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Levels of immunoglobulins (G, A and M) and circulatory immunocomplexes in Nigerians with joint pains

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Summary

Serum immunoglobulin (G, A and M) and circulating immune complex concentrations were measured in eighty-three Nigerians having joint pains and forty apparently healthy Nigerians by the single radial immune-diffusion and polyethylene glycol precipitation methods respectively. The IgA level was significantly lower and IgM statistically higher in patients with joint pains than the controls. There was however no significant difference between the mean IgG concentrations in patients with joint pains and controls. The observed low circulating IgA levels could be as a result of depressed thymic activity, development of autoimmunity or utilization in immune complex formation. The mean concentration of soluble immune complexes was significantly higher in patients with joint pains than in the apparently healthy subjects. These immune complexes some of which may remain localized in the surface of cartilages, ligaments and menisci would cause activation of complement resulting in persistent inflammation and joint pains in these patients.

Résumé

Le sérum transporteur de l'immunoglobuline (G, A et M) et les concentrations du complexe immunisé en circulation, furent mesurés chez quatre-vingt-trois Nigérians souffrant des douleurs de l'articulation, et chez quarante Nigérians apparemment en bonne santé, par le moyen de l'immunodiffusion radiale simple, ainsi que la methode de la précipitation du polyéthylène contenant de la glycérine successivement. Le niveau du IgA était significativement plus bas, tandis que celui du IgM était statistiquement plus haut chez les malades avec des douleurs des articulations que chez les gens en bonne santé. Cependant, il n'y avait pas de différence signicative entre les concentrations moyennes du IgG chez les malades souffrant des douleurs de l'articulation et chez les personnes en bonne santé. Les niveaux bas du IgA en circulation, déjà observés pouvaient être le résultat des activités thymiques en depression, du developpement de l'autoimmunité, ou de son utilisation dans la formation du complexe immunisé. La concentration moyenne des complexes immunisés solubles était significativement plus haute chez les malades sous observation que chez les sujets qui étaient apparemment en bonne santé. Certains de ces complexes immunisés qui peuvent rester sur le surface des cartilages des ligaments et des ménisques pourraient être à la base de l'activation du complément resultant en l'inflammation constante et en douleurs de l'articulation chez ces malades.

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Introduction

Painful joints is a common presentation of many diseases seen in the orthopaedic clinics in Nigeria. Previous works have reported reduced

haemoglobin, elevated uric acid and normal parasitic findings as well as abnormal immunoglobulin levels in Nigerians with polyarthritis (Greenwood, 1969a and b). In the present study, immunoglobulin and immune complexes levels were quantified in the blood of Nigerians who present with joint pains and limitation of movements without any further complications.

Materials and methods

Patients

Eighty-three patients (thirty-three males and fifty females) between the ages of 20 and 70 years) attending the Orthopaedic clinic of the University College Hospital, Ibadan, Nigeria were studied. They were all Nigerians and were referred because of pain in the joints and limitation of movements. Before inclusion, the patients were clinically assessed to determine their suitability for the study and any with infectious articular and periarticular conditions were excluded. All patients had physical examination with particular attention to weight, pulse rate and blood pressure. The intensity of pain and inflammation in the affected joints were also evaluated. In addition, all patients had radiological examination. They had their blood taken for laboratory investigations, differential blood picture, sedimentation rate, fasting blood sugar, liver function tests and the renal function test. All patients had blood analyses for the immunoglublins (G, A and M) and circulating immune complexes the results of which are being presented in this study. All the subjects gave negative slide latex agglutination tests for rheumatoid factor.

Included in the study were forty sex- and age-matched apparently normal subjects with no joint pains as controls. These included patients who had fully recovered from minor surgical operations or were blood donors to the University College Hospital blood transfusion service. They had been screened for absence of joint pains.

Assays

About 5 ml of clotted blood was collected from each subject by venepuncture. The serum was separated after the clot had retracted at room temperature. The serum was separated into two aliquots. The sample for immunoglobulin measurements was stored at −20°C until analysed, whilst the other aliquot was assayed immediately for the level of soluble immune complexes.

Level of circulating soluble immune complexes were quantified in thirty-four patients with joint pains and 101 controls using the polyethylene glycol precipitation method of Haskova *et al.* (1978). Polyethylene glycol (P.E.G.) 6000 solution was added to serum in borate buffer to give a final concentration of 3.7% P.E.G. and 1 and 30 dilution of serum. After incubation at room temperature, the immune complex levels were measured at 450 mμ.

