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Original Research Article

Elevated high-sensitivity C-reactive protein among apparently healthy adults with concomitant prediabetes and latent tuberculosis infection in Nigeria

Benson O. Akinshipe[1]*, Edirin O. Yusuf[2], Alfred F. Ehiaghe[3], Tunde O. Egunjobi[1], Opeoluwa A. Yusuf[4]

[1]Departments of Medical Microbiology, School of Clinical Medicine, College of Health Sciences, Igbinedion University and Igbinedion University Teaching Hospital, Okada, Nigeria
[2]Department of Medical Microbiology, School of Clinical Medicine, University of Benin/ Teaching Hospital, Benin City, Nigeria
[3]Department of Medical Laboratory Science, Nnamdi Azikiwe University, Awka, Nigeria
[4]Department of Chemical Pathology, University College Hospital, Ibadan, Nigeria

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***Correspondence:**
Dr. Benson O. Akinshipe,
E-mail: bensonakinshipedr01@yahoo.com

ABSTRACT

Background: The increasing prevalence and convergence of type 2 diabetes mellitus (DM) and active tuberculosis (TB) comorbidity, especially in adults in the lower-and middle-income countries, demand new approaches to control the 'syndemic'. Consequently, we set out to investigate the possibility of early detection of prediabetes mellitus and/or latent tuberculosis infection using novel method.
Methods: This was a case-control study of 105 adults classified into 4 groups: Healthy Community Controls (HCC, n=30); Prediabetes mellitus (PDM, n=25); Latent Tuberculosis Infection (LTBI, n=23); Individuals with Prediabetes mellitus+Latent Tuberculosis Infection (PDM+LTBI, n=27). Sera collected were assayed for high-sensitivity C-reactive protein (hs-CRP) using the ultra-sensitive Human high sensitivity C-reactive protein ELISA Kit (Melsin Medical Co., Ltd, China). Other ancillary tests and measurements done include Erythrocyte Sedimentation Rate, serum Glycated-hemoglobin (HbA1c), Interferon-gamma (INF-ϒ) and Waist circumference.
Results: A total of 88 (83.8%) of the enrolled participants had full complement of results and were included in the analysis of four study groups: HCC (n=25), PDM (n=21), LTBI (n=19) and PDM+ LTBI (n=23). With respect to the serum biomarkers, isolated PDM and LTBI cases recorded significantly higher HbA1c (%) and INF-ϒ positivity respectively. Predictors of PDM+LTBI show statistically significant higher tertile (T3), representing elevated hs-CRP levels, (OR=6.50, 95% CI=4.83-22.39, p=0.0037).
Conclusions: This study revealed that persons harboring the two associated asymptomatic conditions, PDM + LTBI have higher inflammatory state detectable by assaying the biomarker, hs-CRP, which could be used for 'one-time bi-directional targeted screening' for PDM in LTBI and vice versa.

Keywords: Adults, Biomarker, Highly-Sensitive C-reactive protein, Latent tuberculosis infection, Prediabetes

INTRODUCTION

The global burden of disease from concomitant type 2 diabetes mellitus (DM) and pulmonary tuberculosis (TB) is immense. The increasing prevalence and convergence of DM and TB is an emerging public health problem, especially among adults in the lower- and middle-income countries (LMICs) where both disorders are common.[1,2]

For instance, Nigeria, the most populous country in Africa and currently rated 6th globally among 22 high TB-burden countries (HBCS), has been noted to be facing an increasing rate of DM among its adult population; this carries with it a concomitant increase in the prevalence of TB.[3,4] The reason for the increased risk of DM in TB-endemic areas and vice versa is not yet clear. In the majority of cases, both DM and TB are preceded by asymptomatic latent preliminary stage, prediabetes (PDM) and latent tuberculosis infection (LTBI), respectively.

These two chronic occult conditions can coexist undetected for many years, causing irreversible damage to vital body organs.[5] The increasing convergence and harmful confluence of DM and TB demands new approaches to PDM and/or LTBI detection in order to stem the tide of the emerging 'syndemic' in Nigeria.

