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Cyto-Pathological Diagnosis of Post Kala-Azar Dermal Leishmaniasis (PKDL) in Tertiary Care Hospital, At Patna, Bihar

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Abstract
Objective: *Post–kala-azar dermal leishmaniasis (PKDL) has potential for transmission of visceral leishmaniasis (VL) in endemic population which is dermatosis after cure of VL. It has importance for public health problem for elimination of visceral leishmaniasis (VL). The aim of present study was to evaluate the cyto-pathological diagnosis of post kala-azar dermal leishmaniasis.*
Material and Methods: *A total of 122 patients attending in Medical and Skin & VD OPD for different PKDL lesions were included in study.*
Result: *Out of 122 patients, 102 patients showed that median age of males and females at the time of diagnosis was significantly different (P = 0.013). A significant association was observed between family history of VL and sex of PKDL patients (c^2 = 5.72, P < 0.01). 33% of the VL patient's development PKDL within one year of treatment. The appearance of lesions and diagnosis is an important factor in VL transmission. There was a significant association between type of lesions and duration of appearance after VL treatment (c^2 = 6.59, P = 0.001). Diagnosis of PKDL is still difficult in the field condition; however in tertiary care centres NMCH Patna demonstrated presence of LD bodies in macular lesion 39% and papulo-nodular 100%.*
Conclusion: *Majority of macular lesions were still undiagnosed even by advanced techniques which can hamper the national elimination programme for VL. India is a developing country and has high endemicity of VL particularly north Bihar, which contributes more than 80% of total VL and PKDL of India. Most sophisticated tests available today for the diagnosis of PKDL have their own challenges like lack of limited resources, lack of skills, lack of interpretations, lack of infra-structures; poorly contribute to diagnosis and delay the treatment among poor populations.*

Introduction

Post–kala-azar dermal leishmaniasis (PKDL) is a neglected dermatosis caused by Leishmania donovani parasites in the skin that develops as a sequel to kala-azar after apparently successful treatment of patients with visceral leishmaniasis

(VL) or those without a history of VL. Being a non life threatening condition,the patients often delay the treatment, thereby maintaining the reservoir of infection. The diagnosis of PKDL rests on the demonstration of the parasites in the tissue smears, immune diagnosis by detection of parasite antigen or antibody in blood or detection and quantitation of parasite DNA in tissue specimen. Clinically it is characterized by macular, maculo-papular, and nodular lesions in patients recovering from VL who are otherwise healthy. It occurs in nearly 10–20% of patients cured of VL within 1–5 years in India, and approximately 50% of patients in Sudan within six months.(Zijlstra EE, et al , 1994., Am J Trop Med Hyg 51: 826–836)

In the Indian sub-continent, kala-azar appears to occur at an interval of 10– 15 years (WHO.1984). Patients with PKDL have immense public health importance because they act as reservoirs of infection. (Sen Gupta PC, 1968.). N0 animal reservoirs of L. donovani, in asia specific sub-continent, it is important to diagnose and treat as early as possible to prevent future outbreaks of VL(Thakur et al, 2008.)

As the sophisticated molecular tests are not only expensive but also need skilled hands and expensive instruments. So to be useful, the diagnostic method must be accurate, simple and affordable for the patients and for the population, with screening intention especially in endemic areas. The slit skin smear examination is an old standard and reliable technique for the diagnosis of PKDL that can be performed by the pathologists as an OPD procedure. Still this is considered as the gold standard tests because parasites are detected directly under the microscope, along with culture. (Verma et al 2013).

It has been reported that a large proportion of VL patients treated with sodium antimony gluconate (SAG) have a high probability of development of PKDL as well as in other anti-leishmanial drugs like Am Bisome, Miltefosine, paramomycin, amphotericin B and combination therapy. Clinical and epidemiologic aspects of PKDL are important for understanding transmission dynamics and defining VL control strategies. Because there is no mechanism currently available to detect PKDL cases at the community level. So the control strategies currently operational in disease-endemic areas would fail to achieve their goal of complete elimination of Kala-azar. Therefore, we studied neglected cases of dermatosis of Kala-azar endemic areas either with past history or family history of kala-azar as well as those cases which were reluctant to conventional treatment of dermatosis for the detection of PKDL cases by identifying clinical and epidemiologic features that are important to public health.

