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Original Article

Pathology Section

Morphologic Spectrum of Duodenal Biopsies in Malabsorption: A Study from Southern India

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ABSTRACT

Introduction: Duodenal endoscopic biopsy is a common investigation for various non-neoplastic conditions. Malabsorption is a common indication for duodenal biopsy in our setting.

Aim: Our study was undertaken to study the morphologic spectrum of non-neoplastic conditions of duodenum emphasizing on Intraepithelial Lymphocytes (IELs) and to have a clinico-pathologic correlation.

Materials and Methods: This was a prospective descriptive study. Duodenal biopsies from 101 patients with symptoms of malabsorption were studied according to inclusion and exclusion criteria. Informed written consent was taken. Clinical, laboratory, endoscopic, and serological parameters were collected wherever available. Histomorphological parameters were studied on Haematoxylin and Eosin (H&E) stained sections. Intraepithelial lymphocyte counts were done on CD3, CD4 and CD8 Immunohistochemical (IHC) stained sections and correlated.

Results: We studied 101 duodenal biopsies. Our spectrum included 16 patients of celiac disease (CD) (15.8%), 15 autoimmune duodenitis (14%), 13 nutritional deficiency associated duodenitis (12.8%), five infectious duodenitis (5%) and 41 patients of non-specific duodenitis (40.6%) and 10.9% miscellaneous causes of duodenitis. Villous crypt architecture, IEL counts; villous tip IEL counts were statistically significant between CD and other disease groups.

Conclusion: A constellation of clinical, serological, endoscopic and histopathologic features is essential in diagnosing CD and autoimmune duodenitis. Biopsy is also a useful tool in diagnosing infectious duodenitis that are missed in other investigations.

Keywords: Celiac disease, Duodenum, Intraepithelial lymphocyte

INTRODUCTION

The common causes of malabsorption syndrome in the Western population are CD, Crohns disease, cystic fibrosis etc., [1].

Tropical Sprue (TS), parasitic infestations, intestinal tuberculosis, and primary immunodeficiency syndromes are commonly seen in the developing countries like India according to older literature. CD and TS present with similar clinical and histologic features. This emphasizes the need to know the subtle histologic features to differentiate between CD and other close mimics [2]. TS is the most common cause of malabsorption in our setting and form the most important differential diagnosis of CD in its clinical manifestations and in biopsy interpretation.

CD is commonly reported in Northern India, mainly in children [3]. However, in Southern India, exact prevalence of CD is not known and is reportedly rare in view of predominantly rice based diet.

IELs are T cells, seen in between the intestinal epithelial cells. Most T-IELs express alpha/beta T-cell receptor; however 5% have surface gamma/delta T-cell receptors which are seen in CD [4].

Rapid mobilisation of people with changing diet patterns and cosmopolitan life style with fast food habits prevailing at the moment, prompted us to study the causes of malabsorption in the Southern part of country. We intended to study the morphologic spectrum of non-neoplastic conditions of duodenum emphasizing on IELs and to assess the clinico-pathologic correlation.

MATERIALS AND METHODS

It was a prospective descriptive study done in the Department of Pathology and Department of Medical Gastroenterology, from May 2012 to June 2014. Institutional Ethical Committee clearance was obtained and informed consent was taken from all participants. There was a follow up period ranging from six to 14 months for recruited study participants. During the two years study period, 101 patients who presented to medical gastroenterology outpatient department with symptoms of malabsorption and who underwent endoscopic examination were studied.

The criteria for malabsorption were defined as follows: Patients with chronic diarrhoea (diarrhoea lasting for more than four weeks) with malabsorbtive manifestations. The diagnosis of malabsorption was made when any of the following clinical features were present; diarrhoea, steatorrhoea, weight loss, anaemia, oedema and/or clinical features suggestive of nutritional deficiencies (iron, folate, vitamin D) [5]. IELs from villous tip and base of villi were studied in 86 endoscopic biopsies of the total 101 patients studied as IHC for IELs could be performed for all the markers only in that subset. The remaining tissues were tiny and were exhausted.