It is well known that inflammation plays a central role in the initiation and progression of both PDM and LTBI. Most tissue damaging processes such as inflammatory conditions are associated with a major acute phase protein, the C-reactive protein (CRP), which is primarily produced by interleukin-6 (IL-6) dependent hepatic biosynthesis.[6]

The elevation of CRP is frequently the first evidence of inflammation or an infection in the body and precedes clinical symptoms. Elevation of CRP has been documented in a wide range of inflammatory conditions and its serum concentration is frequently determined for the assessment of the grade of systemic inflammation.[7]

The concentrations of the liver-synthesized CRP have been noted to be unaffected by potential confounders such as anemia, protein levels, red blood cells shape or patient age or sex.[8] It has also been observed that CRP concentrations remain relatively constant in individual over time and that there is no marked diurnal variation.[9] However, intense physical exercise has been noted to cause variations in CRP level.[10-12]

C-reactive protein has historically been measured in the clinical laboratory for the detection and monitoring of in-apparent inflammation and infection, using immunoturbidimetric and immunonephelometric techniques. However, standard CRP assays lack the sensitivity required to determine accurately low levels of early inflammation.[13]

In recent years, high sensitivity assays for CRP (hs-CRP) have been developed for sensitive quantification of CRP that can detect minimal variations of serum CRP levels even in the range of normal limits and is of special value that may point to low-grade inflammatory lesions; and as applicable in PDM and LTBI measurement.[13,14]

The American Diabetic Association (ADA) has recently declared that hs-CRP can be used to indicate PDM (normal serum value ranged 0.0-0.3mg/l) and that elevated level can indicate systemic inflammation such as DM and the associated risk of heart disease.[15]

The results of an increasing number of studies suggest that hs-CRP is a sensitive and reliable marker of low-grade systemic inflammation. Elevated hs-CRP has recently been associated with PDM and adiposity in a Korean pediatric population, and its level has been employed in the follow-up of children with LTBI to evaluate response to isoniazid prophylaxis and the level of disease activity.[16,17]

It has been shown that low-grade inflammation, as measured by hs-CRP level, may have an important role in the development of type 2 diabetes among African Americans with a lesser degree of insulin resistance.[18] It has also been observed that serum hs-CRP concentration is increased in elderly prediabetics compared with normal subjects and correlated with HbA1c and albuminuria; and that estimation of hs-CRP levels could be helpful in the early intervention of complications in prediabetics.[19]

The recent commercial availability of automated high sensitivity assays could enable the measurement of low-grade C-reactive protein (hs-CRP) at levels previously unattainable on a routine basis and to explore its clinical utility in asymptomatic conditions in apparently healthy individuals. Previous studies on hs-CRP association with PDM and/or LTBI have limitations: very few were carried out in LMICs, which have high DM- and TB-burden, and none reported from Africa.

Therefore, there is a knowledge gap regarding the concurrence and relationship between these two low grade inflammatory conditions, PDM and LTBI. The authors, therefore, set out to investigate the association(s) between PDM and LTBI in the context of the baseline highly-sensitive C-reactive protein (hs-CRP) result, and to also determine its diagnostic applicability as a marker for PDM and/or LTBI in presumably healthy adults in a high DM- and TB- laden setting.

METHODS

This case-control study was nested within a larger population of presumably healthy adults from a cross-section of three different communities representative of the Nigeria society, namely: Usen village (rural), Okada 'University' town (semi-urban) and Benin City (urban).

The cohort members were volunteers participating in a DM-TB control program that started in 2016. Details of the study design and recruitment process have been published elsewhere.[20,21]

Initially, the study population consisted of 105 adults classified into four groups: 1) Clinically Healthy Community Controls (HCC, n=30); 2) Prediabetes Mellitus (PDM, n=25); 3) Latent Tuberculosis Infection

(LTBI, n=23) and 4) Those with concomitant Prediabetes Mellitus and Latent Tuberculosis Infection (PDM+LTBI, n=27).