Material and Methods

The present study was conducted in the Department of Pathology, Nalanda Medical College Patna, India; with the help of Department of Microbiology, Skin & VD and Medicine during the period of January 2014 to December 2017. A total of 122 PKDL suspected cases of skin lesions were send to our department for cyto- pathological evaluation. Out of which 102 patients with PKDL were enrolled in the study after providing informed consent. Each patient were evaluated and epidemiological data were collected in required format.

All the persons were clinically examined for the identification of lesions on the face, trunk, arms, and legs and type of lesions such as macular, papular, nodular, or mixed. Skin slit smears as well as FNAC of nodular lesions of patients were collected for detection of L. donovani using the standard microscopic procedure. Only parasitologically confirmed patients were considered as patients with PKDL in this study. Initial diagnosis of PKDL was based on the clinical examination and positive immuno-chromatographic rk39 strip test. The diagnosis was confirmed by visualization of Leishmania Donovan bodies obtained through slit skin smear and stained by Field or Giemsa stain and examined under the microscope.

Skin slit scrapings were taken under full aseptic precaution. The chosen lesion, preferably an

indurated one, was cleaned with spirit, mopped with 10% Betadine solution, and allowed to dry. The lesion was gently pinched between index finger and thumb for 1-2 minutes, exerting enough pressure to blanch it. A clean cut of 5-8 mm long and 3 mm deep was made with a sterile disposable scalpel blade to reach the infiltrated layer of dermis. The sides of the cut were scraped by blunt end of the scalpel for many times to obtain adequate sample material. The scraped material was thinly spreaded on clean glass slides using a circular motion working outwards to avoid damaging any parasites. When the smear was dried, slides were fixed and stained with either giemsa or field stain. In some cases of macular lesion, tissue biopsy was taken in a fixative, dehydrated and then embeded in paraffin. The embedded tissue was sectioned into very thin sections (2-4 microns) using the microtome and stained for microscopic examination. Such tissue biopsy was taken as an additional measure for the diagnostic purposes although present study was based on cytological detection of Leishmania Donovan bodies by either skin slit smear examination or FNAC from the suspected skin lesion.

In the present study, the slit skin smear was utilized for the diagnosis of PKDL. This procedure can yield results within an hour, minimally invasive and can be performed in poor resources settings with routine stain and simple light microscope .It is suitable for both children and adults even with facial lesions who may be unwilling for tissue biopsy. Even expert technicians may be trained to look for the L D bodies under the microscope. The reagents used are stable at room temperature and need minimum maintenance. Skin slit smear samples are stable at tropical temperatures and can easily be transported even from the remote areas.

Microscopic examination was performed more than 500 oil immersion high power fields and minimum of 15 minutes zigzag examination before declaring the smear as negative sample.

Results

Out of 102 patients with PKDL, 61 (60%) were males and 41 (40%) were females (Table 1). The overall median age of PKDL patients was 23 years (range = 5–60 years), which was significantly different (P = 0.013) between males (25 years, range = 6–60 years) and females (16 years, range = 5–51 years). The median ages of male and female VL patients were 18 years (range = 4–56 years) and 13.5 years (range = 2–52 years), respectively. In patients 5–14 years of age, out 0f 41 female cases, 49% of PKDL patients were female, although 42% of patients with VL were female. Out of 61 male patients, 18% of PKDL patients were male in 5-14 years age group, although 41% of VL patients were male. Such findings indicated that more female PKDL patients seek early treatment for PKDL. We did not observe any PKDL patients in persons < 4 years of age.

There was no significant association of past history of VL with PKDL by sex (c^2 = 0.399, P = 0.5273) (Table-2). Nearly 85 (83%) of PKDL patients had a history of VL, of whom 80 were treated with a single full-course regimen, and 5 received multiple regimens at different times and combination of two drugs. 38 cases (37%) had a family history of VL and 64 (63%) did not have a family history of VL. A significant association was observed between family history of VL and sex of patients with PKDL (c^2 = 5.72, P < 0.01). 83% patients of PKDL with past history of VL and 38% patients of PKDL with family history of VL indicated a possible synergistic effect of these two factors on PKDL (Table-3).

