

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Histopathological Study of Non Healing Ulcers of Skin in Patients Attending Tertiary Care Hospital.

Brij Mohan Kumar Singh^{1*}, Arijit Bishnu¹, and Barnini Banerjee².

¹Department of Immunoematology & Blood Transfusion, Kasturba Medical College, Manipal, Manipal University, Karnataka, India.

¹Department of Pathology, Melaka Manipal Medical College, Manipal Campus, Manipal University, Karnataka, India.

²Department of Microbiology, Kasturba Medical College, Manipal, Manipal University, Karnataka, India.

ABSTRACT

Clinico-pathological correlation of non-healing ulcer provides an utmost important clue in the spectrum of this debilitating condition. Little information is available in the outcome of the ulcers when they underwent surgical biopsy for determination in the causation of these conditions. Therefore, the relative incidence and etiology of non-healing ulcer was assessed retrospectively after retrieving the cases of non-healing ulcer from the department of Pathology, Kasturba Medical College, Manipal. A total of 75 cases were studied in this regard and were compared with their Clinical presentation which were retrieved from their case files. As compared to the west, venous and diabetic ulcer predominate, in the developing nations the most frequent cause of a non-healing ulcer are malignancy (n=25) followed by chronic diseases like tuberculosis (n=20). In this study, various risk factors involved in the causation of non-healing ulcer were also compared and was found to be having a greater impact and should not be kept aside. Thus beside a good and accurate clinical diagnosis, early biopsy to rule out malignancy and identify the etiology is mandatory for a better clinico- pathological.

Keywords: Non-healing ulcer, Malignancy, Histopathology, Infections

**Corresponding author*

INTRODUCTION

Skin is the single largest organ of the body that protects against mechanical trauma, radiation and infection. Because of its complexity a wide range of diseases can develop from the skin ranging from infectious to malignancy. The vast diversity of these lesions combined with a body of descriptive data, often overlapping (clinical, histological) produces confusion in the area of nomenclature and difficulty in diagnosis.

General surgical referral guidelines defines any non-healing ulcer as that which has been present for 3-4 weeks duration and has not responded to conventional therapies [1]. Sometimes a need of surgical intervention is forced upon. Although histopathology remains the gold standard for most dermatologic diagnoses, it must be recognized that not all lesions are amenable to definitive "specific" histologic diagnosis.

However a combination of histological details in correlation with clinical data, culture & use of special staining (viz. ZN stain and PAS) and other ancillary investigations help in arriving at a more specific diagnosis.

Thus, the study of non-healing skin ulcers is perhaps intriguing, fascinating, challenging and at times even frustrating than any other skin lesions. This study was in need in the present scenario were patient with non-healing ulcer go unnoticed for a long time without biopsy resulting in uncertainty of developing malignancy which can be identified at a very early stage.

MATERIALS AND METHOD

This study was conducted retrospectively in the department of pathology, Kasturba Medical College, Manipal University, where the archives of department of Pathology was searched for cases of non-healing ulcer for a period of 4 years(2010-2013). Total of 75 cases were retrieved for this study. This group included all ulcers that had not healed 3-4 weeks after conventional therapies of both sexes and all ages.

The detailed histories and clinical examination findings were retrieved from the case files with emphasis on morphological features of ulcers i.e. number, distribution of ulcer (site) and associated diseases were noted. The surgical specimen which was received in our department was consisted of punch biopsy, shave biopsy, incisional biopsy, excisional biopsy and curettage. All glass slides and block were retrieved, including routine Haematoxylin and Eosin stain, Ziehl-Neelsen stained, and Periodic-Schiff's stained slides.

RESULTS

Of the 75 cases, 50 were diagnosed as benign Ulcers and 25 as malignant ulcers (Table 1). The benign ulcers constituted 66.67% and malignant ulcers constituted 33.33%. Out of the 75 cases, 20 cases (26.67%) were found to be Tubercular ulcers. These ulcers were found to be fairly common below 50 years of age. Lupus Vulgaris was found to be the commonest form among the tubercular ulcers constituting 75% of the tubercular ulcers followed by Scrofuloderma accounting for 25% of the cases.

Table 1: Etiological types of ulcer.

<i>Etiological types</i>	<i>Number of patients</i>	<i>Percentage (%)</i>
Tubercular ulcers	20	26.67
Malignant ulcers	25	33.33
Diabetic ulcers	13	17.33
Infectious ulcers	12	16
Other ulcers	5	6.67
TOTAL	75	100

Of the total cases, ulcers associated with diabetes accounted for 17.33% cases. From the study it was observed that diabetic ulcers were relatively common in males accounting for 61.5% and less common in females accounting for 38.5%.