The immunoglobulin levels in eighty-three patients and forty controls were measured by the single radial immuno-diffusion technique of Fahey and McKelvey (1965) as modified by Salimonu (1976) and Salimonu *et al.* (1978). A volume of an optimally diluted monospecific antiserum was mixed with noble agar and poured on glass plate. Wells of equal diameter were cut in the antibody/agar mixture. The wells were filled with test or standard serum (Rowe, 1970). The plates for IgG measurement were incubated at 37°C for 3 h. Those for IgA and IgM were placed at room temperature (18°C) for 18 h. After the incubation, the diameters of the precipitin rings were measured using a Hyland viewer with a micrometer eye piece. The standard errors of the mean for repeated sample measurements were observed to 7.2, 2.3 and 1.0 respectively for IgG, IgA and IgM.

Results

Eighty-three patients (thirty-three males and fifty females) were studied for levels of immunoglubulins (G, A and M). Sixty-five of the patients (78.3%) were within the age range 40–60 years. The mean age range of the whole group was 52 years. Twenty per cent of patients were overweight for their age and 10% had raised blood pressure.

Diagnosis

Osteoarthritis was the commonest condition

(Table 1) being present in sixty-three (75.9%) of all patients. In forty-two cases (51%) it was affection of the knee (Fig. 1), and ten cases (12%) the hip. The shoulder was affected in six cases (17%) and in the ankle five cases (6%). Ten cases had low back pain with evidence of lumbosacral spondylosis, cervical spondylosis was diagnosed in six cases and frozen shoulder with four cases.

Soluble immune complexes

The mean level of soluble immune complexes was significantly higher in the patients with joint pains than in the controls ($t = 7.3870$, $P < 0.01$; Table 2). Out of the 101 healthy controls analysed fifty-three had no detectable levels of polyethylene glycolprecipitable soluble immune complexes in their sera. The rest had values that ranged between 2–10.5 mg/ml except two cases where the values were 17.5 and 27 mg/ml respectively. All the patients with joint pains had detectable levels of soluble immune complexes. Twenty of the thirty-four patients had values that were equal to or greater than 15 mg/ml.

Table 1. Conditions of joint pains

Diagnosis	No. of cases	Percentage
Osteoarthritis		
Knee	42	51
Hip	10	12
Shoulder	6	7
Ankle	5	6
Lumbosacral spondylosis	10	12
Cervical spondylosis	6	7
Frozen shoulder	4	5

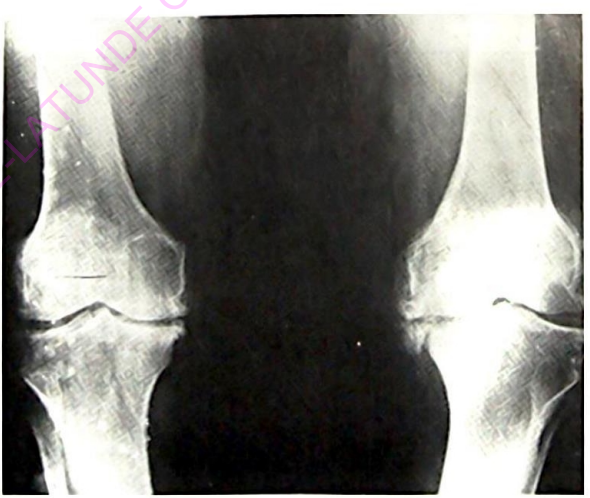

Fig. 1. Illustrates the X-ray of the patient with advanced osteoarthritis of the knees with painful swelling. Left knee, more severe than right knee.

Table 2. Soluble immune complex levels (mg/100 ml) in patients having joint pains and controls

	Patients with joint pains	Control
n	34	101
Mean	25.29	2.996
1 s.d.	17.396	4.582

Immunoglobulins

There were no significant differences between the mean IgG concentration in patients with joint pains and the apparently healthy controls (Table 3). There is also no appreciable difference in the proportion of subjects having high IgG levels between the patients and the apparently healthy subjects (Table 4).

The IgA levels were significantly lower ($t = 2.4055$, $P < 0.02$) in the patients with joint pains than the controls (Table 3). In addition, a larger proportion of the healthy subjects had high IgA levels than in patients with joint pains ($\chi^2 = 6.3806$, $P < 0.025$).

