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CLINICOHISTOPATHOLOGICAL EVALUATION OF SKIN BIOPSIES IN HANSEN'S DISEASE- A PROSPECTIVE STUDY AT A TERTIARY CARE CENTRE

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Abstract

Background: Leprosy is the most ancient and widely spread global disease and was described in various articles of ancient civilizations. In 1990, the goal of eliminating leprosy by the end of the 20th century was proposed by the World Health Organization but still leprosy is highly prevalent in India. Leprosy is categorized in various types depending upon clinical features, histopathological findings and the host immunity. Various clinical features and acid fast bacilli demonstration in slit skin smears can help in diagnosis of leprosy but histopathological examination can help to classify and aid in the definitive diagnosis of leprosy and also prognosis of the disease and assessment of regression of the disease in patient undergoing treatment. **Aim:** To classify all skin biopsies having clinical diagnosis of leprosy in to various types according to the Ridley Jopling's classification of leprosy, also to find out the correlation between histopathological diagnosis of skin biopsies with clinical diagnosis of leprosy and to study various clinical presentations of leprosy with respect histopathological diagnosis. **Materials and Methods:** It was a prospective study done in the Department of Pathology, Shri Bhausaheb Hire Government Medical College Dhule over a period of 24 months i.e. from 1st January 2022 to 31st December 2023. Skin punch biopsies from 288 clinically diagnosed patients of leprosy were subjected for routine processing and routine staining with Haematoxyline & Eosin stain as well as Fite-Faraco staining. The lesions were classified using the Ridley-Jopling's classification into Tuberculoid Leprosy, Borderline Tuberculoid, Mid Borderline, Borderline Lepromatous and Lepromatous Leprosy. Few of the lesions were categorized as Histioid leprosy and Erythema Nodosum Leprosum **Result:** There was a male predominance (59.02%) seen in our study. Most common age group affected was 21to 30yrs followed by 31to 40yrs. Most common histopathological diagnosis was Borderline Tuberculoid leprosy followed by Indeterminate leprosy. Most common clinical presentation was hypopigmented patches, hypoasthetic patches, erythematous patches and tingling and numbness. 100% Fite-Faraco stain positivity was noted in Lepromatous and Histioid leprosy. Clinicohistopathological concordance was 100% in Erythema Nodosum Leprosum, followed by Tuberculoid (94%) and Borderline Tuberculoid (90%). The concordance was minimum in Mid Borderline leprosy(33%). **Conclusion:** Despite of implementation several government projects to control leprosy,

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leprosy still remains to be highly prevalent disease in India, also seen in this study, as we got 188 cases of leprosy over a period of 24 months. In our study we got high clinicohistopathological concordance in the diagnosis of Tuberculoid, Borderline Tuberculoid and Erythema Nodosum Leprosum cases. But it is low in the diagnosis of Midborderline and Indeterminate leprosy. There is overlapping of clinical features in various types of leprosy, so histopathological examination should be strongly recommended and considered as Gold standard for the diagnosis of leprosy.

Key words: Leprosy, granulomas, hypopigmented patches, acid fast bacilli, clinicohistopathological concordance.

Introduction

Leprosy is the most ancient and widely spread global disease and was described in various articles of ancient civilizations.[1] Leprosy was discovered by Sir Gerhard Armauer Hansen in 1873 [2] and is also called as Hansen's disease . Leprosy is ubiquitous in underdeveloped and developing tropical countries. India alone is responsible for contributing worlds 60 % cases of Leprosy.[3]

Leprosy is a chronic infectious disease which is caused by the bacteria Mycobacterium *leprae*. *M. leprae*, belongs to the family of Actinomycetales, it is an acid-fast, gram-positive obligate intracellular bacillus that demonstrates tropism for phagocytes in the skin and schwann cells within peripheral nerves. It also affects the skin, muscles, eyes, bone and mucosa of the upper respiratory tract[4]. The transmission of the disease occurs through droplets from the nose and mouth. An untreated patient can transmit the disease to a healthy person if there is history of prolonged contact for months. Initiation of treatment stops the risk of transmitting the disease.