Table 1 Demographic evaluation of Age incidence of patients of PKDL

Age	Male patients(61 cases)	Female patients(41cases)	Total cases
5-14 yrs	11 (18%)	20 (49%)	31 (30%)
15-24 yrs	17 (28%)	5 (12%)	22 (21%)
25-34 yrs	15 (24%)	8 (19%)	23 (23%)
35-44 yrs	9 (15%)	6 (15%)	15 (15%)
>45 yrs	9 (15%)	2 (5%)	11 (11%)
	61(100%)	41(100%)	102(100%)

Table 2 Demographic evaluation of incidence of past history of VL

Total no.of patients included in study (n=102)	Past history of kalaazar (VL) present	No past history of kalaazar (VL)
MALE (n=-61)	52 (85%)	33 (80%)
FEMALE (n=41)	9 (15%)	8 (20%)
Total (n=102)	85 (83%)	17(17%)

Table 3 Demographic evaluation of incidence of family history of VL

Total no.of patients included in study (n=102)	Family history of kalaazar (VL) present	No family history of kalaazar (VL)
MALE (n=-61)	17(28%)	44(72%)
FEMALE (n=41)	21 (51%)	20 (49%)
Total	38 (37%)	64 (63%)

Table 4 Demographic evaluation of time in years between diagnosis and appearances of lesions of PKDL

Time elapsed	Male patients(61 cases)	Female patients(41cases)	Total cases
Within 1 year	29 (47%)	25 (61%)	54 (53%)
2-3 years	23 (38%)	11 (27%)	34 (33%)
4-5 years	6 (10%)	3 (7%)	9 (9%)
>5 years	3 (5%)	2 (5%)	5 (5%)

Table 5 Evaluation of LD bodies detection in different type of skin lesion in same patient (Total 54 cases)

LD bodies present	Macular lesion (54)	Papulonodular lesion(54)
YES	21(39%)	54 (100%)
NO	33(61%)	00
TOTAL	54(100%)	54(100%)

Table 6 Drugs used and PKDL presentation in VL-treated cases, Bihar, India

Anti-leishmanial drug	No. (%) patients(85)	Median time lapse after treatment (months)	Range (months
SSG	9(10%)	23	1-192
Amphotericin B	15(18%)	29	8–106
AmBisome	32(38%)	9	5-13
Miltefosine	20(23%)	31	29-42
Paramomycin	4(5%)	25	19-37
Combination therapy (paramomycin+miltefosine)	5(6%)	14	8-21

Confirmation of the Presence of LD Bodies

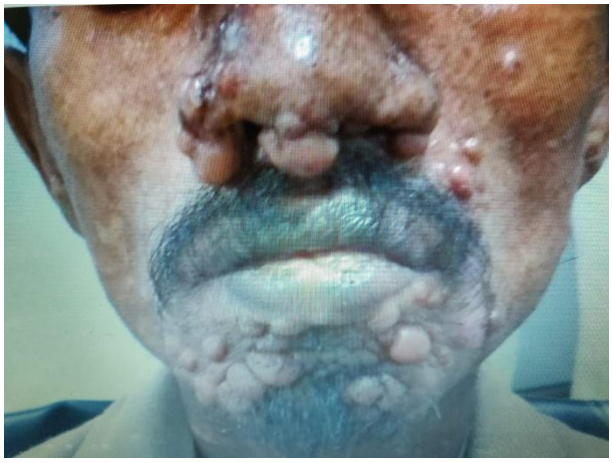

Fig.1: Photo shows typical nodular pattern of PKDL.

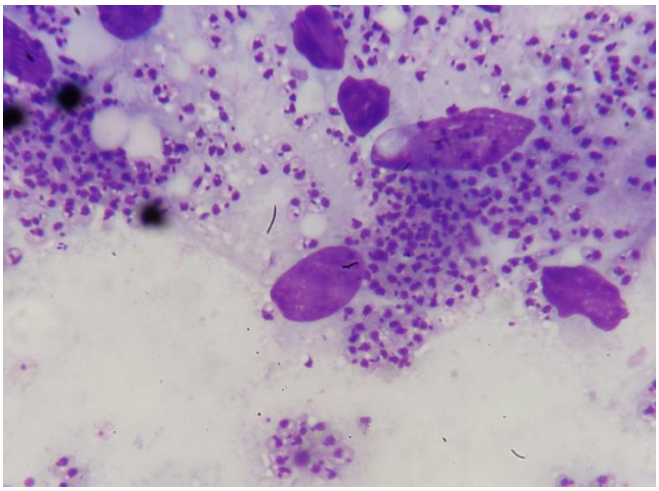

Fig.2: Microscopic picture of LD bodies in slit skin smear.

