Journal of Medical Sciences

ISSN 1682-4474

science alert

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Research Paper

J. Med. Sci., 12 (6): 161-167
15th August, 2012
DOI: 10.3923/jms.2012.161.167

HLA-B27 and its Associated Clinical and Biochemical Presentation among Ghanaians with Ankylosing Spondylitis

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HLA-B27 is a genetic predisposition marker for the development of Ankylosing Spondylitis (AS). AS is uncommon in West-Africa, but due to environmental and lifestyle changes, its prevalence is said to be increasing. This study sought to determine the baseline prevalence of HLA-B27 among Ghanaians presenting with AS, find out their disease activity, clinical presentation, presence of extra-articular manifestations, inflammation and dyslipidemia. In a cross-sectional study, 65 AS subjects were recruited from the orthopaedic departments of two leading Teaching Hospitals and a private laboratory, medilab diagnostics with centres across the country. Fifty healthy blood donors were also recruited as control group. HLA-B27, BASDAI score, Lipid profile, TNF-α and ESR levels were estimated among them. Statistical comparisons were analyzed using the one way ANOVA followed by Bonferroni's Multiple Comparison test. There were four HLA-B27 positives representing 4.6%, the mean BASDAI score was 44.7/100. 48 AS patients had Sacroiliitis in their X-ray reports. None had a family history or any extra-articular manifestations. AS subjects had higher ($p<0.001$) levels of total cholesterol and triglycerides compared to the controls. The HDL level for the patients was significantly lower ($p<0.001$), as compared to the control. The LDL level indicates a higher ($p<0.001$) value as compared to the controls. TNF-α level was 13.11±0.50 pg mL^{-1} compared to 5.70±0.48 pg mL^{-1} of control whiles the ESR was 34.64±1.87 mm h^{-1} as compared to 9.23±0.91 mm h^{-1} of controls. AS patients had moderate disease activity with no extra articular manifestation and a prevalence of 4.6%. Dyslipidemia was prominent and that inflammation plays a pivotal role in the development of atherosclerosis.

Key words: Human leucocytes antigen, ankylosing spondylitis, bath ankylosing spondylitis disease activity index, dyslipidemia, tumour necrotic factor-alpha, prevalence

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JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued eight times per year on paper and in electronic format.

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INTRODUCTION

There is rising number of non-infectious diseases among the Ghanaian populace with rheumatoid conditions contributing 1.9% of the top ten causes of morbidity and mortality (PPME-GHS, 2005). Ankylosing Spondylitis (AS) is an unremitting inflammatory arthritis, affecting largely the spine and sacroiliac joints, causing fusion of the spine (Braun and Sieper, 2007). The Human Leukocytes Antigen, B27 (HLA-B27) is a predisposing marker for the development of AS. This antigen is uncommon in Africans, but due to environmental and lifestyle changes, its prevalence is said to be increasing. The prevalence of HLA-B27 among Caucasian patients with AS is about 90% (Sengupta and Stone, 2007). In most West-African countries however, HLA-B27 prevalence among AS patients is found between 3 to 6%, supporting the theory that some ethnic groups may be genetically protected from spondyloarthritis (Belachew *et al*., 2009). Definitive diagnosis of AS and its subsequent association with HLA-B27 in Ghana has not been evaluated, Moreover, other genetic and environmental factors may be involved in the causation of AS in the HLA-B27 negative population (Lopez-Larrea *et al*., 2002).