It was observed among malignant cases, that Squamous cell carcinoma is the most common variety of malignancy encountered in an ulcer accounting for 80% followed by Basal cell carcinoma 16% and malignant melanoma 4%.

Of the total cases, 30 cases were sent for culture and sensitivity tests, where infectious etiology was suspected. Staphylococcus was found to be the most common pathogen accounting for 50% of the bacteriological isolates. This was followed by klebsiella and Proteus (16.67%) and Pseudomonas & Streptococcus which was seen in 8.33% each.

The group of "other ulcers" consist of venous ulcers which were diagnosed clinically and radiologically but no histopathological evidence could be found; ulcers due to foreign body granuloma histopathologically, and ulcers for which no etiology could be identified histologically. Hence these ulcers could not be typified in definite etiological classes and were grouped together.

From the history and physical examination we identified a number of risk factors such as Increasing fasting blood sugar, bare foot walking, Repeated trivial trauma, Peripheral neuropathy, under nutrition, Overcrowding, Presence of physical deformity, Prolong sun exposure, Smoking etc.

Some risk factors were found to be associated with specific etiologies. Risk factors like: overcrowding & under nutrition were found to be more commonly associated with tubercular ulcers. Malignant ulcers were associated with prolong sun exposure. Diabetic ulcers were found to be associated with increased fasting blood sugar which means poor glycaemic control, thus a worse condition and peripheral neuropathy. Other independent risk factors which were found to be associated with all forms of ulcers reported in the study include repeated trivial trauma. Smoking has been found to be a non-specific risk factor. However most, if not all the risk factors were commonly associated with lower extremity ulcers.

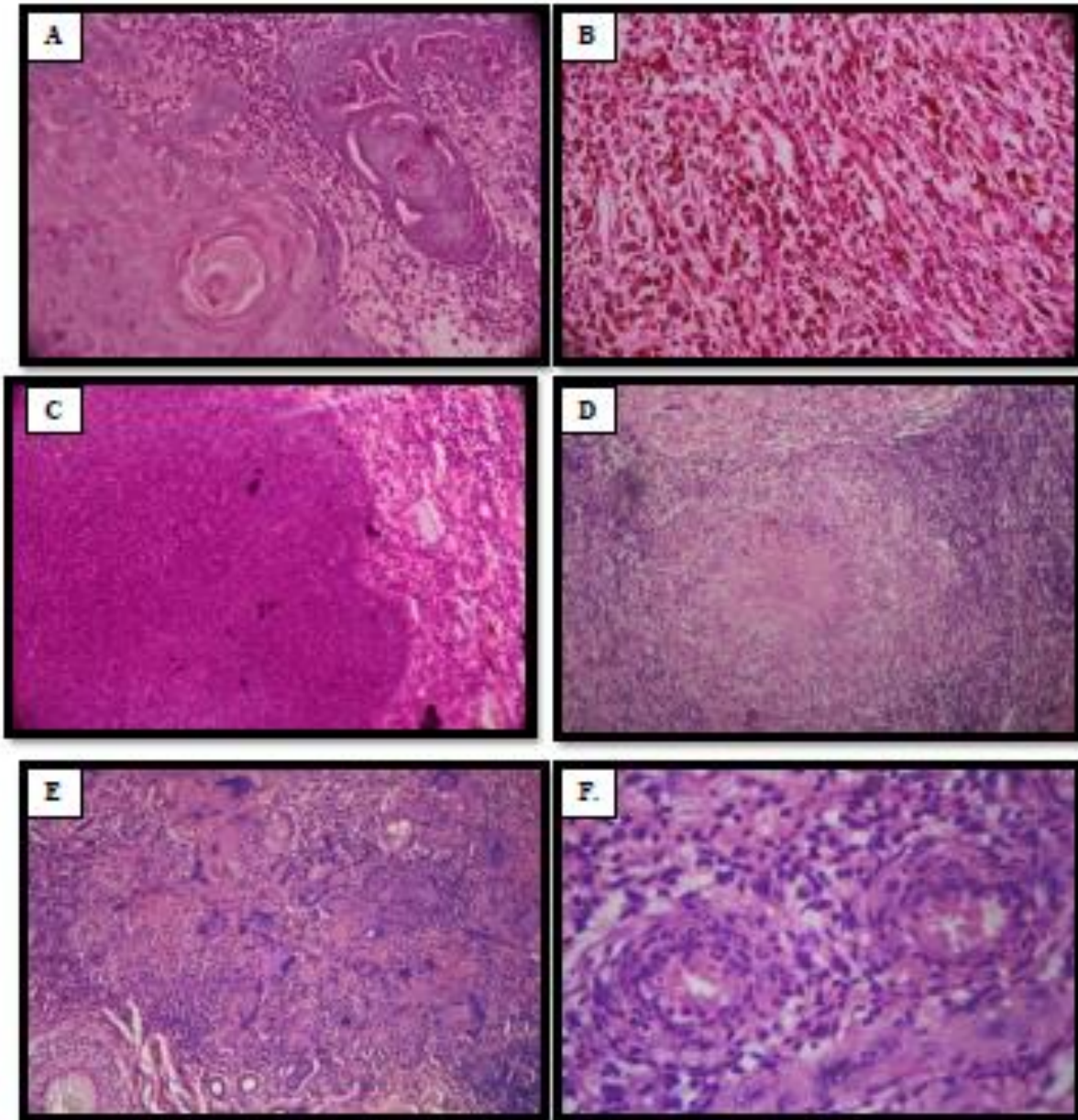
It was also observed that the sensitivity of the clinical diagnosis of malignancy was 84%, whereas the positive predictive value of clinical diagnosis of malignancy was 91.3%; implying a better clinical diagnostic accuracy compared to the non-healing ulcers due to infectious etiology which had a sensitivity of 33.33% and positive predictive value of 50%. The tubercular & diabetic ulcers were found to have sensitivity rates of 75% & 71.42% respectively and positive predictive values of 68.2% and 83.33% respectively.