In the slit skin smear, LD bodies seen as lying singly or in clusters, either intracellurly or within mononuclear macrophages or as extracellular structures. The amastigote forms have the following characteristics

* Round to oval bodies, 2-4 microns in size along longitudinal axis.
* Presence of delicate cell membrane.
* Presence of delicate cell membrane and kinetoplast lying at right angles to each other.
* The nucleus shows approximately 5-6 times the size of kinetoplast.
* The colour of kinetoplast and the nucleus are same as nucleus of histiocytes, although in some cases the colour of kinetoplast appear as slight darker than the nucleus.

The above mentioned characteristics of LD bodies were analysed for each slit smear examined. For the field diagnosis of PKDL in endemic areas, the slit skin smear examination was used as primary confirmatory test in rk39 positive cases. All slit skin smear negative patients in field of endemic areas, clinically suspected as PKDL was considered to be evaluated by molecular methods in tertiary referral centres.

However, we did not observe any significant association between individual history and family history of VL on cases of PKDL (P = 0.126, c^2 = 2.342).

Duration of first appearance of lesions and diagnosis was approximately 12 months (range = 1–144 months). Nearly 54% of persons were given a diagnosis within 12 months after appearance of lesions and 46% were given a diagnosis after one year. Female patients (61%) were given a diagnosis of PKDL comparatively earlier than male patients within a year after occurrence of a lesion, but the difference was not statistically significant (P > 0.05) (Table-4).

The median time of manifestation of PKDL was 24 months (range = 1–192 months). Overall, PKDL developed in 82% of patients within 5 years of successful treatment for VL. In 33% of patients PKDL developed in within one year of successful treatment**.** Regarding the distribution of initial occurrence of type of lesions, 41 (48%) of the PKDL patients had macular lesions and 44 (52%) had either papular or nodular lesions. The most prominent site of initial occurrence of lesions in the cohort were the face and arms in 41 (41%) patients; face, arms, and legs in 21 (21%) patients; and face in 12 (12%) patients. Types of lesions and duration of their appearance after VL treatment was significantly associated (c^2 = 6.59, degrees of freedom = 2, P = 0.001).

Out of 102 cases, in 54 cases where mixed distribution of macules, papules and papulo-nodular lesions found. Skin slit smear taken from both the lesion separately.

Selection of skin lesion for skin slit smear examination for detection of LD bodies is very

important .The conclusion may be drawed that out of 54 positive cases for LD bodies, LD bodies found in 39% only in macular lesion, although all 54 cases were LD bodies positive on skin slit smear examination when the sample was collected from the papulonodular lesions. Of 85 patients, 9 cases (10%) showed complete cure after treatment with a full course of SAG, 15cases (18%) after treatment with amphotericin B, 32cases (38%) after treatment with Am Bisome, 20 cases(23%) after treatment with Miltefosine, 4 cases (5%) after treatment with Paramomycin and 5 cases (6%) after treatment with combination therapy (paramomycin & miltefosine) (Table 7). Most of the patients 80 cases (94%) patients received a single drug and only 5 (6%) received multiple drugs of combination therapy. patients treated with AmBisome, lesions occurred within 5– 13 months,as compared with other regimens, lesions appeared in 2 years after cure of VL(Table-5).

History of VL seems to be more predispose to PKDL than persons without VL,and is an important factor for development of PKDL. It has been reported that 15–20% of PKDL patients had no history of VL suggestive of asymptomatic infection. 17% of patients with PKDL In the present study had no history of VL. Causes of development of PKDL without a history of VL need to be investigated.

The type of anti-leishmanial drugs used for treating VL patients has an implicit association with PKDL. In this present study of PKDL patients, 85 patients was with a history of VL .Previously it used to develop among patients with a past history of VL treated with sodium antimony gluconate (SAG) but it also occurs with all currently used therapies such as Amphoterin B , AmBisome ,Miltefosine ,Paramomycin as well as combination therapy with Paramomycin and miltefosine.(TABLE 7) . It has been reported that most kalaazar patients treated and cured with SAG, but not SAG-resistant cases, showed development of PKDL even after increasing the dose and duration of treatment with SAG.

Discussion

Female Sand fly (Phlebotomus aregentipes) is a vector, responsible for the development of VL and subsequently PKDL has the potential to develop infection by producing promastigotes and is thus capable of transmitting parasites to human by bites .Apart from skin lesion as dermatosis, PKDL patients are healthy and do not have any physical limitations, but PKDL can lead to severe disfigurement, especially the face. The incidence of PKDL is an important public health problem in India because PKDL acts as the reservoir of L. donovani and helps trigger transmission of VL in disease in endemic areas.