Exclusion criteria focused on medical conditions that would potentially confound results or interfere with save completion of the study, including metabolic disorders, active infections, hemoglobinopathies, pregnancy, and recent participation in a clinical trial.

This study was conducted within a larger population-based survey. No formal ethical review was required as the study was partly undertaken with retrospective analysis of routine clinical data which were anonymized before analysis by study researchers. As such, formal research ethic committee review was not required.

Data collection

Previous information supplied by study participants on socio-demographic, life-style and medical history variables were re-assessed through interviews, during clinic visits or at home using pen and paper format.

Trained study assistants and nurses repeated the measurement of anthropometrics, including Body Mass Index (BMI) and Waist Circumference, using standardized approaches as detailed elsewhere.[22]

Participants were re-classified into those that met the WHO minimum recommendations for Physical Activity (PA) (at least 75 minutes of vigorous-intensity, or 150 minutes of moderate-intensity activity per week) and those that did not and as described elsewhere.[23] Some of the questions demanded 'Yes'/'No' answer. Responses of "don't know" or "refused" were recorded as missing for all variables.

In addition, information obtained were cross-checked with the medical records at the respective local Health Centre and the population cohort database for completeness and consistency. Bacille-Calmette- Guerin (BCG) vaccination was confirmed by the presence of BCG scar(s).

Clinical laboratory methods

Blood collection and analysis

Resting peripheral blood samples were collected according to standard clinical laboratory procedures. Clinical hematology laboratory variables, viz. hemoglobin (Hb) level, white blood cell (WBC) counts, lymphocyte counts, CD4+T cell counts, and erythrocyte sedimentation rate (ESR) were re-analyzed at a central laboratory.

The biochemical parameters analyzed included the levels of: lipids, viz. Total Cholesterol (TC), Low Density Lipoprotein (LDL-C3) and High Density Lipoprotein (HDL-C4) and glycated hemoglobin A1c (HbA1c) using the Cobas CIII system Auto-analyzer (Roche/Hitachi Diagnostic) as previously described.[21,24]

Ascertainment of prediabetes

The result of re-assessed HbA1c level provided an estimate of the glucose control of each participant over the previous 3-month period, an advantage over random blood glucose.

Prediabetes mellitus was defined as HbA1c level of 5.7-6.4% following the recommendations of ADA to include HbA1c in identifying individuals at increased risk for future diabetes, and never been diagnosed of DM as per standard Nigeria Diabetic Society guidelines, for 12 weeks or less prior to this study.[15,25]

Ascertainment of latent tuberculosis infection

Individuals with LTBI were free of clinical signs and symptoms suggestive of pulmonary TB and never been diagnosed with TB as evaluated according to the Nigeria National Tuberculosis and Leprosy and Buruli ulcer Control Program (NTLCP) guidelines.[26]

The LTBI individuals had positive (≥10mm) Tuberculin Skin Test result. Mycobacterium tuberculosis (MTb) antigens positive individuals were re-evaluated for interferon gamma (IFN-ϒ) positivity (≥0.35IU/ml) as determined with the aid of the Quantiferon-TB Gold In-Tube (QFT-GIT) test kit (Cellestis Ltd., Carnegie, Australia) and described by Akinshipe.[20,27]

The calculation of the result of INF-ϒ produced was based on the QFT-GIT Analysis Software Program provided by the kit manufacturer. Samples with indeterminate results, i.e. INF-ϒ 0.35IU/ml for TB antigens and 0.5IU/ml for the positive control were not repeat-tested since test had deployed.[27]

Measurement of serum high-sensitivity C-reactive protein

For the precise measurement of serum High-Sensitivity C-Reactive Protein (hs-CRP) level in the present study, resting blood sample was collected after instructing participants to abstain from acute/strenuous exercise for at least 1 hour prior to sampling. The harvested sera were stored at -20^{0}C until tested. The stored sera were thawed at room temperature (RT^0).