Table 2: Comparative study of the findings by different researcher(Causes of Non Healing Ulcer)

<i>Workers</i>	<i>Mekkes et al</i> ⁵⁴	<i>Zeegelaar et al</i> ⁸	<i>Saraf et al</i> ⁵⁵	<i>Present study</i>
Causes of non-healing ulcers	1.Venous insufficiency 2.Peripheral vascular disease 3.Diabetes 4.Pressure ulcers 5.Infection(mostly Streptococcus)	1.infection(mostly Staphylococcus) 2.malignancy 3.trauma 4.Nonspecific ulcers	1. leprosy 2.diabetes 3.trauma 4.venous ulcers	1.malignancy 2.tubercular ulcers 3. diabetes 4. infection 5.miscellaneous

Table 3: Comparative study of the various risk factors identified by different workers

Ch.Manes et al [32]	Gaur et al [35]	Shetty et al [50]	Vijay Vishwanathan& colleagues [53]	Present study
1.FBS levels 2.Peripheral neuropathy	1.Ill fitting shoes and shoe related factors 2.Repeated trivial trauma 3.Bare foot walking	1.Overcrowding 2.Undernutrition 3.Bare foot walking 4. Low education level	1. increasing age 2.undernutrition	1. Fasting blood sugar 2.Bare foot walking 3.Repeated trivial trauma 4..Peripheral neuropathy 5.Undernutrition 6. Presence of physical deformity 7.Smoking 8.Prolong sun exposure. 9.Overcrowding



(A) Well differentiated Squamous cell carcinoma, (H&E, X200) (B) Malignant melanoma showing the pleomorphic melanocytes infiltrating the dermis, (H&E, X400) (C). Basal Cell Carcinoma showing the peripheral palisading arrangement of the basaloid cells, (H&E, X200) (D) Caseating Granuloma in Scrofuloderma, (H&E, X200) (E) Lupus vulgaris: showing Langhans giant cells and Non necrotising Granuloma., (H&E, X200) (F) Polymorphonuclear leukocyte inflammatory infiltrate around blood vessels to bacterial infection (H&E, X400).

DISCUSSION

Non healing ulcers are an important cause of morbidity in developing countries [2, 3]. This may be explained by the poor socioeconomic conditions, poor health services in remote areas and lack of public awareness.

In contrast with the developed countries, little is known about the prevalence and etiology of non-healing ulcers in these areas. One study from Nigeria reported that the most common etiologic factors were

trauma, diabetes mellitus and sickle cell disease [4]. Depending on the region, infectious causes such as cutaneous Leishmaniasis or Buruli ulcer may be encountered [5, 6]. In contrast venous ulcers are most common cause of ulcers in developed countries but are reported to be uncommon in tropical countries [7]. Zeegelar et al [8] also reported that venous ulcers and diabetic ulcers are common in developed countries. In his study we found that the most common cause of ulcer was bacterial infection (59%) followed by a surprisingly large number of malignancies (55%)

Patients in the present study who had an ulcer with a mean duration of 6 ± 2 years were diagnosed as malignant. This is in correlation with the observation of Mandong et al, [9] who reported mean duration of malignant ulcer is 5-10 years.

