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Interleukin 6 as a Biomarker of Ischemic Heart Disease

Martin Joseph Sto. Tomas, Patricia Megan Baluyot, Ana Loraine Delos Santos, Jenelle Faustino, Eunice Licudine, Ella Marie Mendoza, Steven Truelen, Ma. Jennifer Tiburcio

Department of Medical Technology, University of Santo Tomas, Manila, Philippines

Abstract- Interleukin 6 (IL-6) is a multifunctional 21-27kDa glycopeptide with 184 amino acid residues. IL-6 is also responsible for various proinflammatory and anti-inflammatory processes (Agorastos *et al*., 2014). This study aimed to determine if Interleukin 6 can be used as a biomarker of Ischemic heart disease (IHD). The study employed twenty-four (24) patients and was divided into two groups: IHD patients (n=12) and patients without IHD (n=12). Plasma samples were obtained and IL-6 levels were determined through an Enzyme-Linked Immunosorbent Assay. Results show that there is a significant difference (p<0.001) in the IL-6 levels of IHD patients (median of 33 pg/mL) from patients without IHD (median of 3.8 pg/mL). Based on these findings, those with an increased IL-6 level ($\geq$ 5 pg/mL) are 25.0 times more likely to have IHD than those without increased IL-6 levels. Furthermore, it was found that for every 1 pg/mL increase in IL-6, the chances of developing the disease increases by 1.24 [$CI_{95\%}$: 1.03 to 1.48]. From these findings, it can be inferred that Interleukin 6 can be used as a biomarker of ischemic heart disease.

Index Terms- Interleukin 6, Ischemic Heart Disease, Biomarker, Predictor

I. INTRODUCTION

Since the discovery of Interleukin 6 (IL-6), it has been known as a main pro-inflammatory and pleotropic cytokine along with Interleukin 1 (IL-1) and Tumor necrosis factor alpha (TNF-α), exhibiting a wide range of cellular and humoral effects associated with inflammation, host defense, and tissue injury (Croker *et al*., 2012). It is also said that IL-6 is significantly involved in both physiological and pathological processes such as granulocyte production and maturation, T cell differentiation and acute phase protein production. Lind (2003) clearly states that IL-6 plays a vital role in acute phase response. It also plays an important part as a primary mediator of C-reactive protein production. As what Silva and Pais de Lacerda (2012) state, the production of C-reactive protein is mainly done by the liver and is induced by the secondary inflammatory cytokine, Interleukin 6.

The National Institute of Diabetes and Digestive and Kidney Diseases in 2013 defined ischemic heart disease as a disease caused by the thickening of the walls of the coronary arteries. It may lead to heart attack once deposits of cholesterol, which result in the narrowing of arteries, block the supply of nutrients and oxygen in the blood. Ischemic heart disease (IHD) is also the leading cause of cardiovascular mortality worldwide, with greater than 4.5 million deaths occurring in the developing world (Okrainec *et al*., 2004). According to the Global Burden of Disease Study, the developing countries contributed 3.5 million of the 6.2 million global deaths from IHD in 1990 (Backer, 2009). While in 2008, the World Health Organization (WHO) reported an estimated death of 17.3 million people from cardiovascular *disorders* (CVD), representing 30% of all global deaths. Of these deaths, an estimated 7.3 million were due to ischemic heart disease. In the year 2010, approximately 3.8 million men and 3.4 million women worldwide died from the said disease. The projections estimate that the developing countries will account for 7.8 million of the 11.1 million deaths due to IHD in 2020 (Backer, 2009). In the Philippines, cardiovascular disorders are among the leading causes of deaths among Filipinos. The latest WHO data published in April 2011 state that deaths due to Ischemic heart disease reached 57,864 or 13.73 % of total deaths. While in the year 2012, the Philippine National Statistics Office (NSO) stated that five (5) out of ten (10) Filipinos die of heart disease (Sindico, 2012).

According to Rimmerman (2013), certain markers are usually treated as detectors for inflammatory processes such as ischemic heart disease. Some of these markers, which provide significant information regarding a patient's diagnosis and prognosis, are Troponin I and C-reactive protein (Zakynthinos and Pappa, 2009). Recent researches, however, suggest that Interleukin 6 (IL-6) is a better and a more specific marker for this disease. Lee *et al*. (2007) further conclude that IL-6 values were independent of CRP, but not vice versa, suggesting that IL-6 increases at an earlier atherosclerotic state. It is for this reason that the principal investigators focused on Interleukin 6 as a predictor for the leading cause of cardiovascular mortality worldwide, ischemic heart disease.

