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Low-Density Lipoprotein Concentration is an Independent Predictor for Oxidised LDL in Patients with Familial Hypercholesterolaemia

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ABSTRACT

Familial hypercholesterolaemia (FH) is an autosomal dominant genetic disorder characterised by severe hypercholesterolaemia leading to premature coronary artery disease (CAD). Oxidised low-density lipoprotein (ox-LDL), F2-isoprostanes (ISP) and malondialdehyde (MDA) are established oxidative stress biomarker, but the status of oxidative stress in FH is not well studied. The aim of this study is to investigate oxidative stress status among FH patients and normocholesterolaemic control (NC) subjects. Ninety-eight FH patients and 100 (age, gender and BMI matched) NC subjects were recruited in series of health screening programmes across the country. Fasting blood samples were analysed for serum ox-LDL, ISP and MDA. Ox-LDL, ISP and MDA concentrations were higher in FH groups compared to NC (mean±SEM: 63.0±6.5 vs 25.5±1.2 (U/l), $p<0.001$); 749.7±74.0 vs 354.2±18.1 pg/ml, $p<0.0001$; 342.4±46.0 vs 162.7±13.5 nmol/g, $p<0.0001$). Ox-LDL showed correlation with glucose ($p<0.05$), TC ($p<0.001$), LDL-c ($p<0.001$) and HDL-c ($p<0.01$) in all subjects. LDL-c was associated positively with ox-LDL concentration ($p<0.001$). LDL-c was an independent predictor for ox-LDL concentration ($p<0.001$) after adjustment for the various confounding factors. In conclusion, FH patients have higher oxidative stress status which contributes to the greater risk of developing atherosclerosis and its related complications. LDL concentration is an independent determinant of ox-LDL, suggesting that both the proatherogenic quantity and quality of LDL coexist in FH which enhance the risk of premature CAD.

Keywords: Familial hypercholesterolaemia, atherosclerosis, F2-isoprostanes, malondialdehyde, oxidised low-density lipoprotein

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INTRODUCTION

Familial hypercholesterolaemia (FH) is a group of genetic disorders involving several genes including low-density lipoprotein receptor (*LDLR*), apolipoprotein B (*APOB*), proprotein convert asesubtilisin/kexin type 9 (*PCSK9*) and other possible genes yet to be described, resulting in severe elevations of blood cholesterol levels (Goldberg et al., 2011). The prevalence of homozygous FH is generally about 1:1 million (Soutar & Naoumova, 2007). However, heterozygous FH is a common genetic disorder occurring in one in every 300 to 500 people in most areas of the world (Hopkins, Toth, Ballantyne, & Rader, 2011). With an approximate population of 30 million in Malaysia, it is estimated that 60,000 individuals are affected and a majority of whom are under diagnosed (Alicezah et al., 2014).

FH is characterised by acute increase of low density lipoprotein (LDL) cholesterol and total cholesterol in the circulation, deposition of cholesterol in peripheral tissues, the presence of tendon xanthomata and accelerated atherosclerosis which leads to the increased risk of premature coronary artery disease (CAD) (Lye et al., 2013). The range of total cholesterol concentrations in heterozygous FH patients are typically in the range of 9.1 to 12.9 mmol/L and in a homozygous range from 16.8 to 25.9 mmol/L (Goldberg et al., 2011). There is evidence that other diseases with increased risk for developing atherosclerosis have also increased status of oxidative stress (Martinez-Hervas et al., 2008; Stephens et al., 2006)

As a result of elevated LDL cholesterol concentrations and its prolonged plasma half- life, it is possible that it will lead to increased susceptibility to free radical attack and oxidation. Hypercholesterolaemia is reported to be associated with enhanced oxidative stress, related to increased lipid peroxidation (Palombo, Lubrano, & Sampietro, 1999). Endothelial cells, smooth muscle cells, neutrophils and monocytes all have the potential to oxidatively modify the LDL, leading to the generation of lipid peroxidation products such as oxidised LDL (ox-LDL) and reactive oxygen species (Nourooz-Zadeh, Smith, & Betteridge, 2001).

