Platelet Activity And Some Immunological Findings In Atherosclerosis and Coronary Heart Disease

A.I.S. Al-Assaf
Department. of Biology, College of Education Ibin Al-Haithum, University of Baghdad.

Abstract

The platelet activity and some immunological aspects of coronary heart disease CHD were investigated by employing platelet count and serum levels of IgG,IgM and IgA,C3 and C4.Three groups of patients were investigated : patients with ischemic heart disease IHD, patients with IHD+hypertension and patients with IHD+unstable angina.The results revealed that the mean of platelets (mm³) was significantly reduced in IHD+unstable angina group 2.72±1.12 compared with conrol group 8.13±2.72.

With respect to the immunological study, the results revealed that IgG level (mg/dl) was significantly reduced in IHD+hypertension group 1113.70±8.81 and in IHD+unstable angina 857.85±17.78 compared with control group 1279.33±22.25.The same effect was observed on IgM,in IHDgroup, the mean was 114.47±4.40 and in IHD+hypertension group was 122.10±5.32 compared with control group 177.25±4.86.The mean of complement component C3 was significantly increased in IHD+ hypertension group 197.92±6.56, also in IHD+unstable angina group 150.750±10.78 compared wih control group 102.79±10.38.

Introduction

Coronary heart diseases CHD refer collectively to clinical heart diseases due to lesions of coronary arteries (1,2),and the most common disease is atherosclerosis, which is a systemic disease of the large arteries.The consequential reduction in the blood supply to the myocardium is on essential cause of CHD and activation of inflammatory pathways is important in this pathogenesis (2,3).

* CHD   coronary heart disease.
* IHD   ischemic heart disease.

The formation of atheromatous plaque has been attributed to the response to' injury 'of the endothelium, and the subsequent proliferation of the smooth muscle cells into the intimal cells of the arterial wall, the accumulation of lipids and the deposition of the extra cellular matrix components (4,5).This occurs over a number of years and after about the third decade of life. There is no definite pattern to the amount of atheromatous tissue in which an individual accumulates (6).All individuals possess some degree of atherosclerosis but why some should have an accelerated form remains unknown..

Atherosclerotic lesions are characterized by infiltration of immune component cells such as macrophages and T-lymphocytes (5). Also, there is the influence of various humoral and cellular factors on blood monocyte interaction with arterial intima and on the possible reasons of disturbances of monocyte lipid from vascular wall at atherosclerosis (7).So, the similarity of the cell population of atherosclerotic plaques in human arteries and immune inflammation foci in various diseases allow to regard atherogenesis as a chronic inflammatory reaction similar to delayed-type hypersensitivity reaction (8).

There is some evidence to suggest that coronary heart disease is one condition where there is an increased percentage of 'active' platelets present in the patient's circulation (9).This is supported by the demonstration of an increased rate of platelets turnover (10).When platelets first enter the blood stream they are metabolically very 'active'. This high level of activity is maintained for approximately two days, and 'active' platelets normally constitute approximately 25%of the total. It is generally accepted that an increase in the proportion of these active platelets in the circulation of patients with CHD can be the result of atheromatous disease and accompanying ulceration (9).

The study aims to investigate some immunological aspects (the role of immunoglobulins and complement) of the patients with ischemic heart disease IHD in addition to measure the platelets activity and their function in the same patients.

Materials and Methods
Patients
This is a prospective study conducted at Baghdad Teaching Hospital at Baghdad Medical City involving 50 patients ; 40 with a previous

diagnosis of ischemic heart disease IHD, 4 with IHD + hypertension, and 6 with IHD +unstable angina, with an age range of 50-72 years.Ten healthy patients, aged 38-73 years, were taken as control.

Methods

1. **Collection of blood and serum:** Three milliliters of venous blood were obtained from each patient and healthy individuals for immunological tests. Serum was separated by centrifugation of blood (3000 r.p.m.for10 minutes) and 1 milliliter of blood put in EDTA tube for platelets count.

2. **Assessment of immunoglobulin levels and complement proteins:** The levels of immunoglobulins (IgG,IgM and IgA) and the complement components C3,C4 were measured by using the method of Single Radial Immunodiffusion (RID) (11) with commercially available plates (Biomaghreb).