In the present study, malignant ulcers were found to be the most common cause of non-healing ulcers of skin accounting for 33.33% (Table 2). Of these squamous cell carcinoma is the most common. This finding is in concordance with that reported by Yakubu et al [10]. This was followed by non-healing ulcers due to Tuberculosis which accounted for 26.67%. Diabetic ulcers accounted for 17.33%. Non healing ulcers of infectious etiology accounted for 16% and other ulcers 6.67%. No venous or arterial ulcers were found histologically. However, two cases of venous ulcers were found which were diagnosed clinically and radiologically but no specific histologic features were identified to typify them; hence these were grouped under "other ulcers".

In case if diabetes is considered as a vascular disorder rather than a metabolic disorder, the vascular ulcer will account for 19.33%; which will reduce the discrepancy with other published literature by (Burton S. Claude) [11] (Callum M. J. et al) [12] and (Yound J. R) [13] that reports vascular ulcers as the most common cause of non-healing ulcers. However the concept is controversial as arteriosclerotic lesions in vessels and/or neuropathy is not the only reason for causing ulcers in diabetes rather it is a combination of factors.

The present study observes that, among the malignant ulcers Squamous cell carcinoma is the most common. Similar conclusions were drawn by Singh et al, [14] Talvalkar et al, [15] Schreiber et al [16] and Adinarayan M et al. [17]. In the present study 6 patients presented with Marjolin's ulcer, and were confirmed histopathologically as Squamous cell carcinoma. This is in concordance with the conclusion drawn by Morra & colleagues [18] and Jellouli & colleagues [19].

It was observed that common age group for malignant ulcer was 60-70 years with a male preponderance. Similar conclusions were drawn by Singh et al [14]. Also malignancy below the age of 30 years was rare, only 3 cases were detected. This matches with that reported by Singh et al [14] who found 4 cases below the age of 30 years.

The 2nd most common cause of non-healing ulcer was found to be due to Tuberculosis accounting for 26.67%. Although this is in contrast to the conclusion drawn by M. Naveduz Zafar et al, [20] Binod Kumar Thakur and colleagues [21] and Padmavathy & colleagues [22] who reported incidence of cutaneous Tuberculosis of 3.62%, 0.25% and 1.6% of all skin biopsies respectively.

But in the recent years, with the emergence of anti-Tubercular drug resistant strains and AIDS epidemic, there is a worldwide rise in the incidence of Tuberculosis. More so ever, in the poverty struck areas of the world due to poor nutrition, poverty, non-availability of diagnostic aids and treatment, overcrowding, ignorance about the disease, rise in immunosuppressive therapy, decline in Tuberculosis control efforts and emergence of resistant strains of Mycobacterium; have amplified the situation [23, 24]. Another reason for the discrepancy of the results may be the following: - in the present study we have included cases which have presented with non-healing ulcers only, unlike the other studies mentioned above which included all skin biopsies (not specifically ulcers) as the sample size for their calculation of incidence and prevalence. Also our study contains a small number of cases which raised our incidence rates.

Among the Tubercular ulcers, patient presented at the 2nd & 3rd decades which was in accordance with Binod Kumar Thakur and his colleagues [21]. Padmavathy and colleagues also reported a similar observation [22]. Lupus Vulgaris was found to be the most common form followed by Scrofuloderma. Similar to this study, M. Naved uz Zafar et al [20] also reported Lupus Vulgaris as the most common form. Similar results were seen by Satyanarayan [25], Singh et al [26] and Kumar et al [27].

In our study we could not demonstrate Acid Fast Bacilli (AFB) by Ziehl Neelsen staining (ZN stain) method in pathological sections. This is not in accordance with the results of Mahaisavariya et al [28] who found AFB in 30.77% cases, M.Naveduz Zafar et al [20] who found AFB in 18.42% cases and Ranjan Agarwal et al [29] who found AFB in 17.19% cases.

However the above figures also indicate that demonstration of AFB is a laborious procedure with a subtle chance of microbiological confirmation by finding AFB in the pathological sections. Hence diagnoses are achieved by the correlation of various absolute and relative criteria.

We observed diabetic ulcers as the 3rd common cause for non-healing ulcers, accounting for 17.33% of the ulcers. This is in accordance with Brossen et al [30] who found that 12% of the population studied had ulcers related to diabetes. Neil et al [31] too, found that approximately 20% of all diabetes related admissions in UK were due to diabetic foot ulcer disease. Alex and colleagues also reported an incidence rate that ranged from 8%-17%. The above studies are in contrast to the figures reported by Ch. Manes et al [32] and Nyamu et al [33] being 4.75% and 4.6% respectively.

The present study also observed two age peaks in presentation of the patients with non-healing ulcers due to diabetes. The first peak is in the 6th decade accounting for 38.5% of the patients and the 2nd peak is in the 5th decade accounting for 30.8% of the patients. The other studies by Bansal et al [34] and Neil et al [31] reported similar age incidences.

