Original Article
Alterations and diagnosis potential of serum lipid profiles in rheumatoid arthritis patients

Shicheng Guo[1,2,4*], Rongsheng Wang[4*], Ting Jiang[4], Fengmin Bai[4], Qin Ding[4], Yanyun Ma[1], Yan Wang[5], Yajun Yang[1,3], Xiaofeng Wang[1,3], Lixing Tan[1], Li Jin[1,3], Hong Nie[6], Jiucun Wang[1,2,3], Dongyi He[4]

[1]State Key Laboratory of Genetic Engineering and Ministry of Education Key Laboratory of Contemporary Anthropology, Collaborative Innovation Center for Genetics and Development, School of Life Sciences, Jiangwan Campus, Fudan University, Shanghai 200438, China; [2]Institute of Rheumatology, Immunology and Allergy, Fudan University, Shanghai 200040, China; [3]Fudan-Taizhou Institute of Health Sciences, Taizhou, Jiangsu 225300, China; [4]Department of Rheumatology, Guanghua Integrative Medicine Hospital, Shanghai 200052, China; [5]Department of Microbiology, School of Medicine, University of Pennsylvania, 415 Curie Blvd, Philadelphia 19104, USA; [6]Shanghai Institute of Immunology, Shanghai Jiaotong University (SJTU) School of Medicine, Shanghai 200025, China. *Equal contributors.

Abstract: The aim of the study is to compare the serum lipid profiles of rheumatoid arthritis (RA) patients and healthy volunteers and find the association between the serum lipid profiles and RA in Chinese Han population. Blood samples were collected from 2001 patients with early diagnostic RA and 3883 matched healthy controls for their serum lipid profiles including total-cholesterol (T-CHOL), triglyceride (TG), high density lipoprotein-cholesterol (HDL-C) and low density lipoprotein-cholesterol (LDL-C). Associations between RA and alterations of the lipid profiles were estimated with univariable and multivariable logistic regression. T-CHOL (OR = 0.54, 95% CI: 0.50-0.58), TG (OR = 0.4, 95% CI: 0.37-0.44) and HDL-C (OR = 0.01, 95% CI: 0.01-0.01) were significant protective factors, while LDL-C (OR = 5.58, 95% CI: 4.95-6.29) was a significant risk factor to RA. The results from Random Forest revealed that serum lipid profiles have powerful prediction ability to distinguish RA from normal controls with sensitivity of 94.7%, specificity of 90.4% and accuracy of 93.2%, respectively. Risk discriminatory model based on T-CHOL, TG, HDL-C, LDL-C, gender and age in logistic regression further showed strong prediction ability of serum lipid profiles in RA with the area under the curve (AUC) of receiver operating characteristic (ROC) = 0.972 (95% CI: 0.968-0.976). Serum lipid profiles were significantly associated with rheumatoid arthritis and have important implication in RA auxiliary diagnosis.

Keywords: Serum lipid profiles, rheumatoid arthritis, prediction, random forest, diagnosis, rheumatoid factors

Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease characterized by inflammation, symmetric polyarthritis in peripheral joints. RA affects nearly about 1% of the population based on the 1987 American College of Rheumatology criteria [1]. However, the etiology and susceptible factors were not clear yet. Compared with other autoimmune diseases such as psoriasis, Crohn's disease, Type I diabetes, lupus and multiple sclerosis, RA had higher incidence. The incidences of rheumatoid arthritis were dramatically different among different ethnics, such as 0.0158% in Taiwan

(2013) and 0.048% in Italy (2014). Empirically, RA primarily affects joints. However, it also affects other organs in 15-25% of patients. Patients with RA are at increased risk for several disorders, includingcancer, infection andcardiovascular disease (CVD). The severe disability, grave financial and medical costs make RA become one of most substantial burdensfor both people with RA and health services.

The present diagnostic protocol of RA is complicated and time-consuming. The diagnostic pipeline is based on the clinical presentation and serologic tests, while there is no singular test for it yet. Ultimately, rheumatoid arthritis is

diagnosed based on a combination of the presentation of the joints involved, characteristic joint swelling and stiffness in the morning, the presence of blood rheumatoid factor (RF) and citrulline antibody, as well as the findings of rheumatoid nodules and radiographic changes (X-ray testing) [2]. Early detection of the disease and commencement of disease-modifying anti-rheumatic drugs (DMARDs), within 12 weeks of symptom onset can bring better disease outcome, less disability, less joint damage and fewer complications. However, the current diagnostic pipeline is insufficient in the early diagnosis because of the complicated protocol. More diagnostic schemes should be introduced to diversify the diagnosis approach for RA. The present study was designed to evaluate the role of lipid profiles in early diagnosis of the rheumatoid arthritis.