3. **Platelets count:** An aliquot of 0.05 ml blood from (EDTA) tube was mixed with 2 ml of formal citrate and let it stay for about15 minutes.Then examined by haemocytometer (12).

4. **Statistical analysis:** The analysis of variance (ANOVA) test and least significant difference (LSD) test by probability of less than ($P<0.05$) were used to assess significant differences between means.

Results

In table (1), the distribution of platelets count in control and CHD patients are presented .The mean of obtained platelet count in the control was 8.13±2.72 , in patients with ischemic heart disease IHD was 7.22± 2.68, and 9.36±2.99 in patients with IHD + hypertension, while in patients with IHD + unstable angina it was 2.72±1.12. Statistically, there was a significant difference ($P<0.05$) in platelets activity in IHD + unstable angina patients as compared to the control group.

Table (2) shows the mean values of serum IgG, IgM and IgA levels (mg/dl) in controls, which were 1279.33 ± 22.25, 177.25 ± 4.86 and 246.30 ± 18.31,respectively.In patients with IHD they were 1191.66 ± 31.80,114.47 ± 4.4 and 195.60 ± 8.48, respectively. Patients suffer from IHD+ hypertension displayed levels of 1113.70 ± 8.80,122.10 ± 5.32 and 21847 ± 5.19, respectively, while in IHD + unstable angina,

they were 857.85 ± 17.87, 178.58 ± 277 and 195.60 ±388, respectively. Statistically, there was a significant difference (P<0.05) in serum IgM level between IHD with control. Also, there was a significant difference (p<0.05) in serum IgG, IgM levels between IHD+hypertension patients with control group. Patients of IHD +unstable angina showed a significant difference in IgG levels as compared with control group.

Measurements of serum complement components C3 and C4(mg/dl) are presented in table (3). In control group, the mean values were 102.79 ± 10.38 and 39.35 ± 1.50, respectively, in IHD patients were 140.44 ± 5.56 and 39.95 ± 2.09, respectively, while in IHD +hypertension they were 197.92 ± 6.56 and 34.80 ± 2.86, respectively. In IHD +unstable angina group, the means were 150.750 ± 10.78 and 34.06 ± 2.74, respectively.   Statistically, there was a significant difference (P<0.05) in serum C3 level between IHD + hypertension, and IHD + unstable angina patients as compared to control group.

Discussion

The study aimed to investigate the platelet activity and other immunological aspects in patients with IHD,because it is generally accepted that an increase in the proportion of these "active" platelets in the circulation of patients with coronary heart disease could be the result of atheromatous disease and accompanying ulceration (9).

The present results indicate that there is no increase in platelets count in patients as compared with control. This may be due to the old age of our patients and this could be correlated with previous works (13) who postulated that an increase in "active "platelets could be a primary factor in the initiation and/or acceleration of the process of atherogenesis leading to atherosclerosis and its clinical manifestation and the activity of platelets decreases steadily during the age (9).Other studies showed that fibrin clots and thread formation with platelet entrapment in the intermediate stages, and "micro clot" and "micro plaque" formation in late stages can also occur (14).

Immune mechanisms have been suggested to have an important role in the development of coronary atherosclerosis and its thrombotic complications (15).

We have evaluated the predictive value of the levels of three immunoglobulin classes at increased risk of myocardial infarction. The immunological test results revealed that there was no increase as compared to the normal ranges, although in case of IHD +hypertension and IHD + unstable angina there was an increase in their means as compared with control, but still within normal ranges .These findings agreed with (16) who suggested that atherosclerosis, the main lethal disease in the western world, is associated with a cellular immune response in the arterial lesions and a humoral immune response directed towards oxidized lipoproteins, certain microbes and other antigens. The local immune response is dominated by macrophages and T cells, while to date, the role of B cells in lesion is unclear. But our findings about immunoglobulin levels disagree with (15) who suggested elevated levels of IgA, IgE and IgG are associated with myocardial infarction

Also, there is another study which demonstrated that IgG seropositively was not associated with an increased risk of ischemic heart disease as defined by the Rose angina questionnaire in Singapore (17). In addition to the study from American heart journal (18), that in acute IHD neither C pneumonia IgG nor IgA titers were strongly predictive of CHD.