Small intestinal biopsies in resection specimens of non-intestinal pathologies were used for determination of normal range of IELs. Endoscopic small intestinal biopsies from suspected malabsorptive conditions, autoimmune diseases, infections and nutritional deficiencies were included in the study. The endoscopic biopsies from polyps and neoplastic conditions were excluded from the study. Patients with lactose intolerance (based on clinical suspicion and a positive lactose hydrogen breath test) and irritable bowel syndrome (based on Rome II criteria) were also excluded from this study.

All patients were uniformly subjected to five biopsies, two from bulb and three from distal end of duodenum. Histopathological examination was carried out after standard processing and H&E staining procedures. Clinicopathological details and informed consent were obtained from the patients. IHC was done for 72

cases. In the remaining 29 cases, further deeper sections could not be obtained or sections were lost during processing.

Histomorphological parameters like villous architecture, crypt architecture, IEL count per hundred enterocytes (average of three hundred enterocytes) were studied. Villous tip IELs per twenty villous tip enterocytes (average of five villi), inflammatory cells in lamina propria - lymphocytes, neutrophils, eosinophils and epithelioid histiocytes were also analysed and its severity was graded as mild, moderate and severe. Modified Marsh Oberhuber classification was used to classify CD [6]. Interpretation of IEL count and villous tip IEL count was done according to Datta Gupta S et al., [2].

All biopsies were documented for number of biopsy fragments received and site, villous height and architecture (normal/broad or blunted), villous to crypt ratio, crypt hyperplasia, normal, flattened or damaged surface enterocytes, preserved or lost brush borders, IEL count, gastric metaplasia in chronic duodenitis and presence of microorganisms. Biopsy fragments were tested for *Helicobacter pylori, Giardia lamblia, Cryptosporidium species, Strongyloides stercoralis, Ancylostoma duodenale, Entamoeba histolytica, Mycobacterium tuberculosis,* Cestodes such as *Taenia and Diphyllobothrium* species by light microscopy. Appropriate special stains viz., Giemsa, Acid Fast Stain (AFS), Periodic Acid Schiff (PAS) were performed based on the initial findings on H&E stained slides. Diagnosis was based on composite findings with constellation of clinical, biopsy and parasitology work up and was corroborated based on response to gluten free diet on a minimum of six months follow up. Serum tissue Transglutaminase (tTG) levels could not be done for our patients and the levels were not available. Concomitant CD was excluded based on lack of response to gluten free diet.

STATISTICAL ANALYSIS

Statistical analysis was done using IBM- SPSS software version 21.0. All statistical tests were carried out at 5% level of significance and p-value <0.05 was considered as statistically significant.

RESULTS

We studied 101 duodenal biopsies during the two year study period. The various categories were 16 (15.8%) CD, 15 (14.9%) duodenitis associated with autoimmune diseases, 13 (12.8%) nutritional deficiency associated duodenitis, 5 (5%) infectious duodenitis, 41 (40.6%) nonspecific duodenitis and 10.9% miscellaneous causes of duodenitis. The major presenting clinical features in these conditions are presented in [Table/Fig-1].

Sl. No	Group	Diarrhoea n (%)	Anaemia n(%)	Abdominal pain n (%)
1	Celiac disease	14(87.5%)	5(31.2)	2(12.5)
2	Autoimmune duodenitis	10(66.7)	2(13.3)	1(6.7)
3	Nutritional deficiency associated duodenitis	6(46.2)	13(100)	1(7.7)
4	Infectious duodenitis	3(60)	0	1(20)
5	Non specific duodenitis	33(80.5)	2(4.9)	8(19.5)
6	Others	6(54.5)	1(9.1)	2(18.2)

[Table/Fig-1]: Spectrum of major presenting clinical features in patients in the study group.

In all these patients, the primary clinical diagnosis was that of malabsorption. Auto immune aetiology was clinically suspected before serological and autoimmune work up in eight patients, of whom six presented with multiple joint pains and skin rashes, two patients had evidence of vasculitis and three had evidence of dry eyes and biopsy proven evidence of Sjogren syndrome (Focus score > 1). Detailed serological study was performed in all the patients and renal biopsy was available in four patients.

Infectious duodenitis was suspected in five patients clinically and stool examination by microscopy for ova and cyst confirmed evidence of parasitic infestation in two patients before biopsy. The parasites examined were *Giardia lamblia, Entamoeba histolytica, and Cryptosporidium species.*

Nutritious causes were clinically suspected in seven patients and subsequent laboratory work up identified 13 patients with nutritional deficiency while the initial seven patients also had evidence of iron deficiency or megaloblastic anaemia in haematological work up before biopsy. CD was clinically suspected in two patients who had history of intolerance to gluten based diets.