Biomarkers of oxidative stress, are used to measure and evaluate products of lipid peroxidation such as Ox-LDL (Hulthe, Bokemark & Fagerberg, 2001; Stocker & Keaney, 2004), F2-Isoprostane (ISP) (de Faria et al., 2014; Vassalle, Petrozzi, Botto, Andreassi, & Zucchelli, 2004) and malondialdehyde (MDA) (Pirinccioglu, Gökalp, Pirinccioglu, Kizil, & Kizil, 2010). These biomarkers of oxidative stress have been used as indicators of normal biological or pathological process. Therefore, the objectives of this study were to compare the oxidative stress status between Familial Hypercholesterolaemia (FH) patients &normocholesterolaemic control (NC) subjects in a Malaysian cohort and to assess the independent predictors, association and correlation of these oxidative stress biomarkers in all subjects.

MATERIALS AND METHODS

This is a case control, observational study involving 98 FH patients and 100 normal control (NC) subjects conducted in a Specialist Lipid Clinic of teaching hospital. The FH subjects and NC in this Malaysian cohort were recruited from the specialist clinics and from various regions in Malaysia, which include the northern, southern and central region of West Malaysia as well as East Malaysia. All patients gave their written informed consent for participation in the study

and the Institutional Ethical Committee approval was obtained prior to the commencement of the study.

For each subject, a set of questionnaires were completed and relevant clinical data was obtained. All patients and control subjects were screened through a protocol consisting of medical history, physical examination, and laboratory tests including fasting glucose, serum lipids, renal profile, and liver function test. Anthropometry measurement including, body mass index (BMI), waist circumference (WC), smoking habits and history of personal CAD were measured and documented. BP was measured by an automated BP reader (Omron HEM-712C, Japan) with the subject in a seated position and after 5-10-minute rest. The systolic (SBP) and diastolic blood pressure (DBP) was measured to the nearest 1 mmHg. BMI was calculated using the formula: BMI = weight (kg)/height^2 (m^2). Waist circumference (WC) was measured to the nearest 0.5 cm using a measuring tape at midway between the inferior margin of the last rib and the iliac crest in a horizontal plane. Hip circumference measurement was taken from the pelvis at the point of maximal protrusion of the buttock (World Health Organization (WHO), 1995). The presence of CAD was assessed based on the subject family history of CAD, the personal clinical history, and previous medical records. Lipid stigmata such as corneal arcus, xanthelasma, achilles tendon xanthomata and tendon xanthomas were examined and documented.

Diagnosis of FH was made based on the Simon Broome's criteria (Goldberg et al., 2011). Patients with diabetes mellitus, renal, liver, thyroid diseases or any other causes of secondary hypercholesterolaemia (Stone, 1994), those with recent febrile illness, concomitant neoplasm, inflammatory disease or immunosuppressive therapy including steroid usage and those taking vitamin supplements were excluded from this study. Inclusion criteria for NC were TC level of < 6.5 and/or LDL-c < 4.0 mmol/L and those who did not fulfil Simon Broome's criteria for definite or possible FH, as well as clinically well with no chronic diseases or on any medications.

Twenty millilitres (ml) overnight (10-12 hours) fasting venous blood samples were collected into plain tubes of serum for routine biochemical analyses, which are fasting serum lipid (FSL), liver function tests (LFT), renal profile (RP) and Ox-LDL and ISP biomarkers. For the MDA TBARS assay, plasma EDTA tubes were used and for fasting blood glucose (FBG), fluoride tubes were used. Routine biochemical analyses that were performed on all subjects consisting of FSL, FPG, LFT and RP. Fasting serum lipids, FPG, LFT and RP were performed on an automated analyser (Cobas Integra 400, Germany) to identify all the health screening programmes participants that presented with exclusion criteria. All the blood samples were collected by non-traumatic venepuncture in the morning between 0800 and 1000 am. Serum was separated within 1 hour of collection, the biochemical analysis was performed within 24 hours and remaining samples were stored at -20°C until biomarkers analysis were performed within 3 months.

