IMMUNOFLUORESCENCE MICROSCOPY AS A DIAGNOSTIC TECHNIQUE IN DERMATOLOGY

THESIS

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BY
SAFI EDIN ELNUR ALI
(MB.BS.)

SUPERVISED BY

DR. NAGWA M. YOUSSEF
ASSISTANT PROF. OF
DERMATOLOGY & VENEREOLOGY

AIN SHAMS UNIVERSITY
FACULTY OF MEDICINE
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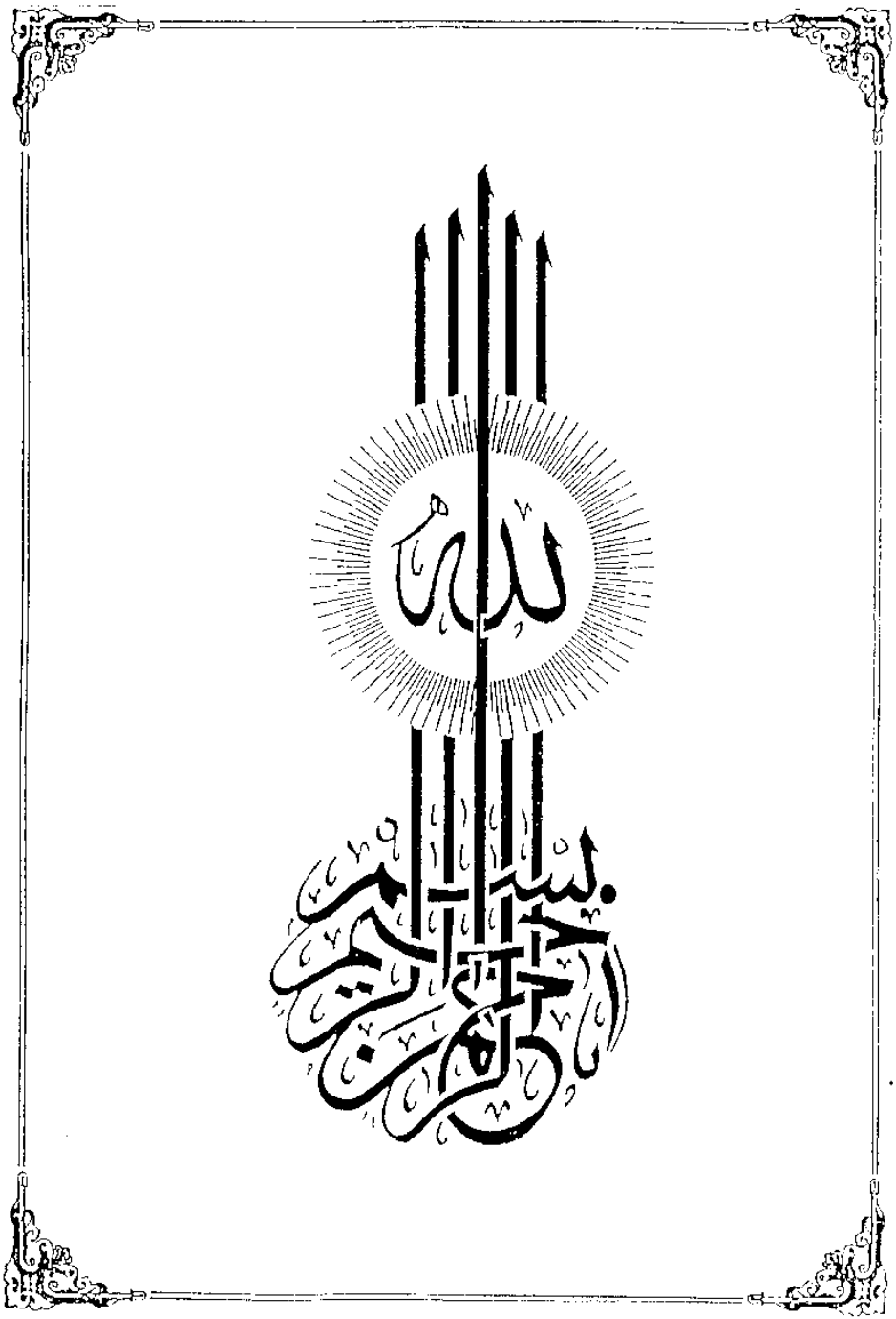