The age group was 15 to 72 years with 43 males and 58 females. The age of CD patients ranged from 15 to 67 years with mean age of 36±17.14 years. There was a female preponderance in autoimmune duodenitis and CD with a male: female (M:F) ratio of 1:4 and 1: 2.2 respectively. Diarrhoea was the most common symptom accounting for 71.3 %, next being anaemia seen in 22.8%. In nutritional anaemia, mean haemoglobin values were lower. Anaemia was seen in 31.3% of CD patients. The mean differences of haemoglobin between various groups were found to be statistically significant by one-way ANOVA test (F statistic 14.34, p-value <0.001). Stool examination was done in 59.4% cases, all were normal except one case which showed hookworm on stool microscopy. Among the CD, 50% had normal mucosa on endoscopy, 37.5% had reduced mucosal folds, and 12.5% had oedematous or ulcerated mucosa.

Villous and Crypt Architecture

Normal villous crypt ratio (V:C) is 3 to 5:1. Mild blunting was given when the ratio was 2:1, moderate blunting when the ratio was 1:1 and flattening when there were no villi seen [6]. All patients of CD had villous blunting, 2 (12.5%) showed mild blunting, 7 (43.75%) each had moderate and severe blunting [Table/Fig-2]. Only nine out of 85 patients in non CD conditions had mild villous blunting. All patients of CD had either total or partial villous atrophy. Crypt hyperplasia was seen in all patients of CD.

IEL Counts

The normal range for upper limit of IEL in villous tip was 20 IEL/100 enterocytes and 18 IEL/100 enterocytes at the base of villi in patients with non-intestinal pathologies.

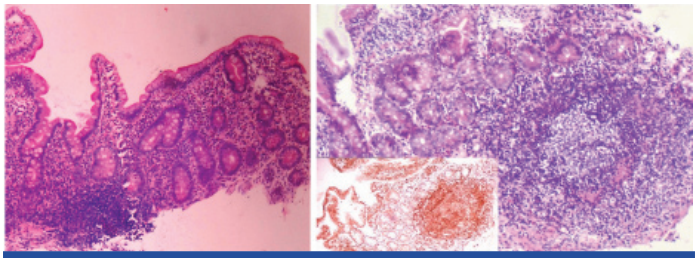
All CD patients had increased IEL. Range was 25-45 IEL per 100 enterocytes and mean IEL counts was 28.2±7.7. Thirty one out of 85 non CD patients had mild to borderline increased IELs. The range of villous tip IEL counts in CD was 3-14 IELs per 20 enterocytes whereas in other diseases, range was 0-12 IELs. Mean villous tip IEL counts for CD was 8.62±3.14 which was higher compared to other groups.

Inflammatory Cells

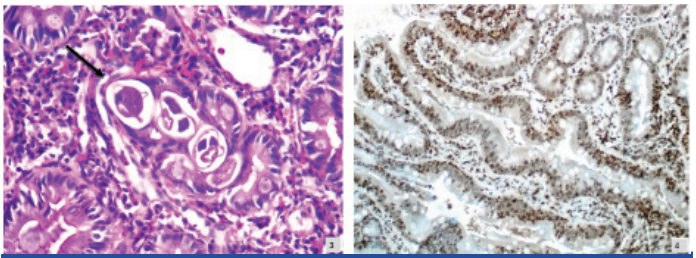
Four patients (25%) had severe increase in lymphocytes and plasma cells in lamina propria and remaining 12 patients had moderate increase among the CD patients. All patients of autoimmune duodenitis had moderate increase. We also observed that 12 of the 15 patients with autoimmune duodenitis had reactive lymphoid follicles in the lamina propria [Table/Fig-3].

Eosinophils in lamina propria were increased in 80% of infectious duodenitis patients. The mean eosinophil count was 33.8±44.7.