Studies concerning the prevalence of HLA-B27 among AS patients in Africa especially Ghana has not been documented. In the general population, dyslipidemia has been shown to be one of the strongest predictors of Cardiovascular Disease (CVD), with elevated levels of Low-density Lipoproteins (LDL) forming the primary treatment target according to national guideline (Szabo *et al*., 2011). In active AS, the bulk of cardiovascular deaths result from accelerated atherosclerosis (Gabriel *et al*., 2003). AS is a chronic condition and hence, the determination of pro-inflammatory and inflammation activities among them will help determine the association between chronic nature of the disease and their risk for developing cardiovascular diseases. Tumour necrosis factor-alpha (TNF-α), is a fundamental cytokine in chronic inflammation and affects lipid breakdown and endothelial function (Garces *et al*., 2008). It promotes the inflammatory response and has been implicated in the pathophysiological processes of rheumatological diseases (Popa *et al*., 2007). Investigations concerning increased Erythrocytes Sedimentation Rate (ESR) in cardiovascular events have already been documented but those regarding ESR and TNF-α as inflammatory component in the development of cardiovascular disease has not been evaluated. The present study was thus set up to determine the baseline prevalence of HLA-B27 among Ghanaians presenting with AS, determine their disease activity, clinical presentation, presence of extra-articular manifestations, inflammation and dyslipidemia. Standardized questionnaires were administered to AS subjects and their blood sample taken for haematological, biochemical and HLA-B27 assays.

MATERIALS AND METHODS

Study design: This was a cross sectional study that was carried out from October, 2007 to June 2009. Ethical approval for the study was obtained from the Committee for Human Research, Publication and Ethics (CHRPE) of the SMS/KNUST/KATH/1423-2006.

Standardized questionnaires were administered to patients diagnosed with AS and those who consented were recruited. The accuracy of the self-reported information on clinical history and socio-demographic information collected from the patients were assessed through record reviews of hospital database with a 100% rate of accuracy.

Subject selection: A total of one hundred and sixty seven (167) patients visiting the orthopaedic Departments of Komfo Anokye Teaching Hospital (KATH), Korle-Bu Teaching Hospital (KBTH) and Medilab Diagnostic Centres were recruited to participate in the study. These patients were from various social and ethnic groups as well as geographically distinct areas of the country. Out of the 167 patients, 65 (42 males and 23 females) were diagnosed with AS using Modified New York criteria. Written informed consent was obtained from each participant and the information regarding the protocol and informed consent was presented to them at the appropriate literacy level. The medical records of each consenting participant were reviewed to ascertain whether they had been previously undergone the tests under investigations. The study was conducted in a confidential manner and random unique study-generated numbers were employed to identify the participants.

Exclusion criteria:

- Patients whose diagnosis could not be confirmed
- Patients with other forms of arthritis
- Confirmed AS patients with Diabetes Mellitus and Hypertension

Control selection: Fifty healthy blood donors from KATH donor clinic who had not been diagnosed with any form of arthritis or chronic joint pain were recruited as the control group.

STANDARDIZED QUESTIONNAIRES ADMINISTRATION

All the consenting participants completed a structured questionnaire assessing socio-demographic characteristics, clinical and extra articular manifestations, family history, presence of other autoimmune conditions and drug history and risk profile for Ankylosing Spondylitis. Physical examination was done to evaluate the general health condition for each participant. Furthermore, the Modified New York Criteria (MNYC) and the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) which access the risk profile and disease activity for Ankylosing Spondylitis subjects were also included in the questionnaire. The BASDAI is user friendly, highly reliable, reflects the entire spectrum of the disease and is sensitive to clinical changes. It is also superior to the Newcastle Enthesis Index (Garrett *et al*., 1994). Calin *et al*. (1999) further assessed the validity of the BASDAI score and confirmed it.

Blood sample collection: Six milliliter of blood sample was collected from each respondent and 3 mL of it was dispensed into a vacutainer containing the anticoagulant, Ethylenediaminetetraacetic Acid (EDTA). This sample was then used for the haematological analysis and HLA-B27 within three hours of collection. The rest of the blood sample was dispensed into a vacutainer to obtain the serum which was stored at -20°C until it was used for the biochemical and serological analysis.

Haemoglobin estimation (cyanmethemoglobin method): Drabkin's reagent was used for the quantitative, colorimetric determination of haemoglobin concentration in whole blood as by method described by Drabkin's (Drabkin and Austin, 1935). The EDTA blood specimen collected were vortexed to obtain homogenous samples, 20 μL of blood was pipetted into 5 mL of Drabkin's reagent, vortexed and allowed to stand for at least 15 min. The mixture was transferred into cuvette of pre-warm spectrophotometer, (Spectrumlab™ 5A, USA) and absorbance read at 540 nm against reagent blank.