In 1990, the goal of eliminating leprosy by the end of the 20th century was proposed by the World Health Organization (WHO) [5]. Despite committed programs of various government and private organizations, disease control is yet to be achieved. In 2016, the WHO launched a new global strategy entitled "The Global Leprosy Strategy 2016–2020: Accelerating toward a leprosy-free world" with the main objectives of reducing the number of children diagnosed with leprosy and presenting visible physical deformities to zero, all countries enacting specific legislation against discrimination, and the reduction of new leprosy cases with grade 2 disability to less than one case per million [5].

Leprosy is categorized in various types depending upon clinical features, histopathological findings and the host immunity.[6] The disease is manifested in two polar forms i.e. Tuberculoid and Lepromatous leprosy. In between these two polar forms lie other forms of leprosy.

Clinical features of Leprosy are [4]

Hypopigmented patches

Partial or total loss of cutaneous sensation in the affected areas.

Thickened nerves

Presence of acid fast bacilli in the skin and nasal smears.

These clinical features and acid fast bacilli demonstration in slit skin smears can help in the diagnosis of leprosy but histopathological examination can help to classify and aid in the definitive diagnosis of leprosy.[7] It will not only help in definitive diagnosis but also prognosis of the disease and assessment of regression of the disease in patient undergoing treatment and also

for research purpose. [7, 8]

Persons affected by leprosy may not only face physical deformity but also face stigmatization and discrimination. Leprosy is curable and treatment in the initial stages can

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prevent disability. So there is rising need to develop more effective tools for early diagnosis and management of leprosy reactions and to prevent nerve damage. [4]

The present study focuses on the importance of histopathological examination for the accurate diagnosis and supporting the clinical diagnosis of leprosy, to guide the clinician for starting of therapy and avoid the untoward effects of treatment.

Aims And Objectives

To classify all skin biopsies having clinical diagnosis of leprosy in to various types according to the Ridley Jopling's classification of leprosy.

To find out the correlation between histopathological diagnosis of skin biopsies with clinical diagnosis of leprosy.

To study various clinical presentations of leprosy with respect to histopathological diagnosis.

Study design

It is a prospective, observational and descriptive type of study

Materials And Methods

It was a prospective study done in the Department of Pathology, Shri Bhausaheb Hire Government Medical College, Dhule over a period of 24 months i.e. from 1st January 2022 to 31st December 2023. The study was ethically approved by institutional ethical committee. All the clinically diagnosed patients of leprosy of all age groups and both sexes were included.

Skin punch biopsies from the lesions were obtained by dermatologist and sent to the histopathology department in 10% formalin solution. Clinical details and provisional clinical diagnosis was provided by the dermatologist. The size of the biopsy was 3-4mm. The biopsies were taken from the skin lesions, it was formalin fixed, routinely processed and routinely stained with Haematoxyline & Eosin stain on automated tissue processor and automated slide stainer available in the department respectively. All the biopsies were also subjected to Acid fast staining using Fite-Faraco stain. The lesions were classified using the Ridley-Jopling's classification into Tuberculoid leprosy, Borderline Tuberculoid, Mid Borderline, Borderline Lepromatous and Lepromatous leprosy. Few of the lesions were categorized as Erythema Nodosum Leprosum and Histioid leprosy which is a rare variant as described by Wade.[9]

Inclusion criteria

All the skin biopsies received in the histopathology section of the department having clinical diagnosis of leprosy.

Exclusion Criteria

1. All the unfixed or autolysed biopsies
2. All the biopsies where clinical diagnosis is not available
3. All the biopsies where histopathology diagnosis was other than leprosy

Following clinical details were taken into account before making clinical diagnosis:

1 Site of the lesion
2 Type of lesion- Flat, plaque, erythema, hypertrophy, nodule
3 Neural involvement- Anesthesia, nerve thickening, tingling, numbness

Histopathological Examination: The classification of leprosy was done based on following histopathological findings:

Location of lesion: Sparing or invasion of epidermis. Involvement of subepidermis,

Presence of granulomas: Whether well formed or ill formed. Extent of granuloma- Involvement of reticular dermis.