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ABBREVIATIONS

BMZ	=	Basement membrane zone.
BP	=	Bullous pemphigoid.
BS	=	Behcet's Syndrome
CD	=	Coeliac disease.
CP	=	Cicatricial pemphigoid.
DEJ	=	Dermal epidermal junction.
DH	=	Dermatitis herpetiformis.
DLE	=	Discoid lupus erythematosus.
EBA	=	Epidermolysis bullosa acquisita.
EM	=	Erythema multiforme.
FITC	=	Fluorescein isothiocyanate.
GS-ANA	=	Granulocyte specific antinuclear antibodies.
GSE	=	Gluten-sensitive enteropathy.
HG	=	Herpes gestationis
LBT	=	Lupus band test.
LE	=	Lupus erythematosus.
MCTD	=	Mixed connective tissue disease.
PBS	=	Phosphate-buffered saline.
PV	=	Pemphigus vulgaris.
RA	=	rheumatoid arthitis.
RAS	=	recurrent aphthous stomatitis.
RNP	=	ribonucleoprotein.
SLE	=	Systemic lupus erythematosus.

Introduction

1. INTRODUCTION

The immunofluorescence technique is a histochemical staining technique for detecting the presence and position of substances e.g. antigens, antibodies, other cell secretions and cell components in cells or tissue sections. The principle of the technique is that certain fluorochrome dyes, exposed to ultraviolet light emit fluorescent radiation, the colour of which depends upon the particular fluoro-chrome. When these dyes are conjugated to proteins that are subsequently added to tissue sections or injected into an animal the position of the proteins can be traced microscopically by the fluorescence they emit under illumination with ultraviolet light With suitable filters (Parish, 1986).

The technique provides tow pieces of information at the same time, 1. evidence that an antigen-antibody reaction has taken place in the tissues. 2. Topographic information about the location of the antigen in the tissues. The technique is very sensitive and has become widely used for detecting biological material. The specificity is naturallly totally dependent upon the specificity of the antisera used (Ullman, 1988).

Many diagnostically useful immunofluoresent tests can be performed on skin and sera taken in the office.

The direct immunofluorescent tests can detect antibodies or other proteins such as complement which have bound in the patient skin. The indirect immunofluorescent tests detect specific antibodies in the patient serum (Eglstein and Pariser, 1978).

The indirect test is less sensitive than the direct, however it may be used as a supplementary test when the direct test cannot be relied upon because the specimen is being mailed over long distances or is in danger of freezing (Lever, 1985).

Clinical indications of immunofluorescence:

1. Blistering or denuding conditions (Pemphigus, Pemphigoid, herpes gestationis, dermatitis herpetiformis).
2. Patches of pruritic, excoriated and reddened skin (dermatitis herpetiformis).
3. Scarring lesions specially on sun exposed areas, on the scalp, or on the lower lip. (Lupus erythematosus, cicatricial pemph igoid)
4. Diffuse red eruptions which are not drug eruptions (L.E., mixed connective tissue disease) (Eglstein & Parsier, 1978).

The aim of this work is to review the value of immunofluorescence as a diagnostic technique in various skin diseases.

2. HISTORICAL REVIEW

The immunofluorescence technique is a histochemical staining technique which was introduced by Coons and cowerkers in 1942, and further developed during the following 10 years (Ullman, 1988).

The first application of the technique in dermatology was reported by Raskin in 1961, with the demonstration of upper dermal fluorescence in skin biopsies taken from patients with experimentally produced Rhus contact dermatitis. In 1963 Burnham et al., published the first report of adermo-epidemal fluorescent band in the lesions of lupus erythematosus, with similar but less well defined findings in other dermatoses. Since then, there has been a burgeoning interest in immunofluorescent techniques in dermatoloty with a rapid increase in reports on its use both as a diagnostic and research tool (Ongley, 1982).

The reports on the characteristic patterns of immunoglobulin deposits in the skin in bullous diseases were of great importance in diagnosing and classifying this group of diseases (Van der Meer, 1969). Also the demonstration of the lupus band in lupus erythematosus was a significant finding (Ullman, 1988).

4. TECHNIQUE AND SITE OFTHE BIOPSY

Four techniques can be used for obtaining a specimen for biopsy: scalpel, punch, shave biopsy,and curettage. A side from excising lesions, scalpel biopsies often are advisable for the study of subcutaneous lesions, since it is usually not possible to obtain adequate amounts of subcutaneous tissue by punch biopsy. (Lever, 1985).

Punch biopsies represent the standard procedure for obtaining specimens of skin for histologic examination. It is important to select a proper site for biospy. In most instances, histologic examination of a fully developed lesion will give more information than examination of an early or involuting lesion. Vesicular, bullous and pustular lesions represent exceptions to this rule. For their histologic examination, a very early lesion is required, otherwise, secondary changes (such as regeneration, degeneration, or secondary infection) may obscure the essential features and make recognition of their mode of formation impossible (Lever, 1985).