Histological parameters like villous blunting, villous atrophy, crypt hyperplasia, IEL counts, villous tip IEL counts was statistically significant by univariate analysis by Fisher's-exact test but not by multivariate analysis. All these parameters were also analysed between CD and non-specific duodenitis, autoimmune duodenitis, and similar results were obtained. Auto immune aetiology was suspected in eight patients clinically, of whom six presented with multiple joint pains and skin rashes, two patients had evidence of vasculitis and three had evidence of dry eyes and biopsy proven evidence of Sjogren syndrome (Focus score > 1). Detailed serological study was performed in all the patients and renal biopsy was available in four patients.



[Table/Fig-2]: Section shows fragment of duodenal mucosa showing moderate to severe blunting of villi. (H&E 10 X). **[Table/Fig-3]:** Section from duodenum shows reactive lymphoid follicles in the lamina propria. (H&E 10X). (Inset) – Section from duodenum shows strong expression of CD20 in the reactive lymphoid follicles. DAB stain, DAKO primary antibody, USA (IHC 10X). (Images from left to right)



[Table/Fig-4]: Section shows infiltration of duodenal glands by adult worms of Strongyloides stercoralis (H&E 60X). **[Table/Fig-5]:** Section shows significant increase in CD3 intraepithelial lymphocytes in duodenal mucosa. Di amino benzidine stain, Immunohistochemistry stain with DAKO antibodies, USA, (IHC 40X). (Images from left to right)

We had two patients of *Giardia lamblia*, two *Strongyloides stercoralis* [Table/Fig-4], one hookworm infestation, one *Helicobacter pylori* diagnosed by demonstration of organisms and one biopsy with microscopic features of tuberculosis. We observed that 80% of infectious duodenitis had increased eosinophils in lamina propria.

Normal IEL counts were found in 65/72 cases on H&E sections. On CD3 IHC, 40/65 cases had normal IEL counts, nine had borderline increased and 16 had increased IEL counts. The level of agreement was done using kappa statistics between IEL on H&E and CD3. Kappa value was 0.207 which was statistically significant concluding that there is no correlation. In our study, 25 patients with normal counts by H&E, had either borderline or definitely increased counts on CD3 [Table/Fig-5-7]. IHC by CD4 and CD8 was done in this group. Mean of CD8:CD4 was taken and it was similar in all groups with no diagnostic utility.

DISCUSSION

In a study by Bai JC et al., estimated prevalence of CD ranged from one in 100 to 300 individuals [7]. In a community based study done by Makharia GK et al., in Northern India in 2011 the overall estimated prevalence of CD was 1.04% [8]. Ganesh R et al., reported four patients of CD in Chennai after evaluating 98 patients with malabsorption [9].

Yadav P et al., studied CD in Northern India on 127 cases, and reported CD in 64.9%, tropical sprue in 22.3% [1]. The high proportion CD can be attributed primarily to dietary practices in the region in addition to increased awareness, better, sensitive screening techniques and understanding the early changes in biopsy. In our study, the CD constituted 15.8%. The other cases in our study were 15 patients (14.9%) with autoimmune duodenitis, nutritional deficiency associated duodenitis in 13 (12.8 %), infectious duodenitis in 5 (5%) while non-specific duodenitis was observed in 41(40.6%). [Table/Fig-8] compares various conditions diagnosed in our study with other studies [10,11].

The mean age was 36±17.14 years with highest burden in 20-59 years. The mean age was 21.8 years in study by Yadav P et al., in Northern Indian population [1]. Thus we found a higher mean age of CD in the adult population in Southern India. Male:female ratio was 1:2.2. The typical presentation of chronic diarrhoea and malabsorption is below 50% in some studies [1,8,11].

In our study, diarrhoea is the most common symptom (87.5%). Most common atypical presentation of CD is Iron Deficiency Anaemia (IDA) [12]. Various studies reported 2.6% to 11.8% of CD in adults with IDA [13,14]. Varma S et al., reported 11 of 19 patients with refractory anaemia had CD in Northern India [15].