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Inflammation: Density of lymphocytic infiltrate, presence of epithelioid cells, presence of Langhans type of giant cells, plasma cells.
Nerve involvement.
Fite -Faraco stain: Presence of lepra bacilli, its density.
Statistical analysis was done using SPSS 16.0.

Observation And Results

We received total 299 biopsies having clinical diagnosis as leprosy over a period of two years. But in 11 cases the histopathology diagnosis was other than leprosy. So we excluded those 11 cases. So the study population in the present study comprises of 288 clinically as well as histopathologically diagnosed cases of leprosy.

Table 1: Age and sexwise distribution of cases

Diagnosis	Male	Female1	Total
0-10yrs	1	6	7
11-20yrs	12	14	26
21-30yrs	42	29	71
31-40yrs	29	24	53
41-50yrs	24	21	45
51-60yrs	33	10	43
61-70yrs	20	10	30
71-80yrs	9	4	13
Total	170(59.02%)	118(40.98%)	288

From table 1 it is clear that the study shows male preponderance (59.02%). The most common age group is 21to30 yrs followed by 31to 40 yrs.

Table 2: Histopathological diagnosis of Leprosy according to age groups

Diagnosis	1-10yrs	11-20yrs	21-30yrs	31-40yrs	41-50yrs	51-60yrs	61-70	71-80yrs	Total
Tuberculoid	1	2	6	9	3	6	4	-	31
Borderline Tuberculoid	2	11	32	20	19	17	6	6	113
Mid Borderline	-	2	1	-	1	3	2	-	9
Borderline Lepromatous	-	2	17	7	5	5	4	2	42
Lepromatous	-	2	-	5	3	4	1	2	17
Histioid	-	1	-	1	2	1	-	-	5
Indeterminate	4	6	13	10	10	5	12	2	62
ENL	-	-	2	1	2	2	1	1	9
Total	7	26	71	53	45	43	30	13	288

From the table it is seen that the most common histopathological diagnosis is Borderline Tuberculoid leprosy followed by, Indeterminate followed by Borderline Lepromatous leprosy.

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Table 3: Clinical features of lesions in various Histologically diagnosed Leprosy cases

HPE Diagnosis	No	Clinical Features							
		Hypopigmented patches	Hypoasthetic patches	Erythematous patches	Tender nodules	Leonine facies, loss of eyebrows	Tingling, numbness	Trophic ulcer	Limb deformities
TT	31	27 (87%)	28 (90%)	3 (10%)	1(3%)	0 (0%)	17 (55%)	8 (26%	0 (0%)
BT	113	96 (85%)	92 (81%)	68 (60%)	3 (3%)	0 (0%)	45 (40%)	20 (18%)	14 (12%)
BB	9	6 (67%)	7 (78%)	2 (22%)	1 (11%)	0 (0%)	6(67%)	3(33%)	0 (0%)
BL	42	27(64%)	8 (19%)	17 (41%)	8 (19%)	6 (14%)	5 (12%)	5 (12%)	7 (17%)
LL	17	2 (12%)	12 (71%)	2 (12%)	4 (24%)	3(18%)	1 (6%)	0 (0%)	1 (6%)
HL	5	1 (20%)	3 (60%)	0(0%)	1 (20%)	1 (20%)	3 (60%)	0 (0%)	0 (0%)
IL	62	43 (69%)	58 (94%)	18 (29%)	4 (7%)	0 (0%)	37 (60%)	6 (10%)	0 (0%)
ENL	9	6 (67%)	6 (56%)	6 (67%)	6 (56%)	1 (11%)	2 (22%)	0 (0%)	0 (0%)

TT- Tuberculoid leprosy
BT- Borderline Tuberculoid leprosy
BB- Midborderline leprosy
BL- Borderline Lepromatous leprosy
LL- Lepromatous leprosy
HL- Histioid leprosy
IL- Indeterminate leprosy
ENL- Erythema Nodosum Leprosum

The above table shows clinical features of the lesions. Most of patients show multiple clinical features. Most patients presented with hypopigmented patches, hypoasthetic patches followed by erythematous patches and tingling and numbness.