In Indian sub-continent, untreated patients with VL and PKDL are considered to be the only reservoirs that provide a source of exposure by disseminating the causative parasite to unaffected persons living in the same community of these two reservoirs of L. donovani, untreated PKDL patients were the easy source for sand flies to acquire parasites. Among all the lesions, nodular forms are probably a prominent source of disease transmission in Indian subcontinents. Because nodular lesions have large concentrations of L. donovani, this finding could be one of the reasons for continued sustainability of VL transmission dynamics in disease-endemic regions.

In this present study of patients with PKDL, approximately 53% had either papular or nodular lesions, among them lesions on the face and arms predominate over the other sites which are usual exposed areas for bites of sand flies.

It has been reported that a small percentage of patients treated for VL show development of PKDL within 2–3 years, but PKDL may develop much earlier (i.e., after 6 months) or much later (up to 32 years later) (Salotra et al 2006). In this study, we observed that the median time of PKDL presentation was 24 months after the end of VL treatment, and in some cases occurred much earlier (within 1 month after treatment) or much later (within 16 years).

In present study 33% of patients had PKDL within a year after VL treatment, and

approximately 49% had PKDL within 2–5 years and In Bangladesh, nearly 38% of PKDL patients had manifestations within one year after treatment for VL compared with 33% in this study, which showed nearly statistical similarity in two regions of disease-endemic areas of VL in the Indian sub-continent. Conversely, in Sudan, the time interval between VL and PKDL is short; all cases occur within 0–13 months after treatment, usually within first 6 months.

Development of PKDL among patients with VL who were treated with other more potent drugs such as Am Bisome, miltefosine, and paramomycin occurred in 2-5%.

The distribution of PKDL patients is similar to that of VL patients because PKDL patients represent a small subset of VL patients with a higher proportion among males (60%) than females (40%). The median age of occurrence of PKDL was significantly different by sex (P = 0.013). The higher proportion of female patients with PKDL in persons 5–14 years of age could be caused by appearance of lesions on face, which are often confused with leprosy, a severe social stigma regarding marriage in communities in India, particularly in rural areas. Because of this finding, parents of unmarried women with PKDL approach health providers seeking diagnosis and treatment comparatively earlier than male patients with PKDL.

The time between appearance of lesions and diagnosis is an important factor in the public health importance of patients with PKDL in terms of transmission of infection in the community. The median time between appearance of lesions and diagnosis was 2 years (range = 1–12 years). A long delay in seeking diagnosis and treatment of PKDL and high densities of Phlebotomus argentipes could provide opportunities for continuation of transmission of VL in the community. New Anti-leishmanial drugs regimens and/or combi-nation therapy with high cure rates needs to be investigated**.**

For diagnostic purposes, skin slit smear examination is still the gold standard test, especially for screening in endemic areas and the lesion selection for the slit skin smear is very important, The Patient must be examined thoroughly and papular as well as papulonodular lesion to be selected for the sample collection , as in comparison to papulonodular lesion, parasites detected in macular lesion was only 39% in comparison to papulonodular lesion which were positive for parasites in same patients in this present study. The sensitivity for the slit skin smear is in the range of 67-100% in nodules,36-69% for papules and 7-33% in macules.(Ramesh,V et al 1995;Sharma M C et al,2000; Verma N et al 2015).

Nevertheless, recognizing L D bodies in a well made smear requires patience, experience and dedication. The organisms, when present, are often found in indurated lesions and even under ideal conditions the success rate has varied from as low as 20% in Sudanese (Zijlstra EE,et al 2000) to approximately 60% in Indian PKDL.(Singh RP;1968). The polymorphic presentation, where the degree of inflammation and parasite burden is least in macules, increasing from papules to nodules may explain the smear negativity.(Beena KR ,et al,2003).

Conclusion

The incidence of PKDL has important public health implications because PKDL acts as the reservoir and continuous source of L. donovani in India and causes transmission of VL in endemic areas. As there is no strategy for elimination of PKDL in Indian sub-continent, untreated patients of PKDL should be considered as the only reservoirs and source of exposure for the causative parasite disseminating VL to unaffected persons living in the same community .Skin slit smear examination, still, the gold standard test for the detection of Leishmania donovan bodies, to be performed in endemic areas for the detection of LD bodies in suspected persons of PKDL for achieving the Goal of complete elimination of Kala-azar from the Asian countries.

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