In our study, five patients of refractory IDA were diagnosed as CD, accounting for 31.3%, which is higher compared to 11.8%, reported

Sl. No	Parameters	Celiac disease (n= 16)	Non celiac disease conditions (n= 85)	p-value	Odds ratio
1	**Age** 13-19 20-39 40-59 ≥60	 2 8 4 2	 7 37 35 6	$	*
2	**Gender** Male Female	 5 11	 38 47	0.318#	*
3	**Symptoms** Diarrhoea + Anaemia +	 14 5	 58 15	 0.143^ 0.378#	*
4	Endoscopy	8	29	0.226#	*
5	**Histomorphology**				
A	Villous architecture Blunting present	16	7	<0.001^	3.286
B	Crypt architecture Crypt hyperplasia	16	0	<0.001^	588
C	IELs/100 enterocytes	16	38	<0.001^	1.42
D	Villous tip IELs more than 5 per 20 enterocytes	14	24	<0.001^	17.79
E	Lamina propria lymphoplasmacytic infiltrate a)Moderate increase b)Severe increase	 12 4	 76 9	$	*
F	Eosinophils in lamina propria, increased	8	33	0.404^	*

[Table/Fig-6]: Comparison between CD and non celiac disease conditions.
$- p-value could not be commented as cells have count less than 5.
*not applicable
By Pearson Chi-square test
^ By Fishers-exact test

Sl. No.	Compared groups	Villous architecture		Crypt architecture		Villous atrophy		IEL count*		Villous tip IEL count**	
		p	OR	p	OR	p	OR	p	OR	p	OR
1	CD Vs NCDC	<0.001	3.286	<0.001	588	<0.001	3.286	<0.001	1.421	<0.001	17.792
2	CD Vs NSD	<0.001	#	<0.001	#	<0.001	#	<0.001	2	<0.001	0.052
3	CD Vs AiD	<0.001	6.333	<0.001	#	<0.001	6.333	<0.001	3.286	<0.001	28

[Table/Fig-7]: Comparison of histological parameters between celiac disease and other groups.
CD- Celiac disease, NCDC- Non celiac disease conditions, AiD- Autoimmune duodenitis, NSD- Non specific duodenitis, NAiD-Non autoimmune duodenitis. IEL-Intraepithelial lymphocyte.
*Per 100 enterocytes
**Per 20 villous tip enterocytes.
OR- Odds ratio
-Risk estimate statistics cannot be computed as two cells have value as 0

Diseases	Study by Williams L et al., [10] n(%)	Study by Mahadeva S et al., [11] n(%)	Present study n(%)
Total no of duodenal Biopsies	408(100)	626 (100)	101(100)
Normal biopsies	354(86.7)	502(80.2)	41(40.6%)*
Increased chronic inflammatory cells in lamina propria	38(9.3)	#	
Celiac disease	6(1.47)	15(2.4)	16(15.8)
Possible CD	5(1.22)	#	#
Giardia	2(0.49)	3(0.5)	2(1.98)
Immunodeficiency	1(0.24)	#	#
Raised IEL counts alone	#	14(2.2)	1(0.9)
CD on follow up	#	23(3.7)	#
Peptic duodenitis/ ulcer	#	55(8.8)	2(1.98)

[Table/Fig-8]: Comparison of distribution of biopsy findings in present study and others [10,11].
*In present study as non-specific duodenitis.
#No specific disease was reported in that study.

by Ackerman Z et al., [16]. Haemoglobin values ranged from 2.3 to 8.5 gm/dl with mean value of 6.4 gm/dl and it is comparable to study by Yadav P et al., who reported a mean of 8.8 gm/dl [1].

We reported IDA in 31.3% of CD patients, with a cut off of haemoglobin<11 g/dl. Yadav P et al., reported 88.5% of CD patients had IDA [1]. But they used a higher haemoglobin threshold of 12.0 g/dl for diagnosing anaemia. Five CD patients in our study had refractory IDA. Thus, a diagnostic work up of CD may be justified in refractory IDA patients.

Stennhammer L et al., in their study reported that 10% of children with unexplained short stature had CD [17]. In the present study, three (18.8%) patients of CD had short stature.

As the mucosal damage in CD has uneven distribution, endoscopy may miss the severely diseased areas [18]. The specific endoscopic findings like loss of folds, scalloping, mosaic pattern has a positive predictive value of 84% with 94% sensitivity and 92% specificity [19].

In our study, 37.5% had reduced or scalloped mucosal folds, 50% endoscopies were normal. Abnormal duodenal folds were seen in 82% in Yadav P et al., study [1].