Table 4: Fite- Faraco Positivity in Histologically diagnosed leprosy cases

Diagnosis	Fite Faraco Positivity(No)	%
Tuberculoid	0	0
Borderline Tuberculoid	10	9
Mid Borderline	0	0
Borderline Lepromatous	19	45
Lepromatous	17	100
Histioid	5	100
Indeterminate	0	0
ENL	4	44
Total	55	49

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Fite- Faraco staining was done in all cases. Lepromatous and Histioid leprosy cases showed 100% stain positivity. Tuberculoid, Mid borderline and Indeterminate cases showed 0% stain positivity.

Table 5: Clinicohistopathological correlation

Histopathological Diagnosis	**Clinical Diagnosis**									**Concordance rate**
	TT	**BT**	**BB**	**BL**	**LL**	**Histioid**	**Indeterminate**	**ENL**	**Total**	
TT	29	1	1	0	0	0	0	0	31	94%
BT	2	102	1	6	0	0	2	0	113	90%
BB	1	3	3	0	0	0	2	0	9	33%
BL	0	4	0	35	1	0	2	0	42	83%
LL	0	1	0	2	14	0	0	0	17	82%
HL	0	0	0	0	1	4	0	0	5	80%
IL	6	9	3	2	1	0	41	0	62	66%
ENL	0	0	0	0	0	0	0	9	9	100%
Total	38	120	8	45	17	4	47	9	288	

Table 5 shows the clinicohistopathological concordance. From the table it is clear that the clinicohistopathological concordance is 100% in Erythema Nodosum Leprosum, followed by Tuberculoid (94%) and Borderline Tuberculoid (90%). The concordance is minimum in Mid Borderline leprosy (33%).

Discussion

Leprosy is the chronic granulomatous inflammatory disease which accounts for myriad of clinical symptoms. On one end of the spectrum lie minor skin lesions like presence of a small hypopigmented or hypoasthetic patch to the other extreme end where person can face physical disfigurement to serious limb deformity. But early and accurate diagnosis and treatment can only not control the disease but even cure it. So early and accurate diagnosis of leprosy is the foundation of an accurate treatment. WHO defined three key objectives in April 2016,
under the motto “2016-2020 accelerating towards a leprosy- free world.” [5]

There are different classification systems like Madrid, India, Ridley-Jopling’s classification etc. The most widely used classification system is the Ridley-Jopling’s classification. This system is based on clinical, bacteriological, pathological and immunological parameters. [10]

Our study showed a male preponderance with M/F ratio as **1.44/1**. Most of the studies showed similar findings. [11, 12] Male preponderance may be due to tendency of more outdoor jobs in males may expose them to the infection. Social restrictions, taboos may be the cause of inhibition in females for their lesser reporting to the hospitals.[13]

There was a wide age range in our study i.e. 4yrs to 87yrs. The most common age group affected was 21 to 30yrs, followed by 31-40yrs as depicted in Table 1. This is the age group which is responsible for building financial backbone of our country. Other studies also reported similar findings.[11, 12]

The most common histopathological diagnosis in the present study is Borderline Tuberculoid leprosy followed by, Indeterminate followed by Borderline Lepromatous leprosy as shown in Table 2. Our finding of most common histopathological diagnosis of BT and BL is correlating with other studies.[13,11] Most of the studies do not show Indeterminate leprosy as

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one of the most common diagnosis, but few of the studies do show Indeterminate leprosy as one of the most common diagnosis as in our study.[13]

In our study histopathology of Tuberculod leprosy showed well formed granulomas with epithelioid cells involving epidermis, and superficial dermis, erosion of epidermis, presence of lymphocytic infiltrate. Borderline Tuberculoid leprosy cases showed perivascular or periadnexal granulomas in superficial dermis, granulomas enchroaching epidermis, presence or absence of Langhans giant cells. Lymphocytic infiltrate. Midborderline cases showed presence of ill defined granulomas in dermis, perineural lymphocytic infiltrate and presence of AFB on Fite Faraco staining. Histopathology of Borderline Lepromatous leprosy showed dermal infiltrate by macrophages and lymphocytes. Fite- Faraco stain showed abundant acid fast bacilli. Lepromatous leprosy cases showed thinning of epidermis, presence of Grenz zone and dermis showing infiltrate by macrophages showing strong Fite- Faraco stain positivity.