The mean IgM levels were significantly more elevated ($t = 5.5725$, $P < 0.01$) in patients with joint pains than in healthy subjects (Table 3). A greater proportion of the patients with joint pains had high IgM concentrations than in the healthy subjects (Table 4).

Table 3. Mean immunoglobulin levels (± 1 s.d.) in the subjects studied

	IgG	IgA	IgM	*n*
Patients with joint pains	1528 (± 1083)	326 (± 134)	267 (± 137)	83
Healthy controls	1493 (± 743)	396 (± 159)	166 (± 64)	40
Comparing patients with controls				
t	0 2096	2.44055	5.5725	
P	< 0.2 (n.s.)	< 0.02	< 0.01	

n.s. = significant

Table 4. Number of subjects with high *(higher than mean + 2 s.d. of healthy controls) immunoglobulin levels

	IgG	IgA	IgM	Total no. *n*
No. of test patients with high Ig levels	6 (0.07)*	—	33 (0.40)	83
No. of healthy controls with high Ig levels	2 (0.05)*	3 (0.08)*	2 (0.05)	40
Comparing the proportion of children with high Ig in the two study gorups				
χ^2 =	0.2205	6.3806	16.0817	
P =	> 0.2 (n.s.)	< 0.025	< 0.005	

None of the subjects had values lower than mean − 2 s.d. of controls.

*Proportions of subjects whose Ig levels are greater than the mean + 2 s.d. are in parentheses.

n.s. = Not significant.

Discussion

We observed that the IgG was only slightly higher in patients with joint pains than in controls. It is not known whether the production of elevated immune complexes demonstrated in patients with joint pains is at the expense of circulating IgG. Financial constraint did not permit the identification of the class of immunoglobulin in the immune complexes.

We observed depressed mean IgA levels and isolated deficiency of IgA levels in five of the patients with joint pains. Such depressed IgA levels have been reported in patients with frozen shoulder (Bulgen *et al.*, 1978). The significance of this finding in our study is uncertain. The most commonly associated disorders with depressed IgA are autoimmune disorders (Amman & Hong, 1971) including rheumatoid arthritis, systemic lupus erythematosus, thyroiditis pernicious anaemia and chronic active hepatitis. IgA synthesis is related to effective thymic control (Lancet, 1975). Thymectomy in neonatal life results in depressed IgA levels in experimental animals (Arnason *et al.*, 1964). It is possible that the depressed IgA production in these patients is due to depressed thymic activity or the development of autoimmunity (Allison *et al.*, 1971). As was speculated for IgG, the possibility of increased serum IgA consumption in the formation of soluble immune complexes by patients with joint pains can however not be ruled out.

IgM levels were observed to be high in the patients with joint pains. IgM concentration is known to be elevated following continuous and direct exposure to particulate antigens (Mattern *et al.*, 1961; Hobbs, 1970) including malaria (Abele *et al.*, 1965; Williams & McFarlane, 1970). The observation of high mean IgM level in patients with joint pains in this study is an indication of previous or current infections. It is not clear, however, whether the joint pains are causally related to infections. The patients were all assessed clinically and found not to have any infection at the time of study. We demonstrated a considerably higher concentration of immune complexes in patients with joint pains. In health, antigen bound to antibodies in the circulation are rapidly eliminated. It is possible that in the cases of patients with joint pains soluble antigens, which are formed as a result of parasitic infections such as malaria, (Lambert & Houba, 1974) combine with the antibodies to form complexes in the blood circulation. The reticulo-endothelial system may not be able to eliminate the free antigen or the complexes. These could diffuse into tissue, bind with free extravascular antibody and produce more insoluble aggregates that are incapable of diffusing out. For example, trapping of immune complexes have been demonstrated in collagenous joint tissues of immunized experimental animals (Jasin, 1975; Fox & Glynn, 1975). Some of the immune complexes may however remain localized in the surface layers of cartilage, ligaments and menisci (Cooke, Hurd & Ziff, 1972; Hollister & Mannik, 1974). The immune complexes activate complement which in turn causes the persisting inflammation (Rawson and Torralba, 1967; Hollister, Liang & Mannik, 1973). Another explanation is that the complexes are formed by previously sequestered antigen that has leaked into the systemic circulation, or as a consequence of local alterations in target tissues that render them susceptible to immunological attack by virus or viral products associated with the cell membrane. Further investigations are necessary to (a) determine the class of immunoglobulins present in the precipitated immune complexes and (b) identify any possible infective agents that could predispose the patients to having joint pains.

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