Many studies have established that biopsy forms the gold standard with positive serology [20-23]. As prevalence of CD is low especially in Southern India and with close mimics, having varied atypical presentations, response to gluten free diet has to be assessed which helps in differentiating CD from other differential diagnosis [20].

Western data shows 95% of CD patients have HLADQ2 and 5% have HLADQ8 haplotype [24]. Peraaho M et al., showed that HLA-DQ2 and DQ8 were negative in 37% [25]. In our study, only in 3 (18.75%) patients HLADQ2 was done and all were found to be positive.

The upper limit of normal IELs in our study was 24 IELs per 100 enterocytes [2]. But increased IEL counts in architecturally normal biopsies ranged from 9% to 40% in various studies [1,26], suggesting latent manifestation.

In our study, we had 37 patients (36.6%) of non CD patients with increased IELs. Other causes of increased IEL counts are six cases of (46.7%) autoimmune duodenitis, six cases of (46.2%) nutritional deficiency associated duodenitis, 60% of infectious duodenitis, and 39% of non-specific duodenitis.

Biagi F et al., stated that villous tip IEL is sufficient for diagnosing CD [27]. In this study, the mean villous tip IEL scores were 4.6. He concluded that counting villous tip IELs is very simple and quite reliable method. Jarvinen TT et al., mentioned that the villous tip IEL counts was significantly higher in early celiac [28]. Also, they reported villous tip IELs to be superior to CD3+ IEL counts.

In our study, the range of villous tip IELs was 3 to 14 with mean of 9.2 in CD compared to non CD conditions where it ranges from 0 to 12 with mean of 4.2 IELs and this was statistically significant. Our findings were similar to the study done by Biagi F et al., [27]. Thus increased villous tip IELs can help to distinguish CD from other close mimics.

In Nasseri-Moghaddam S et al., study [29], the IEL count was slightly higher in IHC than H&E (21 vs 19) and the two methods showed excellent agreement statistically. In Pellegrino S et al., study, there was high correlation of IEL counts between H&E and CD3 [30]. In our study, 16 patients with normal IEL on H&E had increased counts on CD3. This highlights the importance of CD3 which can pick up near normal patients.

In our study, we had forty one patients (40.6%) of non-specific duodenitis. Malabsorptive disorders presenting with chronic diarrhoea is extremely heterogeneous and may lack a specific aetiology. This was much lesser when compared to Williams L et al., study [10], who reported 96% of patients with only increase in lamina propria inflammation and no specific aetiology. In the study by Mahadeva S et al., 82% had non-specific histopathology findings [11].

In our series, we studied CD4 and CD8 in IELs in 73 patients. The average CD8:CD4 ratio was 6.64. In all the conditions, predominant cell population in the intraepithelial lymphocytes were T suppressor group and there was no difference in CD8:CD4 ratio in CD and non CD group.

Also, the changing dietary patterns and increased susceptibility to CD is highlighted in Southern Indian population too and our results are similar to another recent series from Western India [31].

Screening serologic tests were not performed in the population as the incidence of CD is very low due to diet practices in the region. Diagnosis was confirmed with small intestinal biopsy which is gold standard and with clinical follow up of gluten free diet for minimum of six months in patients with biopsy findings of villous blunting and increased IELs.

We also found that no single histological, clinical or endoscopic findings are 100% sensitive or specific in the diagnosis of diseases causing malabsorption [32] except with demonstrable micro-organisms.

The biopsy findings described in nutritional deficiency, iron and vitamin B12 deficiencies are not distinct diagnostic entities. Co-existing morbidities reported in our study are similar to other published literature [33,34].

The major strength of our study is that we are reporting one of the highest proportions of CD patients from South Indian population with good clinical, endoscopic, haematologic and serologic correlation.

LIMITATION

Our study is limited by the number of patients studied in each disease group and by lack of serological testing for tTG (both IgA and IgG) as these screening tests were not performed due to low incidence in the population.

Another major limitation of the study is referral bias which is inherent and the findings cannot be correlated with general population as it is done in a tertiary care centre. Tropical sprue cases were included as they are much more common and we wanted to study IEL in the subset and compare with other groups.

CONCLUSION

The study highlights that subtle histological evidences in correlation with good clinical, haematological, serological and endoscopy findings may be significantly helpful in identifying subclinical CD patients.

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