Samples were tested in batches of 60/run by the quantitative enzyme immunoassay technique (double-antibody sandwich) using the ultra-sensitive Human high sensitivity C-reactive protein (hs-CRP) ELISA Kit (Kit Lot No: P20190419, Melsin Medical Co., Limited, China) according to the manufacturer's instructions.[28]

Briefly, 50µl/well each of the reconstituted hs-CRP Kit standard and test serum samples were dispensed into

appropriately labeled high-affinity 96-well plastic micro-plates pre-coated with the corresponding specific monoclonal anti-hs-CRP antibody and the mixture incubated for 2hrs at 37^0C.

The solution was discarded and the microplates washed x4times with 400µl/well Kit wash buffer with the aid of auto-washer. Next, 100µl/well of hs-CRP conjugate was added to all wells (standard and sample wells) and the micro-ELISA strip plate covered with a sealing tape and incubated for 1hr at 37^0C. The mixture was discarded and microplates washed x4times with Kit wash buffer and the liquid mixture aspirated. Then, 50µl/well each of Kit chromogen solution A and Kit chromogen solution B substrate were added and incubated for 15mins at 37^0C (in the dark) with gentle shaking with automatic shaker and then washed x4times with the Kit wash buffer and liquid aspirated.

Finally, 50µl/well of Kit stop solution was added. The intensity of the final colour (yellow) that developed exactly 15mins after adding the Kit stop solution was measured at 450nm wavelength using Stat Fax 4700 microstrip reader as previously described.[10]

The hs-CRP immunoassay in this study used monoclonal antibody traceable to the WHO reference material and a commercial human control serum was used to verify the assay performance for each run. The inter-assay coefficient of variation for the Kit control serum sample for quality control varied from 2.8-7.6% through the period of the data collection.

Approximately 5% of the samples were measured as masked replicates to assess repeatability of measurements. Reliability coefficient for the masked quality control replicates was 0.90 for the hs-CRP assay. The sensitivity of the assay ranged from 0.0-16 mg/l (Melsin Medical Co., Limited, China).[28]Tertiles: In the current study, hs-CRP result was categorized into one of three (3) groups (tertiles): T1 (Lower tertile, <0.15 mg/l); T2 (Mid-tertile, 0.15-0.24 mg/l) and T3 (Higher tertile, ≥0.25 mg/l).

Statistical analysis

Data were presented as mean±standard deviation (SD), or median (range), interquartile range (IQR), frequencies or percentages. Baseline characteristics and clinical laboratory data were stratified according to diagnostic status. Subjects were divided into tertiles according to their hs-CRP level. Data analysis was done using GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA). Continuous data among different groups were compared with One-way ANOVA and where significant difference occurs, Tukey Post hoc test was used. Comparisons of categorical data were analyzed using Chi square or Fisher's exact tests. Kruskal-Wallis test was used for data that were not distributed normally. P values <0.05 were considered significant.

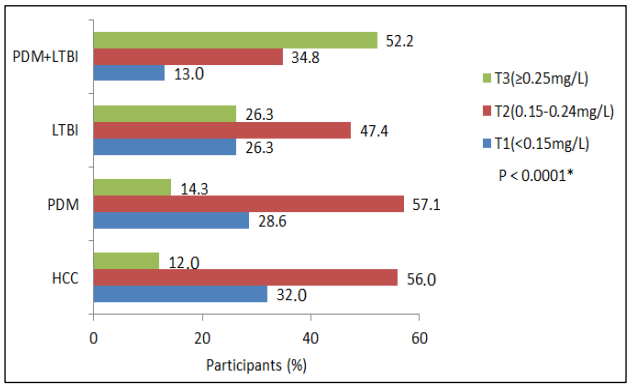
RESULTS

Overall, a final total of 88(83.8%) of the enrolled participants had full complement of results and were included in the analysis of subjects from the four study groups, HCC (n=25), PDM (n=21), LTBI (n=19) and PDM+ LTBI (n=23). Table 1 shows the basic characteristics of the study population stratified by study group. The four study categories presented homogeneity with respect to age, gender, BMI, life-style behaviorals, low rate of hypertension and high BCG vaccination rate. However, participants harboring PDM+LTBI showed slightly lower but insignificantly different measure of the Waist circumference compared with the controls (p=0.0623).

