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# Soluble immune complexes and immunoglobulin (IgG, IgA and IgM) levels in Nigerians with primary liver cell carcinoma

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**Abstract**

Circulating soluble immune complexes and serum immunoglobulins (G,A and M) levels were determined in patients with primary liver cell carcinoma (PLCC) and healthy subjects by the polyethylene glycol precipitation and single radial immunodiffusion methods respectively. A considerably higher proportion of the patients than the controls had elevated levels of soluble immune complexes, IgG and IgM were significantly higher in the patients than the controls, that of IgA was lower. Correlation studies showed association between serum concentration of IgG, IgA and IgM and the levels of circulating soluble immune complexes. Several factors may influence our findings of elevated concentrations of soluble immune complexes and serum immunoglobulins G and M as well as the positive correlations between these indices. It could be as a result of increased rate of production and release of antigen from the tumour; enhanced interaction of antibody with membrane antigens at the tumour cell surface which promoted release of immune complexes or/and decreased rate of elimination of the complexes from the body of phagocytosis. That antibodies are required for the formation of immune complexes may explain our observation of increased levels of IgG and IgM.

**Resume**

Niveaux de complexes immune soluble circulant et serum immunoglobulines (C,A et M) ont ete determine on malades avec primaice Carcinoma de foie Cellules (PLCC) et sains sujects par le polyethylene glycol precipitation et seul radial immunodiffusion methods — respectivement. Un considerablement grandes proportions des maldes que les controles eurent niveaux eleve de complexes immune soluble. En sus pendant quo le moyenne concentrations de complexes immun soluble IgG and IgM etions significativement plus haut en des malades que des controles, cells de IgA a ete inferieuce. Etudes correlation a montre'l'association entre serum concentration de IgG, IgA et IgM et les niveaux de complexes immun soluble circulant. Plusieurs facteurs pouvez influence nos resultats de concentrations eleve de complexes immun soluble et serum immunoglobulins G et M, aussi bein que la correlation positive entre les indices. Cela pourrait etee comme un re resultat de augmente taut de la production et relacher de antigene de la tumeur; Interaction retiausse de anticorps avec membrane antigenes a la surface de cellule tumeur qui fait avancer relacher de complexes immun ou et decroissance taut de elimination de ces complexes de la corps par phagocytose. Ces anticorps etes exige pour la formation de complexes immun pouvez explique notre observation de niveaux augmente de IgG et IgM.

**Introduction**

Increased serum globulins and immunoglobulins in chronic liver disease have been described by several workers[1-4]. However, it has been shown that in some pathological conditions, the liver might be infiltrated by lymphocytes and plasma cells resulting in synthesis of markedly elevated immunoglobulin levels[4]. In none of these studies was the relationship between the most commonly determined immunoglobulins (IgA & IgM) and soluble immune complexes studied. Our study was undertaken to discover whether a possible correlation might exist between these parameters in primary liver cell

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carcinoma (PLCC), a common malignant disease in this environment[5,6]. The factors that may influence our findings are also discussed.

## Materials and methods

### *Patients*

Thirty six patients of mixed sexes aged 15-70 years (mean 48.5 years) with PLCC were studied at the Liver Unit of the University College Hospital, Ibadan, Nigeria. The patients were diagnosed on the basis of the clinical features such as icterus, ascites, hepatomegaly and histological findings of PLCC at liver biopsy as suggested by Scheuer[8]. The patients were not on any cytotoxic drugs at the time of study. Forty healthy volunteers aged 15-70 years (mean 46.3 years) served as controls. The patients and the control subjects were of the same socio-economic status.

### *Assays*

On admission, about 10ml of clotted blood was collected from each subject by venepuncture after an overnight fast. After the clot had retracted at room temperature the serum was separated by centrifugation. The samples for immunoglobulin measurements were kept at -20°C until analysed. The soluble immune complex levels were estimated immediately after serum separation.

### *Immune Complex*

Levels of circulating soluble immune complexes were assayed using the polyethylene glycol precipitation method of Haskova *et al*[9] as previously described[10]. 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 in 30 dilution of serum. After incubation at room temperature, the immune complex concentrations were measured at 450mY.

### *Immunoglobulin*

The immunoglobulin (G,A and M) levels were estimated by single radial immunodiffusion technique of Fahey and McKelvey[11] as modified by Salimonu *et al.*[12]. 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. The plates for IgG measurements were incubated at 37°C for 3 hrs. Those for IgA and IgM were placed at room temperature (18°C) for 18 hours. After incubation, the diameters of the precipitin rings were measured using a Hyland viewer with a micrometer eye piece.