Lipid profile test is a routine test for the patients with autoimmune and inflammatory diseases, and several pieces of evidences have shown that active early rheumatoid arthritis is associated with dyslipidemia [3]. In addition, high-density lipoprotein cholesterol (HDL-C) was decreased in the serum of RA patients, and a pro-atherogenic lipid profile has been reported in patients with active and untreated RA [4, 5]. However, the auxiliary diagnostic role of the serum lipid profiles in RA has not been studied before.

In the present study, the lipid profiles which included total-cholesterol (T-CHOL), triglyceride [6], high density lipoprotein-cholesterol (HDL-C) and low density lipoprotein-cholesterol (LDL-C) were collected from 2001 cases with RA and 3883 ethnically matched healthy controls. The associations of lipid profiles with RA patients were investigated and the diagnostic role of serum lipid profiles in RA was evaluated with Random Forest and multivariable logistic prediction model.

Materials and methods

Material and samples

A total of 5884 subjects were enrolled including 2001 cases with RA without any therapy of anti-rheumatic drugs and 3883 ethnically matched healthy controls from Guanghua hospital and Taizhou longitudinal cohort [7] during the period of May 2009-April 2014 with the com-

pleted written informed consent. The study was granted with permission No2013-K-02 by Guanghua Integrative Medicine Hospital, Changning District, Shanghai, China. The individuals were excluded from the study if they have been diagnosed for any other autoimmune inflammatory disease at any time during the above period. The research was approved by both the academic advisory board of Guanghua Hospital and the School of Life Sciences, Fudan University, Shanghai.

Lipid profile detection

Blood samples were obtained from patients before surgical treatment or endovascular management as well as other medicine intervention. Five milliliters of antecubital venous fasting blood was collected from each of the subjects, being allowed to clot and then centrifuged at 3,000 rpm for 15 min within 30 min of sample collection and analyzed within 6 h after the separation. The serum lipid profiles, including T-CHOL, HDL-C, LDL-C, and TG, was measured with the FAITH-1000 automaticbiochemistry-analyzer, according to the manufacturer's instructions (LaoLa Electronic Co., Ltd, Nanjing, China). In addition, ESR (*erythrocyte sedimentation rate*), CRP (*C-reactive protein*), APOA1 (apolipoprotein A-I), APOB (apolipoprotein B), APOE (apolipoprotein E), LPA (Lysophosphatidic acid), IgA, IgG and IgM antiglobulins of RF were measured as our previous work [8]in the RA patients to evaluate the correlation between these factors with lipid profile. Inverse normal transformation were applied to the data toimprove the *normality* of variablesin the process of correlation analysis.

Statistical analysis

All data were analyzed using R. The distribution characteristics of the lipid profiles were expressed as median and interquartile range (IQR). A value of $P < 0.05$ was considered significant. Univariable binary regression was applied to discover the association of lipid proteins with RA one by one adjusted with gender and age. Multivariable binary regression was used to adjust all the confounders and to increase the efficiency of the estimators (adjusted by each other). Odds ratios (ORs) and 95% confidence interval (CIs) were calculated with R code. Correlation between lipid proteins and gener/age were conducted with correlation

Serum lipid profiles in rheumatoid arthritis patients

Table 1. Correlation between lipid profiles and clinical characteristics of RA patients

	ESR (Beta, P)	CRP (Beta, P)	apoA1 (Beta, P)	apoB (Beta, P)	LPA (Beta, P)	apoE (Beta, P)	IgmRF (Beta, P)	IgaRF (Beta, P)	IggRF (Beta, P)
CHOL	-0.22 (1.34E-024)	-0.24 (2.03E-029)	0.44 (2.58E-091)	0.66 (3.25E-255)	0.13 (7.69E-10)	0.41 (2.12E-085)	-0.01 (0.671)	-0.04 (0.0698)	0 (0.845)
TG	-0.18 (2.01E-015)	-0.2 (1.64E-019)	0.06 (0.00988)	0.48 (1.2E-107)	-0.06 (0.0086)	0.35 (3.03E-057)	-0.06 (0.00839)	-0.05 (0.0521)	-0.02 (0.46)
HDL	-0.26 (5.17E-034)	-0.22 (7.24E-026)	0.72 (1.12E-055)	0.04 (0.0657)	0.02 (0.411)	0.21 (1.48E-021)	-0.04 (0.119)	-0.05 (0.0386)	-0.01 (0.659)
LDL	-0.16 (1.48E-013)	-0.22 (1.8E-023)	0.28 (5.25E-034)	0.73 (7.35E-055)	0.17 (1.95E-015)	0.32 (2.4E-048)	0 (0.839)	-0.03 (0.19)	-0.01 (0.733)

