



Study of the relation between serum levels of long acting penicillin and the inflammatory markers: C-Reactive Protein and Interleukin-6 in patients with chronic rheumatic heart disease

Thesis

Submitted in the partial fulfillment for master degree in cardiovascular medicine

Presented By

Ahmad Mahmoud Yousef Mohammad
MB.BCH

SUPERVISED
BY

Prof. Dr

Osama Abdel Aziz Rifaie
Professor of cardiovascular medicine
Faculty of medicine, Ain Shams University

Assistant Prof.Dr

Mohamed Atef Hamza
Assistant Professor of cardiovascular medicine
Faculty of medicine, Ain Shams University

Dr

Sameh Attia Amin
Lecturer of cardiovascular medicine
Faculty of medicine, Ain Shams University

Cardiovascular medicine department,
Faculty of Medicine, Ain Shams University
2019



Dedication

To the soul of my Mother

May ALLAH gather me with here in his paradise.

To my Father

Who lights my life.

To my great brothers and sister

Wael, Moustafa and Noha

My best support in this life.

To my beloved wife, Somaya

The rose of my life

To my sweet daughter, Maryam

The best gift from ALLAH



Abstract

Study of the relation between serum levels of long acting penicillin and the inflammatory markers: C-Reactive Protein and Interleukin-6 in patients with chronic rheumatic heart disease

Ahmad Mahmoud Yousef Mohammad, Osama Abdel Aziz Rifaie, Mohamed Atef Hamza, Sameh Attia Amin

Cardiology department, Faculty of medicine, Ain Shams University, Cairo, Egypt.

Corresponding author: ahmadyousoff@yahoo.com

(Ahmad Mahmoud Yousef Mohammad)

Background

Rheumatic heart disease refers to the functional and structural changes of the heart muscle and valves affected by rheumatic fever

Rheumatic fever has a marked tendency to recur leading to high risk of chronic heart lesions or worsening lesions in patients with previous rheumatic heart disease.

C-reactive protein (CRP) and inflammatory cytokines, as TNF α , IL-8 and IL-6, may play a pathogenic role in rheumatic fever and there levels indicate the activity of the disease.

Efficacy of long acting penicillin for secondary prevention of rheumatic fever has not been widely studied.

Objectives

Our study is a prospective cross-sectional controlled study that aims to study the relation between serum levels of long acting penicillin and the inflammatory markers, CRP and IL-6, in patients with chronic rheumatic heart disease.

Methods

Eighty patients from rheumatic heart disease patients coming to Ain Shams university hospitals' outpatient clinic for rheumatic fever prophylaxis by regular long acting penicillin administration were subjected to the study. Patients were divided into to 2 groups: Group A ; 70 patients with rheumatic heart disease already on prophylactic long acting penicillin, and Group B; 10 patients with rheumatic heart disease who have not started prophylactic long acting penicillin yet in addition to Group C; control group of 10 healthy individuals not known to have rheumatic heart disease.

Venous blood samples were drawn under aseptic conditions, centrifuged and 2 ml serum were collected and were stored at minus 20°C for ELISA at El-Demerdash hospital immunology laboratory. All the serum samples were analyzed for long acting penicillin, CRP and IL-6 at Ain-Shams University Clinical Pathology Department using ELISA techniques using ELISA kit for Benzathine Benzylpenicillin, High Sensitivity C-Reactive Protein ELISA Test Kit and Human Interleukin-6 ELISA Kit.

Conclusion

Study results emphasize the importance of long acting penicillin in secondary prevention of chronic rheumatic heart disease.

Results showed a strong negative relation between long acting penicillin and the rheumatic inflammatory mediators; CRP and IL-6. Also the study results emphasized that chronic rheumatic heart disease is an inflammatory process mediated with some mediators as CRP and IL-6.

More large studies need to be done to prove the rule of long acting penicillin in secondary prevention of chronic rheumatic heart disease and to study the nature of the CRHD as an inflammatory process that may need more control with anti-inflammatory therapies.