The majority of the patients in this study group of diabetic ulcers are males. Male to female ratio being 1.6:1. This is similar to Gaur et al [35] who reported a male to female ratio of 1.2:1. However Ch.Manes et al [32] did not establish different rates between male and female population.

The difference in the results and figures of the various studies including the present study may be because of the following reasons—

1. Regional variation in the incidence of diabetes.
2. Time dependant risk factors and complications in the evolution of diabetic ulcers which again depend upon the age of onset of the disease which varies significantly among different populations and demography.
3. Different qualities of diabetic care offered by the different centres.

In the present study we show, non -healing ulcers due to infection accounts for 16%. Bacteria are the main cause among the infectious ulcers in our setting; Staphylococcus being found as the most common accounting for 50% of the infectious causes followed by Klebsiella & Proteus accounting for 16.67% each, Pseudomonas & Streptococcus accounting for 8.33% each. These results are in accordance with the results reported by J.E. Zeegelaar et al [36].

In similar studies by Tan et al,[37], Erikson et al [38] and Giacometti et al [39] reported Staphylococcus as the most common organism.

However other studies by Gaur et al [35] reported Enterococcus as the most common organism and the study by Bansal et al [34] reported pseudomonas as the most common organism.

The reason for such discrepancy could not be assessed but may be due to the environmental factors and the intrinsic properties of the microorganism themselves [40] as reported by Marek et al [41] who observed different composition of bacterial flora in ulceration of diabetic origin and vascular origin.

30 cases on which culture and sensitivity were performed, 12 cases demonstrated growth of microorganisms. This may be due to the unprescribed and unprecedented use of antibiotics in the remote, poverty struck areas where access to valid health care is limited. Also, the fact of lack of isolates from as many as 18 ulcerations does not signify absence of microorganisms in these ulcers. In such cases anaerobic bacteria may be present, which usually reside deep at the base of the wound or under necrotic tissue, and are very difficult to access for adequate collection and culture [41].

Since numerous factors affect the ulcer healing process it is difficult to determine what effects, if any, are due to microorganism. However, possible explanation to the effects of microorganism may be—

1. Bacterial adhesions, proteins present on the surface of bacterial cells which are responsible for their adhesion to host cells, allowing colonization of the ulceration.
2. Exoenzymes decomposing cell material such as collagen and fibrinogen, allowing deeper penetration to tissues and modifying bacterial resistance.
3. Excess of cytokines, exoenzymes, bacterial toxins and proteolytic enzymes destroys growth factors, proteins, decreases collagen synthesis, increases its lysis and destroys freshly produced extracellular matrix. On top of that, these substances inhibit migration and fibroblast activity. Moreover, infection decreases the amount of oxygen that is available for the process of collagen synthesis. In extreme cases, where there is lack of proper treatment, significant colonization of the wound may take place, which leads to infection of the ulceration [41].

Bansal et al [34] observed that most ulcers had polymicrobial infection. This is in contrast to our study which observed isolation of one organism in all the 12 cases. This is similar to that reported Mazur et al [42] in his research. Soneczko et al [43] also reported single isolates in most of the patients.

Various risk factors which were identified from the history and clinical examination of the patients are (Table 3), Increasing fasting blood sugar, bare foot walking, repeated trivial trauma, peripheral neuropathy, undernutrition (BMI=18.5 -22.9 kg/m²), overcrowding, presence of physical deformity, smoking and prolonged sun exposure were in agreement with the studies which identified increasing FBS values as a significant risk factor. This is related to poorer glycemic control which again increases the susceptibility of a diabetic individual to ulcers [32]. Also, as reported by V. Manda et al [44] the greatest risk factors for developing foot ulceration are neuropathy, arterial disease, foot deformity. In a similar study by Alex and colleagues [45] in 2010 reported that peripheral neuropathy patients with hypertension are more exposed for non-healing ulcer. Vijay Vishwanathan and colleagues also implicated risk factors such as increasing age, body mass index of 18.5- 22.9 kg/m² (undernutrition) and family history of diabetes; in their survey. Our study shares a few of the above mentioned risk factors. Various other studies observed undernutrition and/or malnutrition as a risk factor [46, 47, 48].

Other factors such as overcrowding, under nutrition and bare foot walking as risk factors were in concordance with study by Padmavathy and colleagues [22] and Shetty et al [49].

Jayasinghe et al. [50] observed in his research that those using footwear for a longer duration (>10 hours per day) had lower rates of ulcers than those who used footwear <10 hours, this observation conclusively indicate bare foot walking as a potential risk factor.

In our study smoking has been found as an independent risk factor. Smoking delays wound healing leading to non-healing ulcers. Smoking inhibits healing through the effects of anoxia, hypoxia, impaired epithelialisation, vasoconstriction and enzymatic system toxicity. Moreover, smoking has been considered as an important risk factor for skin malignancies (malignant ulcers included) since long.