Table 2. Association of lipid profiles with rheumatoid arthritis

	RA (IQR)	Normal (IQR)	Odds Ratio[1] (95% CI)	*P*-value[1]	Odds Ratio[2] (95% CI)	*P*-value[2]
T-CHOL	4.11 (1.19)	4.51 (1.20)	0.54 (0.50-0.58)	7.9×10^{-71}	0.06 (0.05-0.08)	2.41×10^{-130}
TG	1.07 (0.61)	1.55 (1.22)	0.4 (0.37-0.44)	1.27×10^{-85}	0.27 (0.23-0.31)	2.66×10^{-64}
HDL-C	1.12 (0.41)	1.59 (0.46)	0.01 (0.01-0.01)	6.65×10^{-256}	0.05 (0.04-0.0.07)	3.86×10^{-56}
LDL-C	2.22 (0.91)	1.61 (0.67)	5.58 (4.95-6.29)	2.52×10^{-174}	194.7 (128.2-252.8)	3.79×10^{-184}

Odds Ratio[1] and *P*-value[1] were derived from univariable logistic regression adjusted with gender and age. Odds Ratio[2] and *P*-value[2] were derived from multivariable logistic regression adjusted with gender and age. IQR: interquartile range.

Table 3. Performance of the prediction model with different features

	AUC[1] (95% CI)	AUC[2] (95% CI)	AUC[3] (95% CI)	Features
Model A	0.674 (0.659-0.688)	0.631 (0.613-0.648)	0.676 (0.645-0.707)	Gender and Age
Model B	0.734 (0.721-0.747)	0.705 (0.689-0.721)	0.737 (0.709-0.766)	T-CHOL, Gender and Age
Model C	0.779 (0.766-0.791)	0.753 (0.737-0.768)	0.795 (0.769-0.821)	TG, T-CHOL, Gender and Age
Model D	0.915 (0.908-0.923)	0.898 (0.888-0.908)	0.947 (0.934-0.961)	HDL-C, TG, T-CHOL, Gender and Age
Model E	0.965 (0.961-0.970)	0.965 (0.960-0.971)	0.961 (0.951-0.972)	LDL-C, TG, T-CHOL, Gender and Age
Model F	0.972 (0.968-0.976)	0.969 (0.964-0.974)	0.976 (0.968-0.985)	LDL-C, HDL-C, TG, T-CHOL, Gender and Age

Prediction model were trained with logistic regression. AUC[1] was constructed with total dataset, AUC[2] was constructed with data from female, AUC[3] was constructed with data from data of male.

analysis. R packages "PredictABEL" and "pROC" was used for ROC construction.

Results

Characteristics of lipid profiles of rheumatoid arthritis and normal individuals

The levels of T-CHOL, TG, HDL-C and LDL-C (median ± IQR) in rheumatoid arthritis group were 4.11 (1.19), 1.07 (0.61), 1.12 (0.41) and 2.22 (0.91), while in control group were 4.51 (1.20), 1.55 (1.22), 1.59 (0.46) and 1.61 (0.67), respectively. The lipid profiles of RA patients and normal individuals changed along with age. Additionally, the profiles were also different between male group and female group. In detail, T-CHOL (r = 0.16, *P*-value = 9.84×10^{-14} in RA; r = 0.13, *P*-value = 1.33×10^{-15} in normal) and LDL-C (r = 0.13, *P*-value = 1.38×10^{-9} in RA; r = 0.14, *P*-value < 10^{-19} in normal) were significantly positive correlated with age in both RA patient group and normal group. T-CHOL (r = -0.14, *P*-value = 2.51×10^{-10} in RA vs r = -0.06, P *P*-value = 6.01×10^{-5} in normal) and HDL-C (r = -0.22, *P*-value < 10^{-17} in RA vs r = -0.14, *P*-value < 10^{-17} in normal) were significant negatively correlated with gender in both RA and normal group, indicating higher T-CHOL and HDL-C level in female populations (**Table 1**). On the other side, correlation analysis within the patient group showed that both ESR and CRP were significantly negatively correlated with each elements of lipid profiles (P<1.48×10-13, 1.64×10-19) while APOA1, APOB, APOE were significantly postively correlated with each elements of lipid profiles (P < 0.009, 0.006, 1.48×10-21, respectively). However, in the correlation analysis between lipid profile and anti-globulins of RF, only IgM and IgA were found to be faintly significantly correlated with TG and HDL (P = 0.008 and 0.03, respectively), indicating lipid profile might be an independent biomarker for the diagnosis of RA patient (**Table 1**).