Sample size estimation was calculated using Open Epi software, within the power of study 80% and α-level was set at 0.05 of the difference in mean between two groups to have clinical importance and the standard deviation of lipid profile which were 0.8 and 1.3 mmol/L, respectively in study done by Khoo et al. (2000). At 95% confidence interval, a proposed minimal sample size was 84 for the lipid profile using the formula for case control studies (Charan & Biswas, 2013).

Ox-LDL concentrations in each subject were determined using oxidised LDL ELISA kit (Mercodia, Sweden). In the ELISA plate, 25 µL of calibrators, controls and serum samples were added, followed by 100 µL Assay Buffer. The plate then was incubated for 2 hours on a shaker at room temperature. Next, the plate was washed by 6 times and 100 µL enzyme conjugate solution were added. The plate then was incubated for 1 hour on a shaker at room temperature. Next, the plate was washed 6 times before 200 µL Substrate TMB were added. The plate then was again incubated 15 minutes at room temperature. Fifty microlitres of Stop Solution were added, and the plate wasshaken for approximately 5 seconds on shaker before absorbance reading using a spectrophotometer. The detection limit of the kit was <1 mU/l with absorbance reading of optimal density at 450 nm.

Plasma ISP levels were analysed by Liquid chromatography-tandem mass spectrometry (LCMSMS) which was performed using a Shimadzu LC system consisting of a binary pump (LC-20AD), an autosampler SIL-20AC (set at 4°C), a column oven (set at 40°C) and a system controller (CBM-20A) which were interfaced with 4000 QTRAPTM (Applied Biosystems, Canada). The procedure of LCMSMS and calibration curve and pre-treatment of samples were optimised by a technical representative from the manufacturer. The analyte was detected by Multiple Reaction Monitoring (MRM) mode and each analyte was monitored for two MRM transitions. Mass spectral data were analysed using Analyst 1.4.2 software. A six-point calibration curve was included with each assay using calibrator concentrations from 100, 250, 400, 500, 1000 and 10000 pg/mL which made up from 3 solutions; solution A = 1µg/mL (1µL reference standard + 999 µL water), solution B = 10 ng/mL (10 µL sol A + 990 µL water), and solution C = 1 ng/mL (100 µL sol B + 990 µL water). Limits of detection were between 116.40–10051.00 ng/mL. Accuracy, range were within 85.5–126%. Separation was performed on reversed phase C-18 ZorbaxZorbax Eclipse 0.5 µL × 150 mm × 4.6 mm I.D column from Agilent coupled to a triple quadrupole mass spectrometer running in negative electrospray ionisation mode with a constant flow rate of 0.5 mL/min was used to separate the analytes. The mobile phase consisted of two eluents, solvent A (water/acetonitrile at 98:2 by volume) and solvent B (100% methanol), both containing 0.01% ammonium hydroxide at pH of 9.5. The pre-treatment consists of liquid extraction of 450 µL serum with diethyl ether with subsequent evaporation with Nitrogen gas steam, followed by reconstitution in 1000 µL of water. Sample injection volume and total run time were 50 µL and 9 min respectively.

Malondialdehyde (MDA) was measured by the TBARS assay method adapted from (Ledwozyw, Michalak, Stepien, & Kadziolka, 1986). This assay was based on the reaction of a chromogenic reagent, 2-thiobarbituric acid, with MDA at 25°C. One molecule of MDA reacts with 2 molecules of 2-thiobarbituric acid via a Knoevenagel-type condensation to yield a pink chromophore the last product of fatty acid peroxidation in absorbance maximum at 532 nm. Sodium hydroxide, 1 1, 3, 3-tetraetoxypropane (TEP), n-butanol and acid trichloroacetic (TCA) were obtained from Sigma-Aldrich, USA. Hydrochloric acid (HCl), 2-thiobarbituric acid (TBA), Sodium chloride, Potassium dihydrogen phosphate and dipotassium hydrogen phosphate were purchased from Merck, Germany. For MDA standards preparation, 40.5 nmol/ml of MDA stock solution was prepared by the addition of 0.01 ml origin standard stock with 1 litre of distilled water. Then, a series of 9 different concentrations (0.2, 0.4, 0.8, 1.0, 5.0,