II. MATERIALS AND METHODS

A. Research Locale

Ischemic heart disease (IHD) respondents were gathered from patients of the Our Lady of the Angels, Multi-specialty and Diagnostic Clinic in Sta. Maria, Bulacan. On the other hand, non-IHD respondents were recruited by the principal investigators.

B. Methods of Screening Participants

The researchers asked permission from the attending physician to conduct a study involving patients with IHD as participants. Respondents, including those recruited by the researchers as non-IHD participants, were given consent forms

asking for their voluntary participation in this study. Questionnaires were distributed to gather information regarding the patient's lifestyle including demographic data, body mass index (BMI), smoking status, and medical history which includes a family history of IHD, presence and duration of IHD, presence and duration of diabetes, and history of myocardial infarction. These important data gathered were evaluated based on constructed inclusion/exclusion criteria and those who passed the screening process were accepted as part of the sample population.

C. Inclusion and Exclusion Criteria

Inclusion and exclusion criteria were constructed to select appropriate participants for the study. The following criteria were based on the study of Xiao *et al.*, (2011) among coronary angiographic patients.

Inclusion Criteria

- Age Range: 30 years old and above
- Gender: Male/Female
- Willing to participate in the study and has given a consent form

Ischemic Heart Disease Patients

- Patients who have ischemic heart disease as evidenced by their electrocardiogram (ECG) results

Patients without Ischemic Heart Disease

- Those who are free from ischemic heart disease based on their ECG results

A cardiology consultant was the one who validated, upon reading of the ECG, whether or not the patient have IHD.

Exclusion Criteria

- Presence / history of liver disease
- Women on hormone replacement therapy
- Patients with lymphoma, lupus, giant cell arteritis, rheumatoid arthritis, inflammatory bowel disease, osteomyelitis, and Crohn's disease
- Pregnant women
- Patients with other infections, cancer, and immunologic diseases (rheumatic fever)
- Patients with decreased perfusion pressure, hypotension, and hypovolemia
- Patients with decreased blood oxygen content, anemia, and pulmonary diseases leading to impaired oxygenation of blood by the lungs
- Patients with tachycardias

Ischemic Heart Disease patients

- Patients with cardiovascular disorders other than IHD

Patients without Ischemic Heart Disease

- Those who have abnormal ECG results suggestive of IHD

D. Methods of Selecting Accepted Respondents from Screened Participants

Those who passed the inclusion and exclusion criteria were eligible to be part of the sample population. Accepted respondents were grouped as patients having IHD and patients without IHD.

E. Blood Collection

Collection was done in the morning since Interleukin 6 exhibits a diurnal variation (Agorastos *et al.*, 2014).

F. Blood Sample Processing and Clinical Laboratory Analysis

All samples were taken in the laboratory within the day of collection to separate the plasma from whole blood. Afterwards, the plasma was placed in their respective eppendorf tubes and then stored at a - 20° C frost-free freezer until such time that Interleukin 6 levels were measured through an Enzyme-Linked Immunosorbent Assay (ELISA) test kit (Ebioscience Human IL-6 ELISA Ready Set Go; *cat # 88-7066-86*). The ELISA reader available at the Medical Technology Research Laboratory of the University of Santo Tomas was used. Excess specimens were placed in the infectious waste and disposal was coursed through the University of Santo Tomas, Faculty of Pharmacy laboratory where the ELISA procedure took place. Total cholesterol levels of ischemic heart disease patients were tested at the Our Lady of the Angels Multi-specialty and Diagnostic Clinic. Total cholesterol levels of patients without IHD, on the other hand, were tested at the New World Diagnostic Laboratory.

III. RESULTS

Table 1. Demographic Profile of Ischemic and Non-Ischemic Patients

Demographics	With IHD	Without IHD	*p*-value
Age	55.7 ± 4.2	49.0 ± 2.3	0.180
Gender: Male	4 (33.3%)	6 (50 %)	
Female	8 (66.7%)	6 (50%)	0.680

Values expressed as mean ± SEM, n=12. *P*-values are based on Independent T test & Fisher's exact test.