Key words: CRP, IL-6 LAP, RF, RHD, CRHD

LIST OF CONTENTS

Content	Page
List of Abbreviations	II
List of Tables	IV
List of Figures	V
Introduction	1
Aim of the Study	2
Review Of Literature	3
- Immunological Background	3
-Interleukin-6	4
-C-Reactive Protein	6
- Rheumatic Heart Disease	9
-Pathophysiology	11
-Diagnosis	13
-Prevention	20
Patients and methods	21
Results	24
Discussion	46
Conclusion and Recommendations	52
Summary	53
References	55
Arabic Summary	

LIST OF ABBREVIATIONS

Abbreviation	Title
AF	Atrial fibrillation
CRHD	Chronic rheumatic heart disease
CRP	C-reactive protien
ESR	Erythrocyte sedimentation rate
HS-CRP	High sensitivity C-reactive protein
IFN	Interferon
IL	Interleukin
LAP	Long acting penicillin
MR	Mitral regurgitation
MS	Mitral stenosis
MV	Mitral valve
MVA	Mitral valve area
MVR	Mitral valve replacement
P-value	Calculated Probability
r (statistics)	Pearson correlation coefficient
RF	Rheumatic Fever

LIST OF ABBREVIATIONS (CONTINUE)

RHD	Rheumatic Heart Disease
SD	Standard Deviation
TNF	Tumor Necrosis Factor

LIST OF TABLES

Table	Description	Page
Table 1	Revised Jones criteria	14
Table 2	Doppler Findings in rheumatic valvulitis.	17
Table 3	Morphological Findings on Echocardiogram in rheumatic valvulitis	17
Table 4	Basic demographic and clinical data for all patients and both groups	25
Table 5	Long acting Penicillin regimen for group A patients	25
Table 6	Long acting Penicillin compliance for group A patients	26
Table 7	Comparison between IL6 and CRP between control subjects and subgroups.	28
Table 8	Positive and negative CRP values for all patients and subgroups	31
Table 9	Correlation between IL-6 (ppb) and CRP(mg/dl)	2
Table 10	Correlation between LAP (ppb) and CRP(mg/dl)	34
Table 11	Correlation between LAP (ppb) and IL6 (ppb)	36
Table 12	Correlation between compliance and LAP, IL-6 and CRP	38
Table 13	Correlation between penicillin regimen and LAP, IL-6 and CRP values	41
Table 14	Correlation between rhythm and LAP, IL-6 and CRP	44

LIST OF FIGURES

Figure	Description	Page
Figure 1	Map showing reported worldwide prevalence of RHD from 1991 through 2011	10
Figure 2	One Way ANNOVA test for IL-6 groups	29
Figure 3	IL-6 Mean and SD between groups	29
Figure 4	One Way ANNOVA test for CRP groups	30
Figure 5	CRP Mean and SD between groups	30
Figure 6	CRP between groups	31
Figure 7	Correlation between IL-6 and CRP	33
Figure 8	Linear regression between LAP and CRP	34
Figure 9	Correlation between LAP and CRP	35
Figure 10	Linear regression between LAP and IL-6	36
Figure 11	Correlation between LAP and IL-6	37
Figure 12	Mean and SD of LAP compliance groups	39
Figure 13	Mean and SD of CRP compliance groups	39
Figure 14	Mean and SD of Il-6 compliance groups	40
Figure 15	One Way ANNOVA test between for CRP between penicillin regimen groups	42
Figure 16	One Way ANNOVA test between for LAP between penicillin regimen groups	42

LIST OF FIGURES (CONTINUE)

Figure 17	One Way ANNOVA test between for IL-6 between penicillin regimen groups	43
Figure 18	Mean and SD of CRP between Rhythm groups	44
Figure 19	Mean and SD of LAP between Rhythm groups	45
Figure 20	Mean and SD of IL-6 between Rhythm groups	45

Introduction

Rheumatic fever is the most important cause of acquired heart disease in children and young adults worldwide. It is an inflammatory reaction that occurs approximately 10 to 21 days after throat infection with virulent strains of Group A beta-haemolytic streptococci. It affects large joints (arthritis), the heart (carditis) and less frequently the brain (chorea), skin (erythema marginatum) and subcutaneous tissues. Rheumatic heart disease refers to the functional and structural changes of the heart muscle and valves affected by rheumatic fever (**Manyemba J. *et al.* 2002**).