Clinical laboratory measurements

The outcomes of selected clinical laboratory measurements of study participants according to the study groups are presented in Table 2. There was no significant difference among the participants with respect to the WBC, CD4+T lymphocyte counts, total lymphocytes, lipid profiles, and hemoglobin levels.

However, with respect to the serum biomarkers, the hs-CRP level was significantly higher among the PDM+LTBI group compared to the other study categories (p<0.0001), while the isolated PDM and LTBI cases recorded significantly higher HbA1c (%) (p=0.0215) and INF-ϒ (p=0.0172), respectively. However, the ESR was slightly decreased among the HCC subjects compared to the other study groups (p=0.0383).



*P<0.05 considered as statistically significant. HCC- Healthy Community Control; PDM - Prediabetes Mellitus; LTBI- Latent Tuberculosis Infection; PDM+LTBI - Prediabetes Mellitus and Latent Tuberculosis Infection Combined Disorder

Figure 1: Proportion of participants in the studied groups by Hs-CRP tertiles.

Figure 1 shows the distribution of hs-CRP levels by grade of study tertiles: Low (T1) (<0.15mg/l); Moderate (T2) (0.15-0.24mg/l) and Higher (T3) (≥0.25mg/l). Bivariate analysis showed that T3 hs-CRP was associated with the PDM+LTBI group (52.2%) compared with LTBI (26.3%), PDM (14.3%) and HCC (12.0%), p<0.0001.

Table 1: Basic characteristics of the participants stratified by study group.

Variable	HCC	PDM	LTB	PDM+LTBI	P value
Overall population (%)	25 (28.4)	21 (23.9)	19 (21.6)	23 (26.1)	
Age (Median) years (IQR)	53 (40-49)	55 (48-57)	51 (45-56)	57 (48-59)	0.5393
Male/Female (%)	13 (52.0)	10 (47.6)	11 (57.9)	12 (52.2)	0.9353
Body mass index (Range) kg/m^2	23.6 (19.2-25.8)	25.1 (19.3-26.2)	24.2 (18.5-26.5)	23.6 (18.9-26.2)	0.1525
Waist circumference (Range) cm	79.4 (78-95)	82.8 (81-95)	82.2 (81-96)	79.1 (78-94)	0.0623
Adequate physical exercise (%)	19 (76.0)	12 (57.1)	11 (57.9)	10 (43.5)	0.1498
Alcoholism (%)	4 (16.0)	4 (19.0	3 (15.8)0.9916	4 (17.4)	0.9916
Tobacco smoking (%)	3 (12.0)	3 (14.3)	3 (15.8)	4 (17.4)	0.9605
Hypertension (%)	2 (8.3)	2 (9.5)	2 (10.5)	2 (8.7)	0.7544
BCG vaccinated (%)	25 (100.0)	20 (95.2)	18 (94.7)	21 (91.3)	0.5462

HCC- Healthy Community Control; PDM - Prediabetes Mellitus; LTBI- Latent Tuberculosis Infection; PDM+LTBI Prediabetes Mellitus and Latent Tuberculosis Infection Combined Disorder; BCG- Bacille-Calmette-Guerin. P<0.05 was considered statistically significant.

Table 2: Frequency of different types of asterion on right and left sides.