Erythrocyte sedimentation rate estimation (ESR): Using vortexed sample of the EDTA blood collected, the ESR was determined using the Westergren tube method (Bull *et al*., 1993).

HLA-B27 estimation: HLA-B27 IMS-sandwich ELISA has high sensitivity and specificity, easy operation and clear interpretation. It combines Immuno-Magnetic Separation (IMS) and ELISA techniques for the rapid detection of HLA-B27 antigen in whole blood samples. The HLA-B27 IMS ELISA kit was purchased from Taiwan Advance Bio-Pharmaceuticals, Taiwan. Tests were performed according to the manufacturer's instructions.

Total cholesterol: Total Cholesterol (TC) was assayed using a kit from Human Diagnostic™ (Wiesbaden, Germany) which employs an enzymatic colorimetric method with lipid clearing factor. The cholesterol is determined after enzymatic hydrolysis and oxidation (Allain *et al*., 1974). The indicator quinoneimine is formed from hydrogen peroxide and 4-aminophenazone in the presence of phenol and peroxidise (Tamaoku *et al*., 1982). The absorbance of the standard and the samples were read at 550 nm against the reagent blank.

Triglycerides: Triglycerides (TG) were assayed using a kit from Human Diagnostic® (Wiesbaden, Germany) which employs an enzymatic colorimetric method with lipid clearing factor. The present method uses a modified Trinder (Barham and Trinder, 1972; Trinder, 1969) colour reaction to produce a fast, linear, endpoint reaction. The intensity of the colour produced is directly proportional to the concentration of triglycerides in the sample.

HDL and LDL cholesterols: High Density Lipoprotein (HDL) Cholesterol was assayed using a kit from Human™ Diagnostic (Wiesbaden, Germany) which employs a precipitant and a standard. The chylomicrons, Very Low Density Lipoproteins (VLDL) and low density lipoproteins (LDL) are precipitated by the addition of phosphotungstic acid and magnesium chloride (Stein and Myers, 1995; Warnick *et al*., 1985). The absorbance of the standard and the samples were read at 550 nm against the reagent blank.

The LDL-Cholesterol Concentration (LDL-C) was calculated from the total cholesterol concentration (TC), HDL-Cholesterol Concentration (HDL-C) and the triglycerides concentration (TG) according to Friedewald's equation (Friedewald *et al*., 1972).

TNF-α estimation: Human TNF-α TiterZyme® Enzyme Immunometric Assay (Assay Design, USA) test kit was used. It is a complete test for the quantitative determination of human TNF-α in biological fluids. It uses a monoclonal antibody to human TNF-α immobilized on a microtiter plate to bind the human TNF-α in the standards or sample, the colour generated is read at 450 nm. The measured optical density is directly proportional to the concentration of human TNF-α in the standards and samples.

RESULTS

Demographic and clinical characteristics of AS subjects: The average age of the AS patients was 46 years of which four (4) were positive and sixty-one (61) were negative for

Table 1: Demographic and clinical characteristics of AS subjects

Parameters	Values
Mean age	46 years
Mean symptom duration	>8 months
Male to female ratio	3:1
Hb (g dL^{-1})	11.27
ESR (mm h^{-1})	34.64
HLA-B27 positives	4.61%
Mean BASDAI score	44.7/100
Sacroiliitis (X-ray)	73.80%

HLA-B27: Human leukocytes antigen, BASDAI: Bath ankylosing spondylitis disease activity index, ESR: Erythrocytes sedimentation rate, Hb: Haemoglobin

Table 2: Comparison of inflammatory markers among AS subjects

Parameter	Subjects (n = 65)	Control (n = 50)	p-value
HB (g dL^{-1})	11.27±0.17	13.99±0.15	0.001
ESR (mm h^{-1})	34.64±1.87	9.23±0.90	0.001
TNF-a (pg mL^{-1})	13.11±0.50	5.70±0.48	0.001