The presented results demonstrated that there is no increase in complement component C3 and C4 levels in IHD, but there was some degree of elevation in C3 level in IHD+hypertension and in IHD+unstable angina as compared to control group. However, there are many results strongly implicate the active participation of the complement system of the inflammatory proteins in the pathogenesis of myocardial tissue injury following coronary occlusion in Baboon (19).

References

1.   Friedbrg,C.K.(1966).Disease of the heart.Philadelphia:Saunders.
2. Lutgens,E;Gorelik,L.;Doemen,M.J.;deMuinck,E.D.;Grewal; Koelia- nsky,V.E.and Flavell,R.A.(1999).Requirement for CD154 in the prog- ression of atherosclerosis.Nat-Med.Nov., 5 (11) : 1313-6.United States
3.Anthony,C.P.and Hibodeau,G.A.(1979)Text book of Anatomy and phy-siology,10th edn. St.Louis : Mosby.
4.Chesterman,C.N.and Berndt,M.C.(1986). Platelet and vessel wall

inter- action and the genesis of atherosclerosis.In :Clinics in Haemato- logy (C.N.Chesterman,ed). 15 : 323-353. London : Saunders.

5.Mach,F.;Schonbeck,U.and Lippy,P.(1999).CD40 signaling in vascular cells: A key role in atherosclerosis.Apr. 137 suppl : S 89-95.Ireland.

6.Woolf,N.(1977).Atheroma-aspects of atherogenesis.Br.J.Hosp.Med., 18: 286-95.

7. Kondakov,I.K.(1999).The role of blood monocytes in atherogenesis. Tsitol-Gene.Jan-Feb; 33(1):70-80.Ukrania.

8.Nagornev,V.A.(1998).Kinetics of vascular wall cells and atherogenesis.   Arkh-patol.Jan-Feb; 60 (1): 39-43.Russia.

9.Rees,B.W.G.(1987).Hypothesis supporting the primary role of increased platelet activity in atherosclerosis and coronary heart disease.Medical Laboratory Sciences.,44: 267-271.

10.Triplett,DA.;Harms,C.S.; Newhouse,P.and Cark,C. (1987).Med.Lab.Sci., 44.

11. Mancini,G;Carbonara,AO.;and Hesemans,J.F. (1965). Immunochemical quantitation of antigen by single radial immunodiffusion.Int.J. Immu- nochem., 2 :235.

12.Dacie,J.V.andLewis,F.M.(1995).Practical haematology.8th edition. Churchil.Liverig Stone:63.

13.Firkin,B.G.(1984).The platelet and its disorder.Lancaster: MTPPrees.

14. Ali,M.and Ali,O.(1997).AA Oxidopathy : The core pathogenetic mechanism of ischemic heart disease.J.Integrative Medicine.,1:1-112.

15. Mänttäri,M.;Palsuo,T.;Manninen,V.andAho,K.(1998).Predicaion of myocardial infarction in dyslipidemic Men by elevated levels of immunoglobulin classes A,E and G,but not M.Vol.158(B):July.

16. Zhou,X.and Hansson,G.K.(1999).Deecion of B cells and proinflamm- atory cytokines in atherosclerotic plaques of hypercholesterolaemic a poliprotein E in knocku mice.Scand.J.Immunol.Jul, 50 (1) :25-30.

17.Koh,W.P.;Taylor,M.B.;Chew,S.K.;Phoon,M.C.;Kang,K.L.and Chow,T.K.(2003).Chlamedia pneumonia IgG seropositively and clinical His- tory of ischemic heart disease in Singapore. J.Microbial.Immunol. Infect.,36 :169-174.

18. WWW.med scape.com./viewaricle/460029-38K-Cached-Similar
    pages.1994-2005.
19. Pinckard,R.N.;O'Rourke,R.A;Crawford,MH.;Cover,F.S.;McManus
    ,LM;Ghidoni,J.J;Storrs,S.B.andOlson,M.S.(1980).Complement
    local-lization and mediaion of ischemic injury in Baboon
    myocardium. J.Clin.Invest.November; 66 (5):1050-1056.

**Table (1) :The mean values of platelets count × 109 in controls
and groups of patients**

Groups of Patients	No.	Mean × 10^{11}	± S.E. × 10^{11}
Ischemic heart disease IHD	40	7.22	±	2.68
IHD + Hypertension	4	9.36	±	2.99
IHD + Unstable angina	6	2.72 *	±	1.12
Control	10	8.13	±	2.72

*The difference between patients and controls is significant at the 0.05
level.