Association of aberrant serum lipid profiles with rheumatoid arthritis

The comparison of lipid profiles between normal controls and rheumatoid arthritis are shown in **Table 2**. We found T-CHOL (OR = 0.54, 95% CI: 0.50-0.58, *P*-value = 7.9×10^{-71}), TG (OR = 0.4, 95% CI: 0.37-0.44, *P*-value = 1.27×10^{-85}) and HDL-C (OR = 0.01, 95% CI: 0.01-0.01,

P-value = 6.65×10^{-256}) were significant protective factors to RA while LDL-C was a significant risk factor to RA in both univariate logistic models adjusted with gender and age. Multivariate logistic regression model with gender, age, T-CHOL, TG, HDL-C and LDL-C showed all of the four lipid factors were significantly associated with RA, which suggested they are independent risk/predictive factors (**Table 2**). Atherogenic index of serum (AIP) which is noted as log (TG/HDL-C) was calculated and the association between AIP and the presence of RA was analyzed by binary logistic regression adjusted with age and gender. The result showed AIP was significantly associated with RA with OR of 30.77 (95% CI: 23.79-39.8) and *P*-value of 4.15×10^{-150}.

Performance of the Prediction model based on lipid profiles

Random forest algorithm was used to distinguish RA from normal samples with the features of age and gender and lipid profiles. Five-fold cross-validation and 1000 iteration were performed in the model construction to evaluate the stability of the prediction model. The result showed the average sensitivity (Sen), Specificity (Spe) and accuracy (Acc) were 94.7%, 90.4% and 93.2%, respectively. In the prediction model, HDL-C, LDL-C, TG, T-CHOL and age have effective contribution of 49.18%, 25.78%, 12.86%, 9.76 and 2.41%, respectively. In the logistic regression analysis, six scenarios with different features were considered (showed as **Table 3**). The most power prediction model was based on age, gender, HDL-C, LDL-C, TG and T-CHOL, in which the AUC = 0.972, and 95% CI ranged from 0.968 to 0.976. The power of the prediction models were also evaluated in male and female population. No significant difference was found between male and female population for the prediction model, suggesting the applicability of the model in general population (**Figure 1**).

Discussion

Our present study showed that the lipid profiles were significantly associated with the susceptibility of rheumatoid arthritis. This lipid profiles is characterized by higher LDL-C and lower TG, T-CHOL and HDL-C levels. Both Random Forest and logistic regression model showed powerful prediction ability in distinguishing rheumatoid
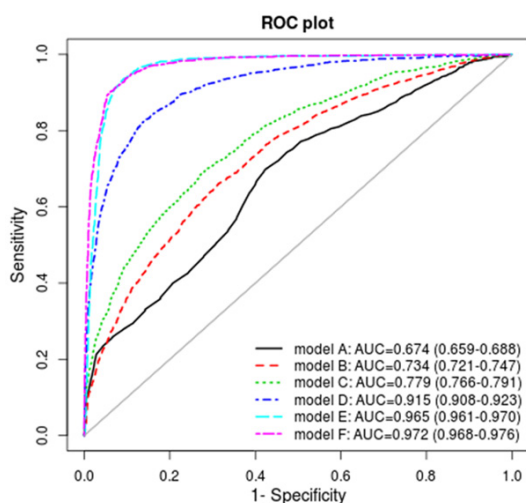
Figure 1. Performance of six prediction model showed with receiver-operation-curve. Model A included feature of gender, age. Model B included the features of gender, age and T-CHOL. Model C included features of gender, age, T-CHOL and TG. Model D features of gender, age, T-CHOL, TG and HDL-C. Model E features of gender, age, T-CHOL, TG, LDL-C. Model F features of gender, age, T-CHOL, TG, HDL-C and LDL-C.

arthritis from normal samples. Therefore, alteration in serum lipid profiles may have a diagnostic role in the future and can be used as a biochemical indicator for the real-time surveillance of rheumatoid arthritis.