10.0, 15.0 and 20.0 nmol/ml) of MDA standards were prepared. An amount of 0.5 ml from each MDA standard was added to 2.5 ml 1.22M TCA in 0.06 M HCl in the test tubes which were then incubated at a room temperature for 15 minutes for protein precipitation. 1.5 ml 0.67% TBA was added into each test tube. The mixture was further heated in a boiling (100ºC) water bath for 30 minutes. After cooling to room temperature, 4 ml of n-butanol was added. The mixture was then shaken for 2 minutes with vortex and centrifuged at 3000 rpm for 10 minutes. The top layer of butanol was pipette in a cuvette and the absorbance was measured at 532 nm. A standard curve graph was plotted after all MDA absorbance were obtained. For MDA measurement of plasma samples, a volume of 0.1 ml plasma samples was diluted with 0.4 ml distilled water. The same procedure as standard curve preparation was used on all diluted plasma samples. Protein concentrations in each plasma samples were measured by Cobas Integra 400, Roche Diagnostics, Germany). The result was obtained using MDA standard curve graph which has been plotted before.

Demographic variables are presented as the mean ± standard deviation (SD) for continuous normally distributed variables, as mean + standard error mean (SEM) for continuous non-normally distributed data, and as percentages for categorical data. Analysis of normality was performed with the Kolmogorov–Smirnov test. Categorical data and proportions were analysed using Chi-square test. Pearson's or Spearman's correlation coefficient was used for analysing the correlation between two variables with normal distribution or non-normal distribution, respectively. A P-value <0.05 was considered statistically significant. The statistical analysis was performed with the Statistical Package for Social Sciences (SPSS version 16.0) software licensed by UiTM.

RESULTS

A total of 99 FH patients from 67 different families (age mean ± SD: 43.9 ± 12.2 years, gender: 38 males and 61 females) and 99 control subjects (age mean ± SD: 44.7 ± 12.2 years, 40 males and 59 females), were recruited into this study. The FH patients (0.84± 0.07) had higher waist-to-hip ratio (WHR) as compared to NC (0.84±0.07) vs (0.82 ± 0.06) (P<0.05).

The levels of TC, TG and LDL-c were significantly elevated in FH patients in comparison with control subjects (8.6 ± 1.7 mmol/L vs 5.4 ± 1.0 mmol/L, 2.0 ± 1.7 mmol/L vs 1.3 ± 0.6 mmol/L, 6.4 ± 1.6 mmol/L vs 3.4 ± 0.9 mmol/L, respectively, P<0.0001). The baseline characteristics of FH patients and control subjects are presented in Table 1.

There were significantly higher ox-LDL, ISP and MDA concentrations in FH compared to controls (63.0 + 6.5 vs 25.5 ± 1.2; p<0.001; 749.7 ± 74.0 vs 354.2 ± 18.1; p<0.001; 342.4 ± 46.7 vs162.7 + 13.5 nmol/g, respectively, p<0.0001) (Figure. 1).

Chi square analysis showed significant association between Ox-LDL with WC (p<0.03), WHR (p<0.05), BMI (p<0.01), Systolic BP (p<0.01), TC (p<0.0001), TG (p<0.05), LDL-c (p<0.0001) and smoking status (p<0.05). F2-Isoprostane was positively associated with WC (p<0.01), TC (p<0.0001), TG (p<0.05), LDL-c (p<0.0001) and smoking status (p<0.01) while MDA was significantly associated with glucose (p<0.01), TC (p<0.0001), TG (p<0.05) and LDL-c (p<0.0001) (Table 2).