**Table (2) : The mean values of serum immunoglobulin IgG,IgM
and IgA levels(mg/dl) in conrols and groups of patients**

Groups of patients	No.	IgG Mean±S.E. (mg/dl)	IgM Mean± S.E. (mg/dl)	IgA Mean±S.E. (mg/dl)
Ischemic heart discase IHD	40	1191.66 ±31.80	114.47± 4.40 *	195.90±8.84
IHD + Hypertension	4	1113.70±8.81 *	122.10± 5.32 *	218.47±5.19
IHD + Unstable angina	6	857.85±17.87 *	178.58±2.77	195.60±3.88
Controls	10	1279.33±22.25	177.25±4.86	246.30±18.31

*The difference between patients and controls is significant at the 0.05 level.

Table (3) : The mean values of serum C3 and C4 levels (mg/dl) in controls and groups of patients.

Groups of Patients	No.	C3 Mean ± S.E. (mg/dl)	C4 Mean ± S.E. (mg/dl)
Ischemic heart disease IHD	40	140.44 ± 5.56	39.95 ± 2.09
IHD + Hypertension	4	197.92 ± 6.56 *	34.80 ± 286
IHD + Unstable angina	6	150.750 ± 10.78 *	34.06 ± 2.74
Control	10	102.79 ± 10.38	39.35 ± 1.50

*The difference between patients and controls is significant at the

فعالية الصفائح الدموية وبعض الجوانب المناعية في امراض تصلب الشرايين وامراض القلب التاجية

انوار ادريس سليمان العساف

قسم علوم الحياة ، كلية التربية – ابن الهيثم ، جامعة بغداد

الخلاصة

هدف البحث الى دراسة دور الصفائح الدموية وبعض الجوانب المناعية لامراض القلب التاجية CHD ، حيث اشتملت الدراسة على ثلاثة مجاميع من المرضى : المجموعة الاولى تعاني من امراض القلب ذات فقر الدم الموضعيIHD والثانية مصابة بارتفاع الضغط IHD + اما المجموعة الثالثة فكان افرادها يعانون من الذبحة غير المستقرة IHD+.قيس مستوى الكلوبيولينات المناعية من نوع IgG,IgM وIgA وكذلك تم تحديد فعالية نظام المتمم complement في هذا المرض من خلال قياس مستوى المكونات C3وC4في مصل الدم .

بالنسبة لفعالية الصفائح الدموية فقد بينت نتائج التحليل الاحصائي عدم وجود فروق معنوية عند مقارنة المرضى مع مجموعة السيطرة ماعدا في حالة مرضى الذبحة غير المستقرة IHD+ حيث انخفض المعدل الى 2.72 ± 1.12 مقارنة مع مجموعة السيطرة 2.72 ± 8.13 وقد يعزى السبب للعمر الكبير للمرضى في العينة المأخوذة، حيث ان نشاط الصفائح الدموية يتناقص مع تقدم العمر .

فيما يخص الفحوصات المناعية، فقد اثبتت نتائج التحليلات الاحصائية عدم وجود فروق معنوية بين مرضى IHD عند مقارنتها مع مجموعة السيطرة، اما في حالة المرضى المصابين بارتفاع الضغط IHD+ فقد بلغ معدل IgG 1113.70±8.81 مقارنة مع السيطرة 1279.33±22.25 كما لوحظ وجود انخفاضا معنويا في مجموعة مرضى الذبحة IHD+ 857.85±17.87 وبالنسبة للIgM فقد انخفض المعدل في مجموعة ال IHD الى114.47±4.40 مقارنة مع مجموعة السيطرة 177.25±4.86 وكذلك حصل

الانخفاض في المرضى المصابين بارتفاع الضغط +IHD 197.92±6.56 مقارنــة مـــع مجموعة السيطرة 102.79±10.38 وكذلك الحال في مجموعــة المرضــى المـصابين بالذبحة غير المستقرة +IHD فقد بلغ المعدل 150.750±10.78 وعموما فان الارتفــاع في المعدلات كان ضمن القيم الطبيعية.