The previous reports on T-CHOL and LDL-C levels in RA patients are conflicting: some studies found similar [4] or lower [9] levels of TC, while others showed increased levels of TC and LDL-C in patients with early RA [10]. This conflict might be the bias caused by small sample size in each study. Our results revealed that the levels of TG, T-CHOL and HDL-C were significantly lower in RA patients than those in normals, while LDL-C was significantly higher in RA patients than that in normals. Atherogenic index of serum (AIP) was significantly higher in patients than that in controls, indicating a more atherogenic lipid profile in rheumatoid arthritis, which is consistent with the previous reports [11].

The cause or consequence of abnormal serum lipid profile in rheumatoid arthritis was not fully defined yet [12]. Treatment of inflammation in RA patients which affecting the levels of lipid profile [13] indicated that aberrant lipid profile in RA might be caused by inflammation.

However, RA susceptibility genes, such as *CTLA-4*, *IL-10*, *PTPN22*, *REL*, *STAT4*, *TNF* and *TRAF1* were found to be significantly associated with lipid profiles in RA patients [14]. Additionally, lipid profile change seemed to be an early event in the process of rheumatoid arthritis [15]. These evidences revealed that lipid profile aberrant play an important role in the development of rheumatoid arthritis. Serum lipids level represents the physiological and pathological status, and comprehensive analysis on the serum lipids profiles could provide abundant information for individuals. It was demonstrated from the present study that serum lipid profiles could be taken as an important auxiliary tool to the diagnosis of rheumatoid arthritis.

Many evidences have showed lipid profile was associated with a number of diseases. The enhanced TG, LDL-C and VLDL-C and decreased HDL-C value increased the risk of coronary heart disease [16], HDL-C level have been shown to be higher in the subject with extensive mammographic dysplasia and family history of breast cancer. In addition, lipid profile has been proposed to be associated with type 2 diabetes, lupus and multiple sclerosis. Furthermore, lipid profiles would also vary in the different conditions which include lipid-lowering medication use, TNFi therapy, age, gender, physical activity [17]. Therefore, comprehensive serum lipid profile determination would be great benefit for the diagnosis or real-time surveillance for the common autoimmune disease. In the next step, we will collect more and more lipid profile data from multiple autoimmune diseases and cancers and apply multi-class prediction analysis to check whether the lipid profile could distinguish multiple classes with high sensitivity, specificity, accuracy and efficiency.

In summary, the present study demonstrated that the lipid profile of rheumatoid arthritis is characterized by higher LDL-C and lower TG, T-CHOL and HDL-C levels in a large Chinese Han population. Regardless the mechanism, serum lipid profile can be taken as an important auxiliary diagnosis method to rheumatoid arthritis.

Lipid profiles are significantly associated with rheumatoid arthritis (RA). T-CHOL, TG and HDL were significant protective factors, while LDL was a significant risk factor to RA. No signifi-

cant correlation between lipid profiles and RF. Lipid profile might be an independent biomarker in RA patient diagnosis.

Acknowledgements

Disclosure of conflict of interest

None.

Address correspondence to: Dongyi He, Department of Rheumatology, Guanghua Integrative Medicine Hospital, 540 Xinhua Road, Changning District, Shanghai 200052, China. Tel: +86-21-55664885; Fax: +86-21-55664885; E-mail: hedongyish@163.com; Jiucun Wang, National Ministry of Education Key Laboratory of Contemporary Anthropology, School of Life Sciences, Fudan University, 2005 Songhu Road, Shanghai 200433, China. Tel: +86-21-65642801; Fax: +86-21-65642801; E-mail: theresajcwang@gmail.com

References

[1] Alamanos Y, Voulgari PV and Drosos AA. Incidence and prevalence of rheumatoid arthritis, based on the 1987 American College of Rheumatology criteria: a systematic review. Semin Arthritis Rheum 2006; 36: 182-188.