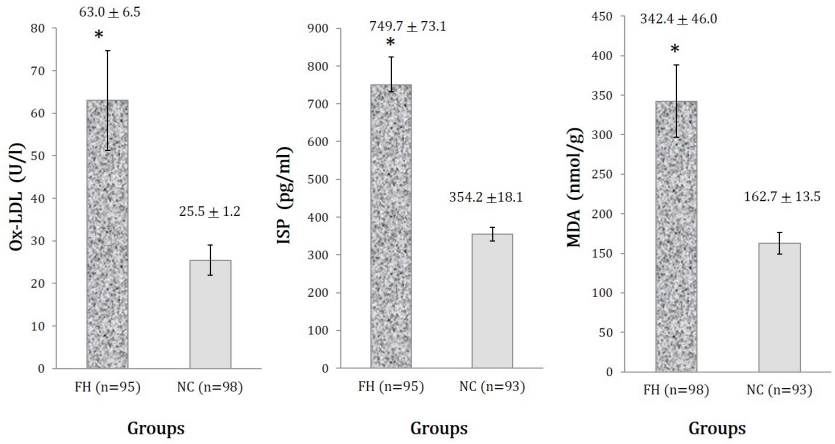

Figure 1. Comparison of Oxidative stress biomarkers concentrations in FH patients and NC subjects. Data were expressed in mean ± SEM.*P<0.001 compared to controls. Ox-LDL: oxidized LDL, ISP: F2-Isoprostane, MDA: Malondealdehyde

Table 1
Clinical and biochemical characteristics of FH patients and controls

Baseline Characteristics	FH (n=98)	Normal controls (NC) (n=100)	P value
[1]Age (years)	44.2 + 12.2	44.7 + 12.2	NS
[2]Gender (%)			
Males	38 (38.3)	40 (40.4)	NS
Females	61 (61.6)	59 (59.5)	
[2]Ethnicity (%)			
Malays	71 (72.4)	91 (91.0)	<0.005
Chinese	26 (26.5)	7 (7.0)	
Indians	1 (1.0)	2 (2.0)	
[2]Current smoker (%)	13(13.1)	17 (17.0)	NS
[1]SBP (mmHg)	133.9 + 21.6	121.2 + 15.6	NS
[1]DBP (mmHg)	76.7 + 10.9	73.0 + 10.3	NS
[2]Hypertension (%)	20 (20.4)	1 (1.0)	<0.05
[2]CAD (%)	20 (20.4)	0 (0)	<0.001
[1]BMI (kg/m^2)	24.5+ 4.7	23.5+ 4.8	NS
[1]WHR (cm^2)	0.84+ 0.07	0.82 + 0.06	<0.05
[1]WC (cm)	84.2 + 11.2	77.6 + 11.1	<0.001
[2]Central Obesity (%)	45 (45.9)	27(27.0)	<0.005
[1]TC (mmol/L)	8.6 + 1.7*	5.4 + 1.0	<0.001
[1]TG (mmol/L)	2.0+ 1.7*	1.3 + 0.6	<0.001
[1]LDL-c (mmol. L)	6.4 + 1.6*	3.4 + 0.9	<0.001
[2]HDL-c (mmol/L)	1.1 + 0.3	1.4 + 0.3	NS
[1]Glucose (mmol/L)	6.2 + 9.0	4.8 + 1.1	<0.01

[1]Data expressed as mean + SD; [2]Data expressed as a proportion (%); NS: Not Significant. SBP: Systolic Blood pressure; DBP: Diastolic Blood Pressure; BMI: Body mass index; WHR: Waist-hip ratio; WC: waist circumference; CAD: Personal History of Coronary Artery Disease

Table 2
Summary of the association between oxidative stress biomarkers and various parameters

Parameters	[1]Ox-LDL (U/L)	[1]F_2-Isoprostane (pg/ml)	[1]MDA (nmol/g)
WC (cm)	p<0.03	p<0.01	NS
WHR (cm^2)	p<0.05	NS	NS
BMI (kg/m^2)	p<0.01	NS	NS
SBP (mmHg)	p<0.01	NS	NS
DBP (mmHg)	NS	NS	NS
Glucose (mmol/L)	NS	NS	p<0.01
TC (mmol/L)	p<0.0001	p<0.0001	p<0.0001
TG (mmol/L)	p<0.05	p<0.05	p<0.05
LDL-c (mmol.L)	p<0.0001	p<0.0001	p<0.0001
HDL-c (mmol/L)	NS	NS	NS
Smoking (yes/no)	p<0.05	p<0.01	NS
CAD (yes/no)	NS	NS	NS

[1]Data are expressed as a p-value of the chi-square test. NS: Not Significant. WC: waist circumference; WHR: Waist-hip ratio; BMI: Body mass index; SBP: Systolic Blood pressure; DBP: Diastolic Blood Pressure; CHD: Personal History of Coronary Artery Disease; ox-LDL: oxidised LDL; MDA: malondialdehyde

Pearson's correlation analysis showed a positive correlation of glucose (p<0.05), TC (p<0.001) and LDL-c 9 (p<0.001) and HDL-c (p<0.01) with ox-LDL. F2-Isoprostane showed significant correlation with TC (p<0.001) and LDL-c (p<0.001) while MDA was significantly correlated with diastolic BP (p<0.05) and LDL-c (p<0.05) (Table 3).