Parameter	HCC (n=25)	PDM (n=21)	LTBI (n=19)	PDM+LTBI (n=23)	P value
Cell counts					
WBC (range) 10^3/µ	(4.4-4.8)	(4.5-4.7)	(4.6-4.8)	(4.5-4.8)	0.0631
Lymphocyte (range) (%)	(37.7-42.0)	(36.9-44.5)	(37.1-43.9)	(35.8-46.5)[a]	0.0884
$CD4^+$T cells (mean)±SD (cells/ µl)	863±185.6	825.1±123.9	858.2±71.8	810.3±55.1	0.4049
Lipid levels					
Total cholesterol(mean)±SD (mg/dl)	144.3±10.6	139.1±9.1	142.4±8.2	138.2±7.6	0.0966
LDL-C3 cholesterol (mean)±SD (mg/dl)	95.3±5.2	97.1±3.8	95.8±3.8	98.2±5.1	0.0615
HDL-C4 cholesterol (mean)±SD (mg/dl)	44.6±2.8	45.8±3.2	43.1±2.6	42.9±2.8	0.0731
Hemoglobin levels					
Hb (Mean)±SD (g/dl)	13.3±0.3	13.1±0.4	13.7±0.7	13.9±0.9	0.1511
HbA_{1c} (Median) IQR (%)	4.9 (4.6-5.1)	6.1 (5.9-6.3)	4.7 (4.5-5.2)	5.8 (5.5-6.4)	0.0215*
Inflammatory markers					
INF-ϒ (Mean) IQR(IU/ml)	0.28 (0.24-0.30)	0.31 (0.26-0.33)	0.39 (0.37-0.52)	0.45 (0.41-0.55)	0.0172*
ESR (Mean)±SD(mm/hr)	18.1±2.1[a]	18.7±1.9[ab]	19.6±3.5[ab]	20.1±2.5[b]	0.0383*
Hs-CRP (mg/l)					
Mean±SD	0.15±0.07	0.18±0.08	0.19±0.08	0.26±0.08[a]	0.0001*
Median	0.16	0.18	0.19	0.26	
IQR	0.10-0.19	0.13-0.22	0.14-0.27	0.20-0.32	

HCC- Healthy Community Control; PDM-Prediabetes Mellitus; LTBI-Latent Tuberculosis Infection; PDM+LTBI-Prediabetes Mellitus and Latent Tuberculosis Infection Combined Disorder; WBC-White Blood Cell; SD-Standard Deviation; LDL-c3-Low Density Lipoprotein; HDL-c4-High Density Lipoprotein; Hb-Hemoglobin; HbA_{1c}-Glycated Hemoglobin; INF-ϒ-Interferon Gamma; ESR-Erythrocyte Sedimentation Rate; Hs-CRP-High-Sensitivity C-Reactive, Protein.groups that share same letter are not.
*Significantly different at p<0.05

Table 3: Predictors of concurrent PDM and LTBI.

Parameters	Odd ratio (95% CI)	P value
Elevated HbA_{1c}	1.45 (0.25-8.51)	0.6791
Elevated ESR	0.73 (0.34-2.17)	0.1894
Elevated INF-ϒ	1.97 (0.31-12.60)	0.4746
Elevated Hs-CRP	6.50 (4.83-22.39)	0.0037*

*Significant at P<0.05; HbA1c-Glycated Hemoglobin; ESR – Erythrocyte Sedimentation Rate; INF-ϒ-Interferon Gamma; Hs-CRP-High-Sensitivity C-reactive protein

Furthermore, multivariate regression analysis of the statistically significant independent variables as possible predictors of PDM+LTBI (Table 3) showed that only higher tertile (T3), representing elevated hs-CRP, remained statistically significant (OR=6.50, 95% CI=4.83-22.39, p=0.0037). Adults with higher tertile (T3) hs-CRP concentration were found to be at least six and half times more likely to harbor the dual PDM+LTBI condition compared to the presence of normal health.

DISCUSSION

Prediabetes mellitus (PDM) and latent tuberculosis infection (LTBI) are two commonly co-existing low-

grade inflammatory conditions that are central to the initiation and progression of type 2 diabetes mellitus (DM) and active Mycobacterium tuberculosis (TB) infection respectively. However, there is paucity of data on PDM and/or LTBI in co-endemic populations to inform recommendations for interventions in those settings.

In this study of presumably healthy adults, a homogenous population was observed with respect to potential cofounding variables, including age, gender, BMI, lifestyle behaviorals, hypertension and BCG vaccination rates (Table 1); but the ESR was found to be moderately elevated among the PDM+LTBI participants(p=0.0383). However, the results of the ultra-sensitive serum high-sensitivity C-reactive protein (hs-CRP) analysis revealed that individuals harboring the twin condition of PDM+LTBI synthesized significantly higher level of hs-CRP compared to the other study groups.