[2] Cornec D, Varache S, Morvan J, Devauchelle-Pensec V, Berthelot JM, Le Henaff-Bourhis C, Hoang S, Martin A, Chales G, Jousse-Joulin S and Saraux A. Comparison of ACR 1987 and ACR/EULAR 2010 criteria for predicting a 10-year diagnosis of rheumatoid arthritis. Joint Bone Spine 2012; 79: 581-585.

[3] Situnayake RD and Kitas G. Dyslipidemia and rheumatoid arthritis. Ann Rheum Dis 1997; 56: 341-342.

[4] Park YB, Lee SK, Lee WK, Suh CH, Lee CW, Lee CH, Song CH and Lee J. Lipid profiles in untreated patients with rheumatoid arthritis. J Rheumatol 1999; 26: 1701-1704.

[5] Choi HK and Seeger JD. Lipid profiles among US elderly with untreated rheumatoid arthritis--the Third National Health and Nutrition Examination Survey. J Rheumatol 2005; 32: 2311-2316.

[6] Livak KJ and Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001; 25: 402-408.

[7] Wang X, Lu M, Qian J, Yang Y, Li S, Lu D, Yu S, Meng W, Ye W and Jin L. Rationales, design and recruitment of the Taizhou Longitudinal Study. BMC Public Health 2009; 9: 223.

[8] Song X, Guo S, Chen Y, Yang C, Ji H, Zhang F, Jiang Z, Ma Y, Li Y, Jin L, Zou H, Zhou X and Wang J. Association between HLA-DQA1 gene copy number polymorphisms and susceptibility to rheumatoid arthritis in Chinese Han population. J Genet 2014; 93: 215-218.

[9] Boers M, Nurmohamed MT, Doelman CJ, Lard LR, Verhoeven AC, Voskuyl AE, Huizinga TW, van de Stadt RJ, Dijkmans BA and van der Linden S. Influence of glucocorticoids and disease activity on total and high density lipoprotein cholesterol in patients with rheumatoid arthritis. Ann Rheum Dis 2003; 62: 842-845.

[10] Georgiadis AN, Papavasiliou EC, Lourida ES, Alamanos Y, Kostara C, Tselepis AD and Drosos AA. Atherogenic lipid profile is a feature characteristic of patients with early rheumatoid arthritis: effect of early treatment--a prospective, controlled study. Arthritis Res Ther 2006; 8: R82.

[11] Plazak W, Pasowicz M, Kostkiewicz M, Podolec J, Tomkiewicz-Pajak L, Musial J and Podolec P. Influence of chronic inflammation and autoimmunity on coronary calcifications and myocardial perfusion defects in systemic lupus erythematosus patients. Inflamm Res 2011; 60: 973-980.

[12] Gonzalez-Gay MA and Gonzalez-Juanatey C. Inflammation and lipid profile in rheumatoid arthritis: bridging an apparent paradox. Ann Rheum Dis 2014; 73: 1281-1283.

[13] Garcia-Gomez C, Nolla JM, Valverde J, Gomez-Gerique JA, Castro MJ and Pinto X. Conventional lipid profile and lipoprotein(a) concentrations in treated patients with rheumatoid arthritis. J Rheumatol 2009; 36: 1365-1370.

[14] Davis LA, Whitfield E, Cannon GW, Wolff RK, Johnson DS, Reimold AM, Kerr GS, Richards JS, Mikuls TR and Caplan L. Association of rheumatoid arthritis susceptibility gene with lipid profiles in patients with rheumatoid arthritis. Rheumatology (Oxford) 2014; 53: 1014-21.

[15] van Halm VP, Nielen MM, Nurmohamed MT, van Schaardenburg D, Reesink HW, Voskuyl

AE, Twisk JW, van de Stadt RJ, de Koning MH, Habibuw MR, van der Horst-Bruinsma IE and Dijkmans BA. Lipids and inflammation: serial measurements of the lipid profile of blood donors who later developed rheumatoid arthritis. Ann Rheum Dis 2007; 66: 184-188.

[16] Kannel WB. Status of risk factors and their consideration in antihypertensive therapy. Am J Cardiol 1987; 59: 80A-90A.

[17] Soptei B, Mihaly J, Visy J, Wacha A and Bota A. Intercalation of Bovine Serum Albumin Coated Gold Clusters Between Phospholipid Bilayers: Temperature-Dependent Behavior of Lipid-AuQC@BSA Assemblies with Red Emission and Superlattice Structure. J Phys Chem B 2014; 118: 3887-3892.