In the present study sun exposure has also been found to be a potential risk factor for skin malignancies. This observation is supported by previous studies by Singh et al. [14], Talvarkar et al [51] and Schreiber et al. [52].

In another study by Gaur et al, [35] the following observations were made: Ill-fitting shoes and the shoe related factors were found to be the most common cause for the development and worsening of the foot ulcers. Habitual bare foot walking was seen in some of the patients. Trivial trauma and in-growing nail was seen in the remaining

SUMMARY AND CONCLUSION

The most common cause of non-healing ulcers in the present study was found to be malignancy. The other causes identified were Tubercular ulcers (26.67%), Diabetic ulcers (17.33%) and Infectious Ulcers (16%) and other ulcers (6.67%)

Among the malignant ulcers, Squamous cell carcinoma is the most common form (80%); followed by Basal cell carcinoma (16%) and Malignant Melanoma (4%). Lupus vulgaris was found to be the most common form (70%) followed by Scrofuloderma (30%) among tubercular ulcers. Diabetic ulcers were found to be more common in the age group of 51-60 years. Males are commonly affected. Staphylococcus was the most common organism isolated (50%) followed by Klebsiella (16.67%), Proteus (16.67%), Pseudomonas (8.33%) and Streptococcus (8.33%).

Various risk factors identified in the present study which have a greater impact on the causation of non-healing ulcer and should not be kept aside. Thus it can be concluded from the study that non-healing of ulcers can be encountered at any age and may be due to a variety of etiology. Thus beside a good and accurate clinical diagnosis, early biopsy to rule out malignancy and identify the etiology is mandatory for a better clinico- pathological correlation and minimize the morbidity and mortality among patient groups.

ACKNOWLEDGMENT

Authors acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript. The authors are also grateful to authors / editors / publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

REFERENCES

- [1] General Surgery Referral Guidelines, Madigan Army Medical Centre, Tacoma, Washington-98431
- [2] Sturm AW, Jamil B, Mc Adam KPWJ, Khan KZ, Parveen S, Chian T, Hussain R. Microbial Colonizers in leprosy skin ulcers & intensity of inflammation. *Int. J Lepr* 1996; 64: 274-281.
- [3] Robinson DC, Hay RJ. Tropical Ulcer in Zambia. *Trans R Soc trop med Hyg* 1986; 80: 1432-137.
- [4] Ohanka EC, O sarenkhoc O. In- patient management of leg ulcers. *East Afr. Med J* 1999; 76: 687-689.
- [5] Herwaldt Bl. Leishmaniasis. *Lancet* 1999; 354: 1191-1199.
- [6] Van der Werf TS, van der Grwaf WT, Tappeco JW, Asiedu K. Mycobacterium ulcerans infections. *Lancet* 1999; 354: 1013-8.
- [7] Sarkar PK, BALLantyre S. Management of leg Ulcers. *Post Grad Med J* 2000; 76: 674-682.
- [8] Jim E Zeegelar, Aimee C Stroink, Willemijn H Steketee, William R. Faber, Allard C Vanderwal, Isaac O O Komolafe, Charles Dzumalale, Cecilia Chibwana , Hans F Wedle, Ed Zijlstan- etiology and incidence of chronic ulcers in Bhatyre, Malawi- *Int. Journal of Dermatology* 2006 Aug; 45(8): 933-6
- [9] Mandong BM, Orkar KS, Sule AZ, Dakun NL. Malignant skin tumors in Jos University Teaching Hospital, Jos, Nigeria (Hospital based study); *The Nigerian Journal of Surgical Research* Vol 3 No 1 March 2001
- [10] Yakubu A, Mabogunje OA. Squamous Cell Carcinoma of the skin in Africans. *Trop Geogr Med* 1995; 2:91-93.
- [11] Burton CS. Treatment of leg ulcers, *Dermatol Clin*, 1993;11: 315-23.
- [12] Callam MJ, et al. Chronic ulcers of the leg: clinical history, *Br Med J*,1987; 294: 1389-91.
- [13] Yound JR, 1983, Differential diagnosis of leg ulcers. *Card Vaso Clin*,13:171-93.
- [14] Rajesh Singh Laishram, Alpana Banerjee. Pukhransan Punyabati, L Durlar Chandra Sharma: Pattern of skin malignancies in Manipur in India: A 5 yrs histopathological review; *Journal of Pakistan Association of Dermatology*, 2010; 20:128-132
- [15] Pandu S. Non-melanoma skin cancer in India; Current scenario. *Indian Journal Dermatol* 2010; 55:373-8
- [16] Schreiber MM, Shapiro SI, Berry CZ, Dahler RF, Friedman RP. The incidence of skin cancer in Southern Arizona. *Arch Dermatol*:1971; 104:124-7
- [17] Adinarayam M, Krishnamurthy SP. Clinicopathological evaluation of non-melanoma skin cancer. *Indian J Dermatol* 2011; 56:670-2
- [18] Mora RG, Perniciaro C, Cancer of skin in Blacks. I.A review of 163 black pts with cutaneous SCC. *J Am Acad Dermatol* 1981; 5:535-43
- [19] Sabin SR, Goldstein G, Rosenthal HG, Haynes KK. Aggressive squamous cell carcinoma originating as a Marjolin's ulcer. *Dermatol Surg*. 2004; 30:229-230
- [20] M Naved uz Zafar, M Arif Memon, M Akbar Asha, Shaheen, Akbar Agha, Yasmeen Hashim, Talat Mirza, Dur Muhammad, Samina Rizvi; Pattern of cutaneous tuberculosis as identified by morphological study of skin lesions at Jinnah Postgraduate Medical Center, Karachi: *Gomal Journal of Medical Sciences* January-June 2010, Vol 8, No.1