A total of 24 patients were included in the study. Those with ischemic heart disease (IHD) have a mean age of 55.7 years and are composed of 4 (33.3 %) males and 8 (66.7%) females. On the other hand, those patients without IHD have a mean age of 49 years and are composed of 6 (50%) males and 6 (50%) females. Furthermore, the mean age ($t_{17} = 1.396$, $p = 0.180$) and gender ($p = 0.680$) of Ischemic and non-ischemic patients do not differ.

Table 2. Interleukin 6 (IL-6) Levels of Ischemic and Non-Ischemic Patients

Interleukin 6 Level	With IHD (n=12)	Without IHD (n=12)	Z-Stat	*p*-value
IL-6 level (in pg/mL)	33 [28.3 to 45.5]	3.8 [1 to 11.94]	3.784	<0.001

Values expressed as median [IQR].

Table 2 summarizes the IL-6 levels of all patients with and without ischemic heart disease. The IL-6 of ischemic patients is significantly higher [Z=3.784, p<0.001] than the non-ischemic patients.

Table 3. Number of Above Normal and Normal Interleukin 6 Levels Among Patients with and without Ischemic Heart Disease

IL-6 level (in pg/mL)	With IHD (n=12)	Without IHD (n=12)	Odds Ratio [CI]	*p*-value
Above normal (≥ 5)	12 (100%)	6 (50%)	25.0 [1.21-516.7]	0.037
Normal (< 5)	0 (0%)	6 (50%)		

Table 3 categorizes the IL-6 levels of both groups into Above Normal (≥ 5 pg/mL) and Normal (< 5 pg/mL) IL-6 levels. This is according to the study done by Mojominiyi *et al.* (2002) which defined that the concentration of IL-6 in apparently normal subjects is less than 5 pg/mL. All 12 (100%) Ischemic heart disease patients have above normal IL-6 levels. Of all patients without ischemic heart disease, 6 (50%) have an IL-6 level above the normal range and 6 (50%) within the normal range. Odds ratio of 25.0 [$CI_{95\%}$: 1.21-516.7] indicates that those with increased IL-6 are 25.0 times more likely to develop ischemic heart disease.

Table 4. Univariate Logistic Regression Results of IL-6 to Ischemic Heart Disease

Variable	Coefficient	*p*-value	ODDS RATIO	
			Estimate	95 % CI
IL-6	0.212	0.023	1.24	1.03-1.48

Table 4 shows that for every 1 pg/mL increase in IL-6, the odds of having ischemic heart disease increases by 1.24 [$CI_{95\%}$:1.03-1.48].

Table 5. Univariate Linear Regression Results of the Variables Considered in IL-6 Level Measurement

Variables	Coefficient	*p*-value
Age	0.056	0.907
Gender	-1.531	0.893
IHD	2.816	0.301
Family Hx	3.028	0.793
CHOLE	3.431	0.342
BMI	0.297	0.794
MI Hx	-0.963	0.800
DM	-0.261	0.887
Smoking	14.000	0.437

Legend: IHD = Presence and duration of Ischemic heart disease (IHD); Family Hx = Family history of IHD; CHOLE = Total cholesterol levels; BMI = Body mass index; MI Hx = History of Myocardial infarction; DM = Presence and duration of type 2 Diabetes mellitus; Smoking = smoking status

As shown in Table 5, age (p= 0.907), gender (p= 0.893), presence and duration of IHD (p= 0.301), family history of IHD (p= 0.793), total cholesterol levels (p= 0.342), body mass index (p= 0.794), history of myocardial infarction (p= 0.800), presence and duration of type 2 DM (p= 0.887), and smoking status (p= 0.437) do not affect the IL-6 levels of patients with and without ischemic heart disease.

IV. DISCUSSION

Results of the conducted study are in parallel with the study done by Tentolouris *et al.* (2004), which states that IL-6 levels are greater in patients with Ischemic cardiomyopathy. In addition, the study of St-Pierre, *et al.* (2005) states that elevated IL-6 level is an independent risk factor of ischemic heart disease in men. The abovementioned study also states that high plasma IL-6 levels were associated with a 70% greater risk of IHD which further supports the findings of the conducted study.

Based from the abovementioned studies, it is inferred that IL-6 is significantly correlated with IHD. This present research however, did not only align to the results of Tentolouris *et al.* (2004) and St-Pierre, *et al.* (2005), but it also showed that IL-6 can be used as a biomarker of IHD among Filipinos. Further, the chances of developing IHD for every 1 pg/mL increase in the IL-6 level and the risk of developing IHD if someone has an above normal IL-6 level were also determined in the conducted research.