Importantly, when the potential predictors of dual PDM+LTBI, including HbA1c, ESR, INF-ϒ and hs-CRP were further analyzed in the context of harboring the twin PDM+LTBI, only elevated hs-CRP was found to be associated. Individuals with the higher level hs-CRP marker (T3) were discovered to be at least six and half times more likely to be harboring concurrent PDM+LTBI compared with the other study categories (OR=6.50, p=0.0037). However, no such correlation was observed for the isolated conditions, elevated hs-CRP level did not correlate significantly with only one condition, i.e. PDM or LTBI; and there was no significant difference in hs-CRP levels between PDM and LTBI groups.

Another finding in this study was that 52.2% of the subjects with co-existing PDM+LTBI synthesized hs-CRP at the higher tertile (T3) concentration i.e. ≥0.25mg/l, however only 13.0% of this same group produced at the low hs-CRP tertile (T1) i.e. ≤0.15mg/l, which are significantly different from the other groups (p<0.0001) (Figure 1).

However, subjects with either PDM or LTBI separately did not present significant differences in hs-CRP compared to controls for the marker hs-CRP≥0.25mg/l (T3). These data indicate a synergistic action of these two precursor stages, i.e. PDM and LTBI, in the occurrence of an increase in the inflammatory process which is accordingly reflected by an increase in hs-CRP synthesis. Pro-inflammatory exacerbation, as seen in elevated hs-CRP, may be the outcome of a mechanism whereby intermediate hyperglycemia, PDM, is synergizing with asymptomatic Mycobacterium tuberculosis infection in LTBI.

A possible pathway for this mechanism is that the hyperglycetide in PDM increased advanced glycation end product that is associated with the failure of blood stream monocytes to absorb Mycobacterium tuberculosis antigens in LTBI due to reduced activation in alveolar macrophages. C-reactive protein (CRP) has been noted as the principal downstream mediator of the acute-phase response following an inflammatory event and is primarily synthesized by IL-6 dependent hepatic biosynthesis.[6]

It has been shown that elevated hs-CRP relative to IL-6 confers an increased risk of ischemic stroke (IS) in a multi-ethnic Northern Manhattan study (NOMAS).[29] Furthermore, Sabanayagam have shown that cytokines such as IL-6, IL-1 and Tumor Necrosis Factor-ϒ (TNF-ϒ) stimulate hepatic synthesis of CRP. Pepys and Hirschfield concluded that CRP is produced as part of pathophysiological conditions including infection and inflammation.[8,19] Taken together, these observations suggest a potential of hs-CRP testing to systematically and simultaneously screen for the commonly co-existing PDM and LTBI, particularly in adults from high DM and TB co-endemic settings.

Previous studies have not focused on the significance of elevated hs-CRP in concurrent PDM+LTBI, and up-to-date, there has been no report on the highly sensitive hs-CRP from the co-endemic populations. However, the current study involved four different categories of presumably healthy adults, including a comparison group of clinically healthy normal control individuals without PDM or LTBI, that are resident in a high DM and TB laden country.

Importantly, unlike earlier reported studies on hs-CRP, the present work involved precise measurement of hs-CRP performed on blood samples collected at a resting state (acute/intense physical exercise was avoided by all the participants at least one hour prior to sampling) in order to prevent unwanted variations in hs-CRP level (possibly encountered in previous works).