Rheumatic fever has a marked tendency to recur following new group A streptococcal upper respiratory tract infection. Recurrence has a high risk of chronic heart lesions or worsening lesions in patients with previous rheumatic heart disease. The severity of rheumatic heart disease and the prognosis depend on the extent of the carditis and the frequency of recurrent attacks. There is much evidence from randomised controlled trials concerning the primary prevention of rheumatic fever or the treatment of pharyngitis caused by Group A beta-haemolytic streptococci (GAS) but less data is available concerning secondary prevention of the disease (**Manyemba J. *et al.* 2002**)

C-reactive protein (CRP) is increased in patients with acute rheumatic fever, High levels of hs-CRP in patients with chronic rheumatic valve disease indicate the persistence of inflammation in the chronic phase(**Golbasi Z. *et al.* 2002**).

Inflammatory cytokines, as TNF α , IL-8 and IL-6, may play a pathogenic role in rheumatic fever (**Yegin O. *et al.* 1997**).

Single monthly injection of 1,200,000 unit of benzathine penicillin confer a high degree of continuous protection against Group A streptococci and afford reliable means of protecting the patient against recurrences of rheumatic fever(**Stollerman G. H. *et al.* 1955**).

Efficacy of long acting penicillin for secondary prevention of rheumatic fever has not been widely studied, consequently the relation between serum levels of long acting penicillin and inflammatory markers CRP and IL-6 is largely unknown.

Aim of the Study:

To detect the relation between serum levels of long acting penicillin and the inflammatory markers C-Reactive Protein and Interleukin-6 in patients with chronic rheumatic heart disease.

**REVIEW
OF
LITERATURE**

Immunological Background:

Interleukins as Inflammatory Mediators:

Immunocytes constantly exchange signals among themselves. Some of these signals are possible due to direct contact between cells. Others are effected through chemical messengers called cytokines which circulate in the blood.(**Morris K. *et al.* 1993**)

Interleukins are cytokines that have very important role in the function of the immune system. The term interleukin is formed from (inter-) "as a means of communication", and (-leukin) "deriving from the fact that many of these proteins are produced by leukocytes and act on leukocytes". It has since been found that interleukins are produced by a wide variety of body cells. The majority of interleukins are synthesized by helper CD4+ T lymphocytes, as well as through monocytes, macrophages, and endothelial cells. They promote the development and differentiation of T, B, and hematopoietic cells.(**Ben Menachem-Zidon O. *et al.* 2011**)

Interleukin-6:

IL-6 is a soluble mediator with a pleiotropic effect on inflammation, immune response, and hematopoiesis. At first, distinct functions of IL-6 were studied and given distinct names based on their biological activity.

For example:

1- The name B-cell stimulatory factor 2 (BSF-2) was based on the ability to induce differentiation of activated B cells into antibody (Ab)-producing cells. **(Kishimoto T. 1985)**

2- The name hepatocyte-stimulating factor (HSF) on the effect of acute phase protein synthesis on hepatocytes, the name hybridoma growth factor (HGF) on the enhancement of growth of fusion cells between plasma cells and myeloma cells.

3- The name interferon (IFN)- β 2 owing to its IFN antiviral activity. When the BSF-2 cDNA was successfully cloned in 1986 **(Hirano T. *et al.* 1986)**

However, it was found that the molecules with different names studied by various groups were in fact identical, resulting in the single name IL-6. **(Kishimoto T. 1989).**

Human IL-6 is made up of 212 amino acids, including a 28-amino-acid signal peptide, and its gene has been mapped to chromosome 7p21. **(Tanaka T. *et al.* 2014)**

Furthermore, IL-6 promotes specific differentiation of naïve CD4 T cells, thus performing an important function in the