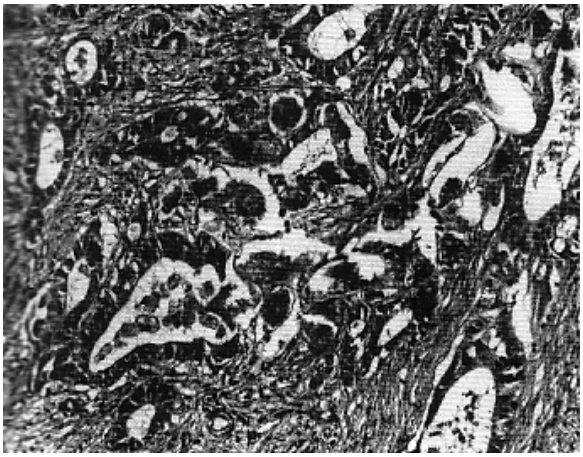


Fig. C

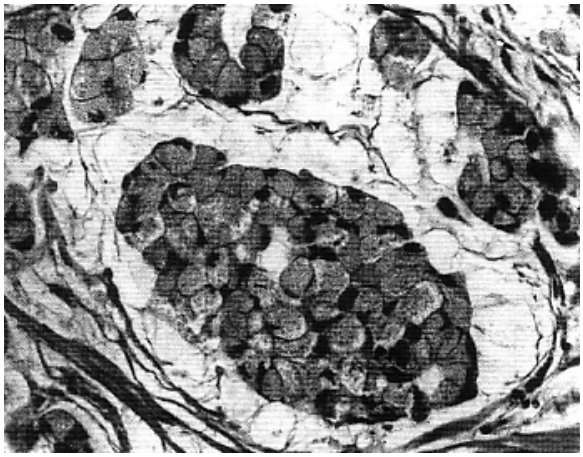


Fig. D

DISCUSSION

The lymph nodes clearance for colorectal cancer specimens was firstly reported by [12], and extensively evaluated in breast cancer staging by Monroe [13]. Also, our study revealed the chemical clearance is an effective method as the mean number of lymph nodes was [21.03] and the mean positive number was [3.09] in contrast to 7 non cleared (hand dissection) centres where the mean numbers were [10.5], [8.44] and mean positive numbers were [1.9], [1.82] respectively [7,14].

The number of lymph nodes recovered to ensure accurate documentation of nodal metastases in colorectal carcinoma was variable in different studies . It was 8 [15] , 14 [16] and 17 [17].

The epidemiologic characters of our patients, their clinicopathologic correlations and survival data are consistent with the most published reports [18,19]. But we confirmed the positive correlation of pathologic grading with the lymph nodes metastases to improve the selection criteria for local rectal resection as the sophisticated investigations can detect local spread but not the nodal status [20] and in our study, the pathologic grading was not only closely related to the nodal status but also with positive nodes level by demonstrating the association of grade III tumors with n3+ class of patients and moreover with n2+ class of single skip metastasis than n1+ with multiple metastases (Fig. C & D).

Also, the present study confirmed that the positive nodes level and their number within every level rather than the total number of positive nodes are more closely correlated with the patient survival similar to Tang et al.,[9]. Moreover the disease free time was significantly correlated with the total positive nodes number, positive nodal level and the number of nodes within every level as described by Yasuda et al., [7].

However, Wohmark et al.,[21] stated that multiple sections of lymph nodes from colorectal specimens leaded to the detection of a small number of additional nodal metastases .

Finally, although the relationship between the level of mesenteric artery ligation and patient prognosis remains unclear [22]. Our data suggested that high ligation might be more beneficial for colorectal cancer patients.

These results persuade us that the tumor grading and positive nodes number, level, their number within every level following chemical lymph nodes clearance should be taken into account in a classification system that more accurately assesses patients prognosis.

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