According to Tousoulis, *et al.* (2006), inflammatory processes are involved in both progression and stability of atherosclerotic coronary plaque formation. Assier *et al.* (2010) further assert that IL-6, being an inflammatory cytokine, is a key player in inflammation since IL-6 regulates the acute phase response, one of the earliest responses to immunological stress. A possible mechanism in which IL-6 contributes to the development of IHD has been suggested. First, IL-6 stimulates acute phase response and the hepatic production of acute phase proteins which include CRP and fibrinogen, proteins both known as strong risk factors for IHD. This in turn increases blood viscosity and promotes platelet proliferation and activity. Next, deposition of fibrinogen in the vessel wall is promoted by autocrine and paracrine activation of monocytes by IL-6. Then, a decrease in activity and plasma levels of monomeric lipoprotein lipase is induced by IL-6, resulting in an increase in lipid uptake of macrophages. Lastly, the hypothalamic-pituitary-adrenal axis is activated by IL-6. This is associated with obesity, hypertension and insulin resistance (Su *et al.*, 2013).

The variables found on Table 5 were based on a study by Dans *et al.* (2005) on the prevalence of atherosclerotic risk factors among Filipinos. The results of this study are in contrast to the studies of Mitchell *et al.* (2007) and Boudi *et al.* (2012) wherein a positive correlation between the presence of increased IL-6 levels and those risk factors mentioned above has been found. The reason for this contradiction is that the number of samples from the computed sample size in this study is much appropriate for establishing an association between IL-6 levels and ischemic heart disease, whether or not IL-6 can be used as a biomarker of ischemic heart disease, and is limited when used for correlating various risk factors to IL-6 levels since, according to Schönbrodt and Perugini (2013), in contrast to associations, correlations generally require larger sample sizes.

V. CONCLUSION

Based on the findings of the study, the researchers hereby make the following conclusions:

1. The Interleukin 6 (IL-6) levels of ischemic patients is significantly higher [Z=3.784, $p<0.001$] than the non-ischemic patients. Thus, there is a significant difference between the IL-6 levels of ischemic heart disease (IHD) patients and patients without IHD.
2. Those with increased IL-6 levels (IL-6 $\geq$ 5 pg/mL) are 25.0 times more likely to develop ischemic heart disease. Thus, Ischemic heart disease is significantly associated with Interleukin 6.
3. Results of logistic regression showed for every 1 pg/mL increase in Interleukin 6, the risk of developing ischemic heart disease increases by 1.24 [$CI_{95\%}$:1.03-1.48]. Therefore, it can be inferred that IL-6 can be used as a predictor for ischemic heart disease.
4. Results of linear regression showed that age (p= 0.907), gender (p= 0.893), presence and duration of IHD (p= 0.301), family history of IHD (p= 0.793), total cholesterol levels (p= 0.342), body mass index (p= 0.794), history of myocardial infarction (p= 0.800), presence and duration of type 2 DM (p= 0.887), and smoking status (p= 0.437) do not affect the IL-6 levels of patients with and without Ischemic heart disease. This can be caused, however, by the limited number of samples used in this study for the correlation of the factors mentioned above with IL-6 level since according to Schondbordt and Perugini (2013), in contrast to associations, correlations generally require larger sample sizes.
5. Research work can further be initiated towards the use of a more intensive screening procedure in addition to the questionnaire in order to classify future respondents in a more distinct way. Also, the IL-6 levels of the patients should be compared side by side with the gold standard (ECG results) so that a more significant finding regarding the use of IL-6 as a biomarker and predictor of IHD could be established. Since this study only considered the total cholesterol levels of both ischemic and non-ischemic patients, the researchers also highly suggest for the future respondents to undergo lipid profile, so that low density lipoproteins (LDL) and high density lipoproteins (HDL) could also be determined. Further, the investigators recommend for the future researchers to go above the minimum number of samples based on the computed sample size, since a larger sample size could not only determine the association of IL-6 with IHD, but it could also provide a better correlation of the various risk factors and IL-6 levels.

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AUTHORS

Correspondence Author – Martin Joseph Sto. Tomas
mjst941811@gmail.com, (+63)9164732676