Table 3
Correlation between oxidative stress biomarkers with parameters in all subjects

Parameters	Ox-LDL (U/L)	F_2-Isoprostane (pg/ml)*	MDA (nmol/g)
WC (cm)	0.123	0.151	0.036
WHR (cm^2)	0.135	0.054	0.87
BMI (kg/m^2)	0.994	0.126	0.098
SBP (mmHg)	0.085	0.105	0.027
DBP (mmHg)	0.124	0.049	0.167*
Glucose (mmol/L)	0.219*	0.128	0.081
TC (mmol/L)	0.552***	0.459***	0.133
TG (mmol/L)	0.130	0.100	0.051
LDL-c (mmol.L)	0.598***	0.492***	0.150*
HDL-c (mmol/L)	-0.208**	-0.164	-0.133

Data are expressed in Pearson's correlation coefficient (r); *p<0.05, **p<0.01, ***p<0.001. WC: waist circumference; WHR: Waist-hip ratio; BMI: Body mass index; SBP: Systolic Blood pressure; DBP: Diastolic Blood Pressure; ox-LDL: oxidised LDL; MDA: malondialdehyde

Multiple linear regression analysis was performed in all subjects to further explore the independent effect of parameters with the biomarkers as dependent variables. LDL-c was shown to be an independent predictor for ox-LDL concentration ($p<0.0001$) after correction for various confounding factors i.e. age, gender, ethnicity, smoking status, BMI, WC, WHR, SBP, DBP, glucose, TC, TG, HDL and LDL (Table 4).

Table 4
Independent predictor of oxidative stress biomarkers in all subjects

Dependent variable	Independent variable	Constant	B	SE	Adjusted OR	95% CI	p-value
Ox-LDL (U/l)	LDL-c	-5.059	0.598	0.991	9.861	7.815, 11.726	0.0001*

Ox-LDL: oxidised LDL; LDL-c: Low Density Lipoprotein cholesterol

DISCUSSION

The purpose of this study was to compare the oxidative stress status between FH and NC subjects in the Malaysian cohort and assess the association and predictability of the oxidative stress biomarkers to ox-LDL level. It was hypothesised that FH patients have higher oxidative stress level as compared to NC subjects due to hypercholesterolaemia. Our case-control observational study demonstrated higher concentration of these oxidative stress biomarkers in FH patients than NC. A similar finding was observed in a case-control study among Greeks on oxidative stress biomarkers which showed greater ox-LDL compared to normolipidemic healthy control (Tsouli et al., 2006). Nourooz-Zadeh et al also illustrated that ISP concentration was greater in FH patients than in healthy controls amongst the British (Nourooz-Zadeh et al., 2001). Subsequently, the finding and consistent with the study of FH and MDA amongst the Turks where MDA was found to be significantly higher in patients with homozygous FH than in heterozygotes group and control (Pirinccioglu et al., 2010). However, these studies only recruited a small number of subjects ranging from 11 to 80 persons per group. Our study included a great number of FH subjects and NC clearly showed significant elevation of all three oxidative stress biomarkers, indicating that FH patients have enhanced oxidative stress status which is one of the key features in the pathogenesis of atherogenesis.

This present study clearly showed highly significant associations between the oxidative stress biomarkers with total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-c), while LDL-c were significantly correlated with all the oxidative stress biomarkers. These findings are parallel with previous reports, which revealed a strong correlation and association between ox-LDL and coronary artery disease (CAD) and concluded that circulating ox-LDL is a sensitive biomarker of CAD (Holvoet et al., 2001). Although the finding was consistent with the previous study where the correlation was seen between ISP and LDL-c and the association between ISP and serum cholesterol level were observed in FH patients, no correlation and association were previously observed in the healthy control group, possibly due to the limited numbers of their subject examined (n=25). For MDA, our finding was similar to Pirinccioglu et al, where MDA was positively correlated with LDL-c and TC suggesting enhanced oxidative

stress status which is important in the understanding of enhanced atherosclerogenesis in FH and related to their increased risk of developing atherosclerosis-related complications (Pirinccioglu et al., 2010).

