DETECTION OF PENICILLIN ANTIBODIES IN PATIENTS UNDER PENICILLIN THERAPY BY PASSIVE HAEMAGGLUTINATION METHOD

THESIS SUBMITTED IN PARTIAL FULFILMENT FOR MASTER DEGREE IN CLINICAL PATHOLOGY

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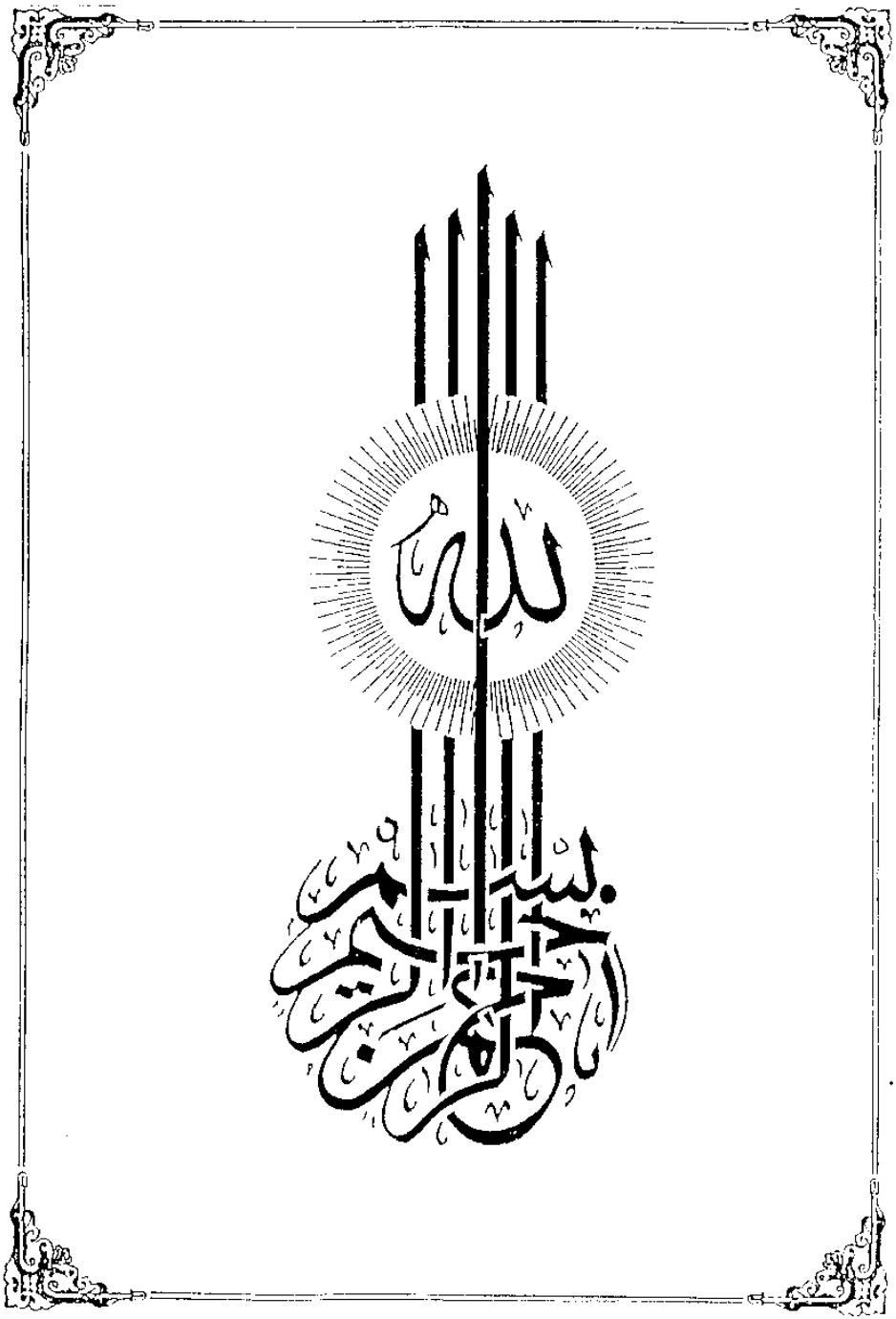
1981

ACKNOWLEDGEMENT

I wish to express my deepest gratitude to Professor Dr. Islah Hassan El Falaky, who has kindly supervised these thesis. Her continuous guidance and help have made this work possible. She furnished me with references, books and materials, which were necessary for the completion of the work. It is a great honour to work under her supervision.