Histopathology of Indeterminate leprosy showed periadnexal, perineural and perivascular scanty lymphohistiocytic infiltrate involving superficial and deep dermis. Histioid leprosy cases showed dermal proliferation of spindle shaped cells arranged in storiform pattern and strongly positive Fite Faraco staining. Patients diagnosed as ENL showed presence of dermal infiltrate by macrophages, dense neutrophilic infiltrate in deeper dermis, Fite- Faraco staining showed large number of acid fast bacilli.

Table 3 shows clinical features of lesions in various histopathologically diagnosed leprosy cases. Clinical features varied in various histopathological types of leprosy. Most patients diagnosed as Tuberculoid leprosy had relatively few asymmetrical lesions and Fite- Faraco stain showed nil or very low bacillary index. Patients diagnosed as Borderline Tuberculoid leprosy showed few hypopigmented and hypoasthetic plaques showing slight elevation. Midborderline cases showed multiple, erythematous, indurated plaques showing asymmetrical distribution. Patients of Borderline Lepromatous and Lepromatous leprosy both showed similar clinical features like presence of multiple, symmetrical or asymmetrical erythematous nodules or papules. Histopathology showed high bacillary index in LL (6+) and BL showed bacillary index as 4-5.[14] The patients diagnosed as ENL showed multiple tender papulonodular lesions. It is a type III humoral hypersensitivity reaction seen in patients on MDT treatment of leprosy[15] usually seen in Lepromatous leprosy and occasionally in BL leprosy. [16] Clinically it is characterized by the rapid appearance of painful erythematous subcutaneous nodules. [16] ENL reaction is characterized by deposition of immune complexes in the tissues, blood, and lymphatic vessels. [17]Histioid leprosy patients showed multiple diffusely distributed papulonodular lesions.

Table 4 shows Fite -faraco stain positivity in different types of leprosy. In our study 100% stain positivity was seen in Lepromatous and Histioid Leprosy cases. Minimum positivity was seen in Tuberculoid, Mid borderline and Indeterminate cases showing 0% stain positivity. Most of our findings are correlating with other studies.[18, 19]

Table 5 shows the clinicohistopathological correlation. In our study maximum clinicohistopathological concordance was seen in the diagnosis of ENL, TT and BT types of leprosy. Our finding of maximum concordance rate in diagnosis of TT and BT Leprosy are matching with other studies. [12, 20, 22]

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Table 6: Comparison of clinicohistopathological concordance rate in various studies:

Type of Leprosy	Present study	Yadav etal [20]	Tilva etal [21]	Kini etal [22]	Atram etal [12]
TT	94	100	77	92	88
BT	90	37	67	82	86
BB	33	-	71	98	96
BL	83	60	63	93	95
LL	82	58	78	97	97
HL	80	-	100	94	98
IL	66	100	-	95	93
ENL	100	-	-	-	-

Our study showed 100% clinicohistopathological concordance in ENL. This may be due to obvious, unique clinical features of the lesions and clear cut histological characteristics for its diagnosis. Clinicohistopathological concordance was minimum (33%) for the diagnosis of Midborderline leprosy. This may be due to the overlap of clinical and histological features for the diagnosis with other types like BT and BL leprosy.

Conclusion

India is successful in achieving elimination of leprosy as a public health problem as per WHO criteria of less than 1 case per 10,000 population at the National level in 2005. The government is implementing various schemes to eradicate leprosy. Leprosy is still a highly prevalent disease in India, also seen in this study as we got 188 cases of leprosy over a period of 24 months. In our study we got high clinicohistopathological concordance in the diagnosis of Tuberculoid, Borderline Tuberculoid and Erythema Nodosum Leprosum cases. But it is low in the diagnosis of Midborderline and Indeterminate leprosy. There is overlapping of clinical features in various types of leprosy, so histopathological examination should be strongly recommended and considered as Gold standard for the diagnosis of leprosy.