Furthermore, in this present study, LDL-c was strongly shown to be an independent predictor for ox-LDL concentration ($p<0.0001$) after correcting for the various confounding factors. The current finding appears to support the previous literature, where ox-LDL was an independent predictor of developing cardiac events (CE) in patients with CAD suggesting measurement of circulating oxLDL may be helpful in the assessment of future CE in patients with CAD (Shimada et al., 2004). In addition, the concentration of LDL is an independent predictor factor of ox-LDL even after correcting for the various confounding factors suggests that both the proatherogenic quantity and quality coexist in FH which is pivotal in the pathogenesis of premature atherosclerosis-related conditions, especially CAD.

It has been well-established that patients with hypercholesterolaemia have an increased risk for atherosclerosis. Ox-LDL is the most used biomarkers in assessing status of oxidative stress in atherosclerotic patients (Garrido-Urbani, Meguenani, Montecucco, & Imhof, 2014) and elevated ox-LDL also was associated with hypercholesterolaemia in general (Garrido-Urbani et al., 2014) as well as many populations (Li, Yang, & Mehta, 1998; Najafi & Alipoor, 2013; Rodenburg et al., 2006; Tsouli et al., 2006; Tsutsui et al., 2002). Morrow (2005) mentioned that ISP represents a biomarker that has the potential to be a great importance in the assessment of human atherosclerotic cardiovascular disease (Morrow, 2005). Isoprostane and also MDA were the two of the most well-studied markers of lipid peroxidation in CAD and CVD (Ho, Karimi Galougahi, Liu, Bhindi, & Figtree, 2013). Since oxidative stress was associated with hypercholesterolaemia, and FH patients have severely elevated cholesterol in their blood, it is quite surprising that studies on these oxidative stress biomarkers in FH have been scarce.

It is important to note that all subjects included in this present study were not subjected to therapeutic intervention with lipid-lowering, antihypertensive and/or antidiabetic medications which are potential confounding factors in oxidative stress.

Although there have been several reports on FH and oxidative stress biomarkers from various countries, the data has been limited and most studies were mostly among the European cohort (Cracowski et al., 2001; Davì, Falco, & Patrono, 2004; Nourooz-Zadeh et al., 2001; Pirinccioglu et al., 2010). Therefore, this present study may fill in the gap and to the best of our knowledge, this is the first report on the association of oxidative stress biomarkers in clinicallydiagnosed FH patients of Asian origin. There have been different population studies on hypercholesterolemic subjects but very scarce, specifically in subjects with clinically -diagnosed FH.

Among the scarce studies relating oxidative stress and FH subjects, most have reported an enhanced oxidative stress status in FH compared to normal controls. The samples used were small between 30 to 80 samples and only covering American and European cohorts (Nourooz-Zadeh et al., 2001; Pirinccioglu et al., 2010; Real et al., 2010; Reilly et al., 1998; Rodenburg et al., 2006). The only previously reported study for an Asian population was reported by Nawawi et al. (2003). This present study may enhance our understanding of oxidative stress in FH patients within the Asian populations which has a different pattern of confounding factors and genetic make-up.

CONCLUSION

In conclusion, FH patients have enhanced oxidative stress status, which in part, may contribute to increased risk of atherosclerosis and its related complications, especially CAD. The concentration of LDL is an independent predictor of ox-LDL even after the confounding factors are removed, suggesting that both quantity and quality of proatherogenic LDL coexist in FH and possibly synergize each other in the pathogenesis of atherosclerosis. There is a potential role for these oxidative stress biomarkers to be used in risk assessment and monitoring of disease progression among individuals with FH. Hence, future studies are required to assess the role of these oxidative stress biomarkers in predicting the risk of coronary events in FH.

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