I wish also to express my thanks and appreciation to Dr. Fadila Hassan Sabry, for her kind help, advice and assistance.



CONTENTS

INTRODUCTION AND AIM OF WORK

INTRODUCTION

Penicillin is one of the most widely used antibiotics, owing to the fact that several forms are present, which have the same general structure, but with different side chains. Its wide use, in addition to its mode of administration, which is mostly by injection, are responsible for the high incidence of sensitization; causing both immediate and delayed reactions, as well as various forms of allergic reactions.

Penicillin is also widely distributed in nature. It may be present in milk of cattle treated for mastitis, in meat of cattle and other animals treated with penicillin. Fish and fowl may be preserved with various antibiotics including penicillin. Penicillin dust may contaminate drugs manufactured in the same plants. These forms of exposure may also lead to sensitization against penicillin.(Criep, 1966).

Owing to the above facts, the subject of penicillin has been widely studied. Studies included its antigenicity, the immune response evoked by its injection, the detection of antibodies produced against penicillin and the significance of these Abs antibodies.

AIM OF THE WORK

Detection of antibodies (Abs) directed against penicillin by passive haemagglutination (HA) had been originally devised by Ley et al in 1958. Further modifications in the technique, were added by Levine et al in 1966. This technique detects IgG and IgM Abs, which are produced as a result of intake of penicillin, or any of its semisynthetic products. Neither IgM nor IgG Abs play a role in mediating immediate allergic reactions or accelerated urticarial reactions to penicillin which are mediated by IgE Abs. Therefore, detection of IgG & IgM Abs by HA technique has no value in predicting , these reactions.

However, IgG Abs were proved to act as blocking Abs; therefore their presence in high titres seems to protect the patient from immediate allergic reactions. A fall in titre of the IgG Abs may be accompanied by the appearance of urticarial manifestations (Levine et al., 1966).

The most important value of detecting IgG titres by HA technique lies in detecting cases of haemolytic anaemia caused by penicillin administration. On the

other hand, IgM titres may be of value in predicting accelerated exanthematous eruptions and cases of agranulocytosis associated with penicillin.

In this work, we aim at detection and estimation of antipenicillin Abs in patients under penicillin therapy and prophylaxis. The method used, is based on that described by Levine et al in 1966, but with some modifications which render it simpler and reproducible. Moreover, a correlation is done between the titre of Abs obtained and the duration of the penicillin intake.

The HA method described in this study may be taken as a model, to be used for detection of Abs against other antibiotics.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Immunogenic Response To Penicillin

As stated by Levine in 1966, three different types of antibodies (Abs) occur in man as a result of injection with penicillin. These are skin sensitizing Abs, IgG and IgM Abs.

The skin sensitizing Abs are two types, the first are benzyl penicilloyl specific (BPO) (of major specificity) while the second are of minor specificity i.e. specific to the minor antigenic determinants benzyl penicilloate & benzyl penamaldate.

Skin sensitizing Abs are detected by direct skin testing of the patient for wheal and flare reactions. Low molecular weight benzyl penicilloyl polylysine conjugate is used to detect skin sensitizing Abs of major specificity, whereas a mixture of benzyl penicillin benzyl, penicilloate and benzyl penamaldate (minor determinant mixture) is used to detect skin sensitizing Abs of minor specificity.

Direct skin tests reveal skin sensitizing Abs already fixed to the skin. These Abs, in the serum, are detected by passive transfer to the skin of normal

recepients, followed by specific challenge of the skin - test site. When they are detected in the serum, they can almost invariably be detected also by direct skin tests.

The IgM and IgG Abs are of benzyl penicilloyl specificity (BPO) and are detected in the serum by a highly sensitive passive haemagglutination method.