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Conflicts of interest: No conflicts of interest
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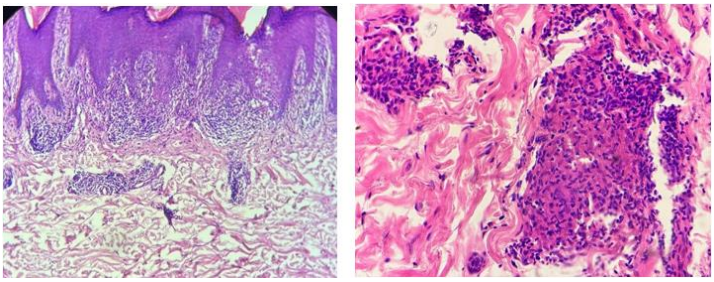

Figure 1: (A&B) Photomicrograph of TT leprosy showing well formed granulomas with dense inflammatory reaction in epidermis and superficial dermis (H&Ex10 and H&Ex40)

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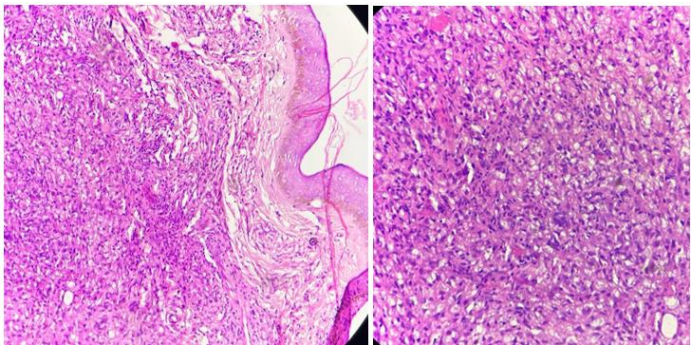

Figure 2: (A&B) -Photomicrograph of LL leprosy showing thinning of epidermis, presence of Grenz zone and dermal infiltrate by foamy macrophages (H&Ex10 and H&Ex40)

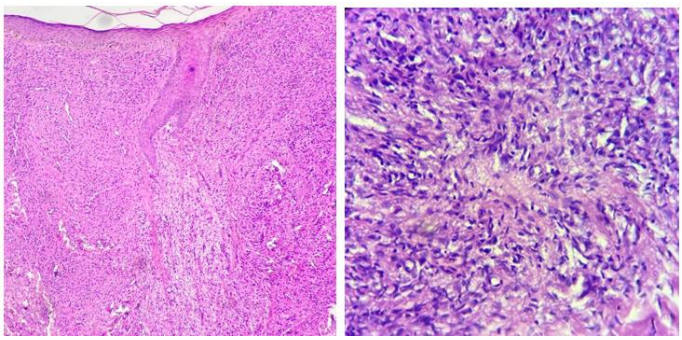

Figure 3: (A&B)- Photomicrograph of Histioid leprosy showing dense dermal aggregates of spindle shaped histiocytes. (H&Ex10 and H&Ex40)

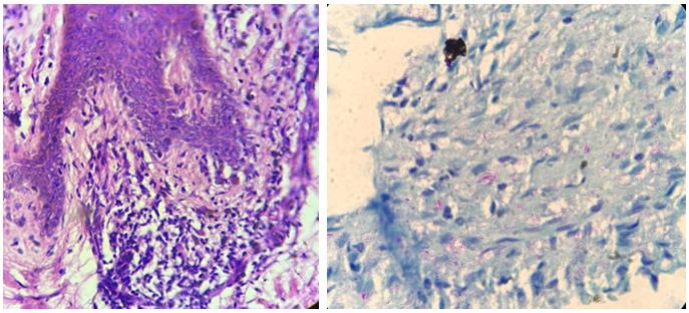

Figure 4: (A&B) A-Photomicrograph of Borderline Tuberculoid leprosy showing ill formed granulomas enchroaching the epidermis along with inflammatory infiltrate. (H&Ex40)
B- Photomicrograph of Lepromatous leprosy showing, numerous collection of acid fast bacilli. (Fite -FaracoX100)

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