For D.I.F. study in bullous diseases, specimens were obtained from an area adjacent to ablister because the pattern of immune deposits deteriorates in

areas where the blister formation is taking place. Biopsies from clincally normal skin were usually taken from the buttock for cosmetic reasons, but more importantly to avoid sun-exposed skin as for example, in patients with lupus erythematosus. Biopsies were not obtained from facial skin because of possible difficulties in interpreting the findings in skin with actinic degeneration. The positive reactions were seen in the following locations: intercellular spaces in epidermis, cell nucleus in epidermis, the dermal=epidermal junction (DEJ), basement membrane zone (BMZ),and the vessel walls in the dermis. The morphology of the deposits in the DEJ and BMZ is so characteristic that the experienced investigater is never in doubt whether they are linear (tubular) or granular. During the first years of the experiments, staining with antifibrinogen was included in the routine procedure. However, fibrinogen is almost always present at the DEJ and in vessel walls in lesional skin of any disease. The information obtained from a positive reaction in relation to a specific diagnosis is therefore limited. Consequently this staining was discontinued as apart of the routine procedure and only performed on special indications (Ullman, 1988).

The serum samples which were examined for the presence of anti inter-cellular or anti-BMZ antibodies, were examined in serial dilutions. Examination of a serum which contains anti-BMZ antibodies in a high titer often demonstrates that the brightness of the reaction increases by increasing dilutions up to a maximum at e.g. 1:160 – 1-320. At further dilutions of the serum, the fluorescence decreases gradually towards the end point. The explanation for this phenomenon (the prozone phenomenon) is not known. The antibodies may form complexes with other serum proteins without binding to their specific antigens. By diluting the serum, these complexes may dissolve again. Serum proteins may cause fluorescence when undiluted sera are examined. Consequently neat sera should not be used in routine investigations for circulating antibodies (Ullman 1988).

The antibodies in sera from patients with bullous pemphigoid and pemphigus are directed against antigens in squamous cell epithelium. Generally they are not species specific. Monkey oesophagus has been reported to be the most suitable antigen substrate. (Chorzelski and Beutner 1969). However monkey tissue is often not available and many laboratories use instead guinea pig lip or oesophagus. In the study by Judd and Mescon

(1979), different epithelial tissues were compared as antigen for detecting pemphigus antibodies. Aguinea pig oesophagus, human foreskin, rabbit esophagus, monkey esophagus and lip were tested. No substrate was found to be superior to the others, since none yielded consistently high or low titers (Lever, 1985). In contrast to the study by Chorzelski and Beutner (1969) monkey tissue failed to detect antibodies in several sera which were positive when examined on other tissues. Ascota and Ivanyl (1982) found that human skin was superior to the other animal substrates. This minor variation in the species specificity of the circulating antibodies may be due to some heterogenecity of the antigens. False positive reactions due to blood group A and B antibodies have never been obtained in the studies, so absorption with blood group A and B substance has not been included in the routine procedure. The abscence of these false positive reactions is probably one of the advantages of not using human or monkey tissues (Ullman, 1988).

Non specific fluorescence and antifluorescence may create difficulties in interpreting the results. The antifluorescence of elastic tissue is yellow and different from the apple-green colour of fluorochrome isothiocyanate. Also stratum corneum, hair shafts and

granules in sweat gland epithelium give a yellow antifluorescence. Eosinophils give a nonspecific fluorescence with different conjugates probably due to non-immunologic binding (Valenzuela et al., 1984).

IMMUNOFLUORESCENCE TECHNIQUE

There are two basic methods used in immunofluorescesnt microscopy. The direct and the indirect methods. The former utilizing patient skin biopsy for study of in-vivo bound antibody, the latter patient's serum for investigation of circulating antibodies. Of the two, the direct method is more widely used for it is more sensitive (orgley, 1982).

The direct method:

Biopsy specimen from the skin of the patient is obtained with a 3 mm punch, snap-frozen, cut in a cryostat at about-20°c, dried and stained without fixation. Direct staining is used to detect substances, including antibody globulins, in tissues or other substrates, by the addition of fluorochrome-conjugated antibodies to the substances, and washing to remove from the preparation all the conjugated antibody except that fixed to the particular substance. The details of the method are:

a- Treat specimen with conjugated antibody for 30 min.

b- Rinse in Physiological saline ph 7.0.

c- Wash in buffered saline 1-2 hours.

d- Mount in glycerol saline.

e- Examine under afluorescence microscope. Rabbit IgG