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RETROSPECTIVE ANALYSIS OF TISSUE FIXED IMMUNOREACTANTS FROM SKIN BIOPSIES OF IMMUNE VESICULOBULLOUS LESIONS MAINTAINED IN MICHEL'S MEDIUM

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ABSTRACT

Diagnosis by direct immunofluorescence (DIF) requires skin biopsies with preserved immunoreactants for which biopsies are snap-frozen in liquid nitrogen or placed in Michel's fixative that facilitates transport. Retrospective study was conducted on 30 perilesional skin biopsies of immune- vesciculobullous lesions in Michel's medium to analyse its efficiency as transport medium. DIF was performed and samples were grouped according to the number of days they had been maintained in Michel's medium. DIF was positive in 28 cases, accounting for 93.33%. DIF was positive in 100%, 92.31% and 83.33% of biopsies even after 5, 10 and 15 days of preservation in Michel's medium, respectively. As the number of days in transport medium increased, the intensity of immunofluorescence decreased. There was no statistical significance (>0.05) between the intensity of immunofluorescence and the number of days the biopsies were in transport medium. Michel's medium is an efficient transport medium, when used within 15 days, in countries where DIF facilities are not available.

KEY WORDS: Michel's medium, DIF, vesiculobullous disorders, immunoreactants

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INTRODUCTION

Vesiculobullous disorders represent a heterogenous group of dermatoses with protean manifestations. The most important techniques for the investigation of patients with vesiculobullous disease are conventional histopathology and confirmative tests like direct and indirect immunofluorescence. [1] Immunofluorescence has greatly contributed to the diagnosis, treatment and understanding of the pathophysiology of vesiculobullous lesions of skin .[2] The demonstration of tissue-bound immunoreactants by direct immunofluorescence microscopy (DIF) is a valuable parameter in the diagnosis of various autoimmune skin diseases and is available only in a few higher centres. [3] Reliable diagnosis by DIF not only requires an experienced observer, but also proper skin (or mucosal) biopsies with well-preserved immunoreactants. For the latter purpose biopsies are usually snap-frozen in liquid nitrogen or, alternatively, placed in Michel's fixative that facilitates transport of biopsies. [4] In this study, we have tried to evaluate the efficiency of Michel's medium as a transport medium for immunofluorescence.

MATERIALS AND METHODS

A retrospective, descriptive hospital based study was conducted on 30 perilesional skin biopsies of immune- vesciculobullous lesions sent in Michel's medium to the department of Pathology over a period of two years. The biopsies were sent to the laboratory in Michel's medium for DIF and were maintained at room temperature and at a pH of 7.0. The specimen was washed three times for 10 minutes in a buffer and then they were frozen, cut using cryostat, incubated with Fluorescein-isothiocyanate (FITC) antibodies IgA, IgG, IgM and C3 and viewed under fluorescent microscope. The samples were grouped according to the number of days they had been maintained in Michel's medium. The time was measured from the date biopsies were obtained from the patient to the date they were analysed by DIF. The intensity of immunofluorescence was assessed subjectively by comparing with dermal collagen which was taken as 3+ and grouped into 1+, 2+ and 3+.

RESULTS

Out of 30 skin biopsies sent in Michel's medium, 28 were positive for DIF accounting for 93.33% and 2 were negative. Pemphigus vulgaris (PV) was the commonest immune-bullous disease accounting for 63.3%, followed by bullous pemphigoid (BP), Pemphigus foliaceus (PF) and Pemphigus erythematosus (PE). Table 1 shows the distribution of immuno- bullous diseases with immuno fluorescence findings. Table 2 shows immune fluorescence findings in comparison with number of days the biopsies were in Michel's medium. As the number of days in transport medium increased, the intensity of immunofluorescence decreased. But they were helpful in diagnosis in spite of decreased intensity. There was no statistical significance (>0.05) between the intensity of immunofluorescence and the number of days the biopsies were in transport medium.