It is well documented that C-reactive protein (CRP) is an acute marker of inflammation and that its concentration increases in circulation during inflammatory events. Based on multiple epidemiological and intervention studies, increased levels of hs-CRP have been found to be associated with future cardiovascular risk (hs-CRP <1.0mg/l=low risk, 1.0-3.0mg/l=intermediate risk, 3.0-10mg/l=high risk, >10mg/l=unspecified elevation).[30] Elevated hs-CRP has been proposed as a reliable indicator for adiposity, prediabetes, and abnormal lipid metabolism in a Korean pediatric population.[16]

One of the very few studies on CRP (in diabetes mellitus) from Africa recently observed statistically different levels in fasting Nigerian diabetics compared with age-matched controls and suggested a possible role of the inflammatory marker in diabetogenesis.[31]

Another study in Thailand, that comprised of 75 cases classified into two groups by their hs-CRP levels, noted that hs-CRP concentrations correlated with HbA1c level in diabetic females and that the mean HbA1c levels were

significantly higher in patients who had hs-CRP levels of≥10mg/l, while other factors such as age BMI, LDL-C, blood pressure and serum creatinine were not correlated with hs-CRP level.[32] Furthermore, hs-CRP, measurement has been employed in the follow-up of children with LTBI to evaluate response to isoniazid prophylaxis and the level of disease activity.[17]

Limitations

A limitation of the present cross-sectional study was that there was only one-time baseline hs-CRP measurement and no follow-up values. However, there are several studies showing that CRP concentrations are relatively constant in individuals over time and there is no marked diurnal variation.[9,13] Moreover, the serum hs-CRP in this retrospective study were analyzed after storage at-20^{0}C for a period of almost 3 months. However, the Reykjavic study confirmed the stability of CRP concentrations in serum preserved at this low temperature for up to 12 years.[33] Although the present study used a cross-sectional design and cannot demonstrate temporality, the study entailed stringent conditions for defining each study. Specifically, HbA1c between 5.7-6.4% was used to define PDM status and as recommended and INF-ϒ group positivity (≥0.35IU/ml) was used to ascertain LTBI.[15] The effect of the small sample size of this study could be minimized by further exploration in future larger observational study.[27]

Added value of this study

To our informed knowledge, the present study is the first to report on the association/synergy between PDM and LTBI in the context of the marker high-sensitivity C-reactive protein (hs-CRP) result, and to also determine the diagnostic applicability of hs-CRP testing in the context of concurrent PDM+LTBI in presumably healthy adults in a resource-limited, high DM and TB setting. If hs-CRP reflects synergistic inflammatory activity in these asymptomatic stages, then this would support its use as prognostic marker and part of clinical evaluation.

For example, a high baseline hs-CRP might suggest that longer treatment duration is necessary (importantly, a high baseline hs-CRP appears to be associated with an increased likelihood of progression to overt DM+TB). The outcomes of the present study support the use of hs-CRP testing to simultaneously screen for asymptomatic people harboring concomitant PDM+LTBI. Thus, a single point-of-care highly sensitive hs-CRP-based PDM+LTBI testing could at once improve the efficiency of intensified case finding in high risk individuals and thereby increase the uptake of DM and TB preventive therapy.

CONCLUSION

The results of this original study of presumably healthy adults revealed that the group of adults harboring PDM and LTBI concurrently were six and half times more likely to synthesize significantly higher level of the high-sensitivity C-reactive protein (hs-CRP) inflammation marker, when compared to normal controls. However, elevated hs-CRP level did not correlate significantly with either of the isolated condition, PDM or LTBI alone. These findings indicated that persons with two associated asymptomatic conditions, PDM + LTBI, have a more active inflammatory state distinguishable by the sensitive high-sensitive C-reactive protein (hs-CRP) marker.

Elevated hs-CRP could help in predicting the risk of concurrent DM+TB early enough in the natural history of the condition (in the pre-symptomatic phase). Clearly, elevated hs-CRP is a novel and effective supplementary diagnostic and prognostic biomarker for DM+TB, and could afford the opportunity to intervene and prevent the overt illness.

However, elevated hs-CRP level might not be useful yet for point-of-care screening. In order for this approach for combined PDM+LTBI detection in the context of elevated hs-CRP marker to be incorporated into clinical practice, agreement among the various hs-CRP methods must be achieved. Factors such as hs-CRP assay calibration and standardization must be addressed adequately by the in-vitro diagnostic industry and the clinical laboratory.

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