- [21] Binod Kumar Thakur, Shikha Verma, Debeeka Hazarika: A clinicopathologic study of cutaneous tuberculosis at Dibrugarh District, Assam. *Indian J dermatol* 2012; 57:63-5
- [22] L. Padmavathy, L.Lakhmana Rao, T. Pari, N. Ethirajan and B. Krishna swamy: Lupus vulgaris and tuberculosis verrucosa cutis-A clinical, pathological, epidemiological study of 71 cases. *Indian J Tuberc* 2008; 55:203-209
- [23] Kumar B, Muralidhar S. Cutaneous tuberculosis: a twenty year prospective study. *The International Journal of Tuberculosis and lung disease* 1995; 3:494-500
- [24] Sehgal VN, Srivastava G, Khurana VK. An appraisal of epidemiological, clinical, bacteriologic, histopathologic and immunologic parameters in cutaneous tuberculosis. *Int J Dermatol* 1987; 26:521-6
- [25] Satyanarayan BV. Tuberculoderma- a brief review together with statistical analysis and observation. *Ind J Dermatol Venerol* 1963; 29:25-42
- [26] Singh G. Lupus vulgaris in India. *Ind J Dermatol Venerol* 1974; 40:257-60
- [27] Kumar B, Muralidhar S. Cutaneous tuberculosis: a twenty year prospective study. *The International Journal of Tuberculosis and lung disease* 1995; 3:494-500
- [28] Mahaisavariya P, Manonukul J, Khemngun S, Cshai prasert A. Mycobacterial skin infections. Comparison between histological features and detection of Acid Fast Bacilli in pathologic sections. *J Med Assoc Thai* 2004;87: 709-12.
- [29] Ranjan Agrawal, Mukta Kumar, Prabodh Kumar: Cutaneous tuberculosis a clinicopathological study- <http://dx.doi.org/10.7713/ijms.2012.0044>
- [30] Brossan B, Bergenheim T, Licher F. The epidemiology of foot lesions in diabetic patients aged 15-50 yrs. *Diabetic Med.* 1990; 7:438-44
- [31] Neil HAW, Thompson AV, Thorgood M et al. Diabetes in the elderly, the oxford university diabetes study. *Diabetic Med* 1989, 6: 608-613
- [32] Ch Manes MD, N. Papazoglou MD, E.Sorsidou, K. Soulis MD, D. Milarkis, A Satsoglou, A Sakallerou: Prevalence of diabetic neuropathy and foot ulceration: Identification of potential risk factors: <http://woundsresearch.com/article/141?page=0,2>
- [33] PN Nyamu, CF Otieno, EO Amayo, SO McLigyeo: Risk Factors and prevalence of diabetic foot ulcers at Kenyatta. National Hospital, Nairobi_ *East African Medical Journal* vol.80 no. 1 January 2003; pp- 36-43.
- [34] Bansal E, Garg A, Bhatia S, Attri AK, Chader J. Spectrum of microbial flora in diabetic foot ulcers. *Indian J Pathol Microbiol* 2008; 51: 204-208.
- [35] Dushyant Singh Gaur, Amit Verma, Pratima Gupta: Diabetic foot in Uttaranchal: Vol.9. No.1, January-March 2007, P.18-20
- [36] Jim E Zeegelar, Aimee C Stroink, Willemijn H Steketee, William R. Faber, Allard C Vanderwal, Isaac O O Komolafe, Charles Dzamale, Cecilia Chibwana, Hans F Wedle, Ed Zijlstan- etiology and incidence of chronic ulcers in Bhatyre, Malawi- *Int. Journal of Dermatology* 2006 Aug; 45(8): 933-6.
- [37] Tan HH, Tay YK, Goh CL- Bacterial skin infections at a tertiary dermatological centre- *Singapore Med J*, 1998, Aug; 39(8): 353-6.
- [38] Gunnel Erikson, Anders Erik Eklund and Lars olof leallings: Clinical Significance of Bacterial growth in venous leg ulcers- *Scandinavian Journal of Infections disease*: 1984, vol, 16, no.2, pages 175-180.
- [39] A. Giacometti, O. Cirioni, AM Schimizzi, MS Del Prete, F Barchiesi, MM D' Errico, E Petrelli and G Scalie-Epidemiology and Microbiology of Surgical wound infections- *J Clin Microbiol.* February 2000, vol 38 no.2 918- 9222.
- [40] Diana C Paltrey, B Rhodes, JG Chatwood- Investigation into microbial flora of healing & on healing decubitus ulcers: *j Clin Pathol* 1981; 34: 701-705
- [41] Mrek Kucharzewski, Jolantri Misztal- Kuyra, Edward Basczak, Andrzej Franek: Analysis of the flora of venous & diabetic ulcerations.
- [42] Mazar E, Niedzwiadek J, klag S, Terlecki P, Zieniba B, Nrowaski J, Kogiol- Motewka M(2000) Bakterie llenowe Z zlynych owrzodzen trofic znych goleni. I I ch wzraliwose na anty biotyki *Przeglad Flebologiczny*; 6; 13: 255-261.
- [43] Saneczko F, Kaszuba A, kozlowska M et al(2003) Flora bakteryjna owrzodzin goleni w przebiegu przewleklej cvzese I. Czestore izolacji I Skald. *Jakosciowy flory bakteryjnej.* *Post Dermatol Aleggol I*; 20: 15-21.
- [44] Venkatramana Manda, Jayadevan Sreedharan, Jayakumary Muttappallymyalil, Rajdeep Das and Emi Hisawatsu. Foot Ulcers and risk factors among diabetic patients visiting surgery dept. in a University