Benzyl penicillin may also be responsible for delayed hypersensitivity reactions, which are detected by skin testing with benzyl penicillin solution injected intradermally. Benzyl pencillin elicits delayed skin reactions by first, forming the appropriate multivalent benzyl penicilloyl protein conjugates in the skin test site, which elicits the reaction. Delayed skin reactions appear to be specific for the benzyl penicilloyl haptenic group, but they also require carrier specificity for elicitation. The term carrier specificity means specificity for the protein or carrier part of a hapten protein conjugate.

From studies conducted by Levine et al in 1966, it was noticed that there is heterogenicity in the immune response to penicillin in man. Meterogenicity was manifested by individual differences in haptenic

specificities of Abs synthesized against penicillin and in the concentration of these Abs in serum.

There are three factors which influence the individual immune response to penicillin.

First, is the duration of intake. It was found that twice as many patients synthesized benzyl penicilloyl specific IgG Abs and skin sensitizing Abs among patients recently treated with pencillin, than among patients, who had not had penicillin therapy for at least two years; whereas, virtually all patients of both groups made IgM Abs. Continued synthesis of small amounts of IgM Abs in the latter group is ascribed to repeated contacts with trace amounts of penicillin present in the environment.

Second is the dosage and route of administration. Treatment with large doses of penicillin given intravenously appeared to decrease the percentage of patients synthesizing IgG and skin sensitizing Abs. This may represent induction of partial immune tolerance.

Third, is the atopic background of the patient. About a three to four fold higher percentage of atopic than non atopic patients were found to synthesize skin sensitizing Abs. The difference was somewhat more

striking for skin sensitizing Abs of minor determinant specificities. The atopic factor appears to be genetically controlled, at least in part, since a family history of atopy occurred four times more frequently in atopic than in non atopic patients.

No significant effect of age, sex or concurrent infection, on the immune response was detected.

A significant linking of the synthesis of various antipenicillin Abs was observed. All patients recently treated with penicillin, who synthesized benzyl penicilloyl specific skin sensitizing Abs, also synthesized benzyl penicilloyl specific IgG Abs. It was also observed that, there is a marked association between skin sensitizing antibodies of minor determinant specificity and benzyl pencilloyl specific skin sensitizing Abs.

Patients with allergic reactions to pencillin made a qualitatively and quantitatively greater immune response to penicillin than did the non allergic reactor controls.

Significance of Abs. Formed Against Penicillin

Significance of IgG& IgM Abs, which were detected by passive HA, was studied by Heggie in 1960. The study was carried on a group of patients showing allergic reactions to pencillin in the form of urticaria, arthralgia and purpuric rash and compared with another group of patients under penicillin therapy, but without allergic sequalee. It was found that circulating Abs were detectable in both groups of patients. However, the frequency was greater in patients experiencing or convalescing from allergic reactions consequent to administration of the drug, than in those who received the drug without mishap.

Studies done by Van Arsdel et al in 1963 on patients with history of penicillin allergy showed that, the relationship between HA Abs and the allergic reaction was puzzling.

A very important question arose, were the serum Abs, the actual mediators of the allergic reaction; or did they merely reflect allergy at the cellular level ?

It was thought possible that HA. Abs appeared only as a response to the allergic reaction and had no significance in predicting a future catastrophe.

Two facts, which helped to prove that HA. Abs had no role in causing allergy, were observed.

The first was that, patients with low titres of HA. Abs before treatment, received penicillin without eliciting any reaction.

The second was that a rise in titre may occur during treatment whereas the skin reaction remained negative.

It was concluded that, although HA. Abs can be associated with clinical sensitivity, yet there is little evidence to show that these Abs are responsible for producing the allergic reaction.

Redmond et al in 1966, studied the relation between HA. Abs, skin tests and allergy to penicillin. They found that, patients with negative skin tests and HA. Abs showed no allergic reactions to pencillin. The HA. Abs in these cases were mostly IgM. Patients with positive skin tests usually had HA. Abs of IgG type. These patients developed accelerated allergic reactions to penicillin only during the time, when their IgG titres were at minimum. Patients with positive skin tests did not develop allergic reactions to penicillin as long as they maintained a high titre of IgG Abs throughout treatment. The association of HA Abs and positive skin reactions may be due to the fact that a common determinant,