Table 1
Distribution of immune-bullous diseases with immunofluorescence findings

Diagnosis	No. of cases	Percentage (%)	Immunofluorescence	
			+	-
Pemphigus vulgaris	19	63.33%	18	1
Bullous pemphigoid	8	26.66%	7	1
Pemphigus foliaceus	2	6.66%	2	0
Pemphigus erythematosus	1	3.33%	1	0

Table2
Immunofluorescence findings in comparison with number of days the biopsies were in Michel's medium

No. of days Biopsies were in Michel's medium	No. of cases	Immunofluorescence	Intensity of immunofluorescence			
		+	–	1+	2+	3+
0-5	11	11 (100%)	0	1 (9.09%)	1 (9.09%)	9 (81.82%)
6-10	13	12 (92.31%)	1	1 (8.33%)	9 (75%)	2 (16.66%)
11-15	6	5 (83.33%)	1	3 (60%)	2 (40%)	0

DISCUSSION

In 1973 Michel et. al developed a fixative solution that preserved immunoglobulins in biopsies. [5] Before the introduction of this transport medium, there were many problems involved in processing of skin biopsies for immunofluorescence. There were also many false negative cases on DIF as immunoreactants were lost during transportation. These were overcome by introduction of Michel's medium as a transport medium. The transport of skin biopsies involves fixing the biopsy in the fixative solution, in which it may be kept up to 10 days, perhaps longer, until processing. This provides sufficient time for the specimen to be sent to any laboratory. Before freezing, the specimen must be washed 3 times for 10 minutes in a buffer, and can then be frozen and processed as usual for immunofluorescence. The fixative Solution contains 55 g of $(NH_4)_2SO_4$, 2.5 ml of 1 M potassium citrate pH 7, 5 ml of 0.1 M C.N-ethylmaleimide, 5 ml of 0.1 M $MgSO_4$ and 87.5 ml of distilled water, with pH adjusted to 7 with 1 M KOH. The wash buffer contains the same constituents, except for $(NH_4)_2SO_4$. [6] Ammonium sulphate has the ability to precipitate macromolecules including antigen antibody complexes without loss of antigenicity, while proteolytic and other depolymerizing enzymes would be precipitated and inhibited. [7] Citrate buffer is used for washing because it is least likely to facilitate epidermal- dermal separation. [8] N ethyl maleimide is used to minimize the proteolytic activity. Magnesium is used in the buffer to neutralize the chelating capacity of citrate. [5] Precautions to be taken in the use of this fixative include a) the tissue should not be frozen before fixation as it leads to loss of immunofluorescence b) in cases of negative DIF on fixed tissue, a frozen control is indicated. Advantages of Michels medium are i) it allows excellent preservation of tissue fixed immunoglobulins comparable to frozen tissue ii) it is a simple method for transport of specimens to specialized laboratories iii) after DIF, the remaining tissue can be used for routine histopathological processing iv) it can be used for transport of other tissues like kidney. [5] In a retrospective study by Vaughan Jones et.al on 656 skin biopsies, DIF gave reliable results on biopsies maintained in Michel's medium for upto 6 months, with no significant alteration in pattern of immunofluorescence. [4] This was similar to our study but we have analysed the efficiency of Michel's medium for 15 days only and further studies are required to assess its efficiency after 15 days. Skeete and Black suggested that intensity of immunofluorescence decreases with long intervals. This is due to catabolism of blood and serum proteins which produces a decrease in pH of the medium. [9] Similar observation was noted in our study. Further research on maintain the pH by inhibiting the catabolism of blood and serum proteins or addition of a buffer are yet to be undertaken. In our study, we have not used other media for comparison. Further studies can be done using other media and our study provides a future scope for research in this direction.

CONCLUSION

We conclude that Michel's medium can be used as an efficient transport medium in countries where DIF facilities are not available when used within 15 days.

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