The data are expressed as Mean±SEM: TNF-α: Tumour necrosis factor, ESR: Erythrocytes sedimentation rate

Table 3: Clinical features of Ghanaian patients with ankylosing spondylitis*

Patients No.	Axial involvements	Peripheral arthritis	Enthesopathy	Extra articular manifestation
15	Bilateral sacroiliitis	Absent	Absent	Absent
48	Bilateral sacroiliitis	Absent	Absent	Absent
94	Bilateral sacroiliitis	Oligoarthritis	Absent	Absent
97	Bilateral sacroiliitis	Absent	Absent	Absent

*Patients were three men and one woman and they fulfilled the New York criteria for Ankylosing Spondylitis. None had a family history of Aukylosing Spondylitis, psoriasis, or chronic bowel disease

the HLA-B27 antigen. Men were twice more susceptible to develop AS than women. The Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) score of 44.7/100 was obtained which denote moderate disease activity with symptom duration greater than 8 months. Forty-eight of the AS subjects had Sacroiliitis in their X-ray reports; one patient had bilateral Sacroiliitis with peripheral oligoarthritis. None had a family history of Ankylosing Spondylitis, psoriasis, extra-articular manifestation or chronic bowel disease as shown in Table 1 and 3.

Lipid profile: AS subjects had significantly higher ($p<0.001$) levels of TC, TG and LDL compared to the controls. The HDL levels for AS subjects was significantly lower (1.07±0.01) as compared to the control (1.52±0.02) with a corresponding increased atherogenic index and ratios i.e., TC/HDL-C and LDL-C/HDL-C as shown in Table 4.

Inflammatory markers: ESR was approximately four times higher among AS subjects than controls. TNF-α level as a pro-inflammatory marker was twice higher among AS subjects compared to the control group (Table 2).

Table 4: Comparisons of lipid profile and atherogenic index between AS subjects and control

Parameters	Subjects (n = 65)	Control (n = 50)	p-value
TC (mmol L^{-1})	5.88±0.06	4.22±0.17	0.001
TG (mmol L^{-1})	0.88±0.02	0.56±0.05	0.001
HDL-C (mmol L^{-1})	1.07±0.01	1.52±0.02	0.001
LDL-C (mmol L^{-1})	3.97±0.04	2.41±0.07	0.001
Atherogenic index (mmol L^{-1})	4.49±0.07	1.77±0.04	0.001
TC: HDL ratio	5.27±0.02	2.77±0.04	0.001
LDL: HDL ratio	3.60±0.05	1.58±.001	0.001
TG: HDL ratio	0.80±0.01	0.36±0.02	0.001

The data are expressed as Mean±SEM, TC: Total cholesterol, TG: Triglycerides, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein, Atherogenic index: (total cholesterol-HDL cholesterol)/HDL cholesterol

DISCUSSION

This study investigated the baseline prevalence of HLA-B27 and its associated clinical and biochemical parameters among AS patients. It was observed that the mean age for AS patients was 46 years and this is contrary to Feldtkeller *et al.* (2003) who reported that in Germany, the mean age for AS patients was 27.7 years. This difference could be attributed to the fact that most of the diagnosis were done late when the disease had already advanced.

The delay in the diagnosis of AS is because there is no unique clinical symptom or laboratory test to make the diagnosis in the early stages of the disease. The presence of radiological sacroiliitis is essential for the diagnosis of AS, according to the modified New York criteria. However, it takes many months of inflammation of the sacroiliac joint before radiological damage can be demonstrated.