- Teaching Hospital in Ajman UAE: International Journal of Medicine and Public health vol. 2/jul-sep, 2012.
- [45] Alex R, Ratnaraj B, Winston B, Samson Davakiruba DN, Samuel C, John J, Mohan VR, Prasad JH, Jacob KS. Risk factors for foot ulcers in pts with DM. A short report from Vellore, South India. Indian J Community Med 2010; 35:183-5
- [46] Emma Johuston: Optimising nutrition to prevent pressure ulcer development.
- [47] Ewan SM S Shehin Bsc, Msc, RN, PhD, J MM Meijers RN PhD, RJG Halfens PhD, T Dassen PhD, RN: The relationship between malnutrition parameters and pressure ulcers in hospitals and nursing homes.
- [48] Wassim Raffoul, MD, Majid Shahin Far MD, Marie Christine Cayenx RN, Mette M. Berger MD PhD: Nutritional status & food intake intake in patients with chronic low limb ulcers and pressure ulcers: importance of oral supplement.
- [49] N. Shetty, M. Shenko, M. Vag, G. D'souza: An epidemiological evaluation of risk factors for tuberculosis in South India: a match case control study. Int J Tuberc Dis 10(1):80-86
- [50] SA. Jayasinghe, I Atukorala, B Gunethilleke, V Siriwardena, SC herath, K De Abrew: Is walking Barefoot a risk factor for diabetic foot disease in developing countries? Rural and remote Health 7: 692.(online), 2007.
- [51] Tahalkar GV. Squamous cell carcinoma of skin; its incidence and etiopathogenesis in 625 cases. Indian Journal Cancer 1970; 7:24-33
- [52] Schreiber MM, Shapiro SI, Berry CZ, Dahler RF, Friedman RP. The incidence of skin cancer in Southern Arizona .Arch Dermatol:1971; 104:124-7
- [53] Vijay Viswanathan, Satyavani Kuempata., Vigneswami Aravindalochanan, Rajeswari Rajan, C.Chinnasamy, Rajan Srinivasan, Jerard Maria Selvam, Anil Kapur: Prevalence of Diabetes and Pre-diabetes and Associated Risk Factors among TB pts in India:PLOS ONE 7(7): e41307.doi:10.1371/journal.pone
- [54] JR Mekkes, MA MCoots, AC Van Der Wal and JD Bos: Causes, investigation ad treatmet of leg ulceration: British Journal of Dermatology 2003; 148: 388-401.
- [55] Saraf SK, Shukla VK, Kaur P, Pandey SS: A clinico-epidemiological profile of non healing wounds in an Indian hospitals.