From this study the prevalence of HLA-B27 was found to be 4.6%. HLA-B27 positivity is one of the factors predicting a bad prognosis (Van Der Heijde *et al.*, 2004) which makes it more important to identify HLA-B27 positivity among patients than those with HLA-B27 negativity. In a study conducted by Allsopp *et al.* (1992) and Hill *et al.* (1991), HLA-B27 was present in 2 to 3% of the western Africa population, however, in a study carried out among 82 inhabitants of Mali, HLA-B27 prevalence was 9.7% (Kalidi *et al.*, 1988). In another study involving 700 people in Gambia, a prevalence of 2.6% was obtained (Allsopp *et al.*, 1992) and 3% was found in Senegal on 100 persons surveyed (Hill *et al.*, 1991). Out of eight subjects examined by Lopez-Larrea *et al.* (2002) in Togo, AS was associated to B27 in only one patient. However, the AS in the remaining 7 subjects was associated with B14 subtype. The prevalence of HLA -B27 among AS in this study (4.6%) is similar to that of the black Americans (3-6%) (Kellner and Yu, 1992; Khan *et al.*, 1977). The

HLA-B27 studies in Black Africa population are fragmentary. None of the seven patients examined by Stein *et al.* (1990) had this antigen which was present only in one patient of Chalmers *et al.* (1977).

One study has shown that 94% of AS patients were HLA-B27-positive among white populace (Khan, 1977). This difference is probably attributed to variations in HLA-B27 distribution among racial groups (Brown *et al.*, 1997). This strongly suggests that the importance of HLA-B27 antigen in the pathogenesis of AS is racially dependent.

The mean BASDAI score from this study was 44.7/100. This BASDAI score of 44.7/100 indicate cervical and lumbar stiffness according to De Diego-Otero *et al.* (2009). This severity seems characteristic of the AS of the black African patient. The result of this study is similar to those used for a review by Mijiyawa (1993) who reported clinical manifestations with spinal and sacroiliac joint involvement in studied patients from Zimbabwe, South Africa and Togo; peripheral joint involvement was present in about 40% of patients; and the disease was in advanced stage and severe in 19% of patients with "bamboo" spine and 23% of patients with hip involvement. In addition, other organs, such as the eyes, lungs and heart, can be also be affected with AS (Inman, 2006).

In the present study, the characteristic early sign of AS which is radiological evidence of sacroiliac joint arthritis was present. Sacroiliitis as shown by x ray examination is still mandatory to fulfil the currently widely used modified New York criteria for the diagnosis of AS (Van Der Linden *et al.*, 1984). Moreover, Sieper and Rudwaleit (2005) revealed that radiological sacroiliitis can be regarded as predictor in about 80% and this findings is similar to that of Van Tubergen *et al.* (2003). Disease activity as determined from the BASDAI score indicates moderate disease activity among AS patients.

In this study, ESR and TNF which measures the level of inflammation and proinflammatory activities were significantly higher ($p<0.001$) among AS patients than the controls (Table 2). This finding is similar to that of Maury *et al.* (2003) who reported that in healthy people, TNF values are maintained at normal levels by a variety of anti-inflammatory cytokines, but people with AS have increased serum TNF levels. TNF-alpha may thus be a major factor in AS related CVD acting both by contributing to hypertriglyceridemia and by promoting atherosclerosis-related inflammation in AS patients. This high TNF-alpha levels results from inflammation in the sacroiliac joints of such patients. Furthermore, Gorman *et al.* (2002) and Weaver (2004) showed that anti-TNF drugs reduce the fatigue, pain, swelling, improve mobility and prevent further damage among AS patients. This anti-tumour-necrosis-factor (anti-TNF) therapy is highly effective in AS.

CONCLUSION

This study has shown that the prevalence of HLA-B27 among Ghanaians with AS is 4.6% and this prevalence is similar to those within the West-Africa sub-region. Their BASDAI scores denote moderate disease activity with no extra articular manifestations. Dyslipidemia is prominent in AS subjects and that inflammation plays pivotal role in the development of atherosclerosis as evidenced in the increase in TNF-α and ESR.

ACKNOWLEDGMENTS

Authors wish to express their profound gratitude to staff of orthopaedic departments of KATH , KBTH and MEDILAB centres nationwide who helped with questionnaire administration and sample collection especially Mr. Ernest Agyei. We are also grateful to all Ankylosing Spondylitis patients who availed themselves for their blood samples to be taken for this study. Sincere thanks go to all my colleagues at the Department of Molecular Medicine.

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