## Results

### *Soluble immune complexes*

A very high proportion of patients (34 out of 36) with PLCC had elevated concentrations (> 12.15mg/100ml) of soluble immune complexes whilst none of the control subjects had increased levels. The differences in proportion between the two study groups are highly significant ($X^2$ = 68.3598, $p < 0.005$). There is also a wide scatter in values giving a mean that is about twenty three times higher than the control values. The differences in means are highly significant ($t$ = 6.6485 $p < 0.005$) as shown in Table 1.

Table 1: Mean (+ I.S.D.) levels of soluble immune complexes in patients with primary liver cell carcinoma

| | Patients with liver cell carcinoma | Control |
|---|---|---|
| *n* | 36 | 40 |
| Mean | 69.98 | 2.99 |
| l.s.d. | 60.3 | 4.58 |

Patients compared with control
$t$ = 6.6485
$P$ = < 0.005

### *Immunoglobulins*

The immunoglobulins G and M levels were significantly higher in patients with PLCC than in controls ($t$ = 4.4495, $p < 0.005$ and $t$ = 3.4313, $p < 0.001$ respectively) — Table 2. Ten out of 36 patients (28 per cent) had elevated IgG levels (> 2980 mg/100ml) whereas only one out of 40 (2.5 per cent)

of the control subjects had IgG levels that was more than the normal level (≤ 2980 mg/100ml). The difference in the proportions of subjects with elevated IgG levels in the two study populations is highly significant ($X^2$ = 9.7802, $p < 0.005$). Seventeen out of 36 (47 per cent) of the PLCC patients had blood IgM values higher than the normal levels whereas 2 out of 40 (5 per cent) of the control subjects had elevated values. This shows that a highly significant proportion of the patients had increased IgM levels ($X^2$ = 18.0148, $p < 0.005$). Unlike the findings in IgG and IgM concentrations, the IgA levels were significantly lower in patients with PLCC than in controls ($t$ = 2.6169 $p < 0.02$) (Table 2). Only one patient out of 36 (2.8 per cent) had IgA values greater than normal and none of the control subjects had elevated level of IgA.

**Table 2:** Mean (+ I.S.D.) Immunoglobulin (G, A & M) levels in patients with primary liver cell carcinoma

| | *n* | IgG | IgA | IgM |
|---|---|---|---|---|
| Patients with primary liver cell carcinoma | 36 | 2381 ± 968 | 308 ± 134 | 397 ± 399 |
| Healthy controls | 40 | 1493 ± 743 | 369 ± 159 | 166 ± 64 |
| Patients compared with controls | | | | |
| *t* | | = 4.4495 | = 2.6169 | = 3.4313 |
| *P* | | < 0.005 | < 0.02 | < 0.001 |

The proportion of subjects with elevated concentrations of IgA in both test patients and controls are therefore similar ($X^2$ = 1.1259 $p > 0.2$).

Correlation studies showed that there was correlation between levels of IgG and serum concentrations of soluble immune complexes in the test patients ($t$ = 2.052, $p < 0.05$) as shown in figure 1. Similarly there is also correlation between the IgA concentrations and levels of soluble immune complexes in the test patients ($t$ = 2.066, $p < 0.05$) — Figure 2. Figure 3 shows that there is good correlation between serum IgM levels in the test patients and their serum concentrations of soluble immune complexes ($t$ = 6.693 $p < 0.01$).

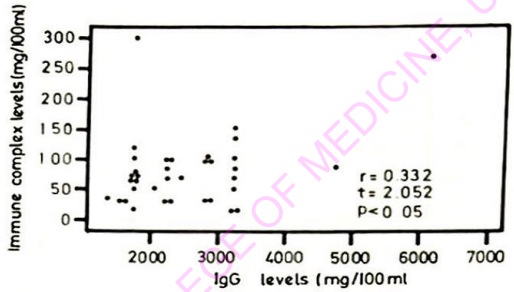


**Fig. 1:** Correlation between Immune complex and IgG levels in patients with primary liver cell carcinoma

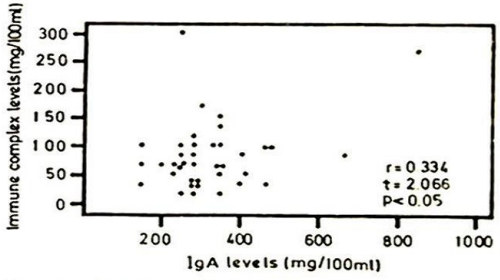


**Fig. 2:** Correlation between Immune complex and IgA levels in patients with primary liver cell carcinoma

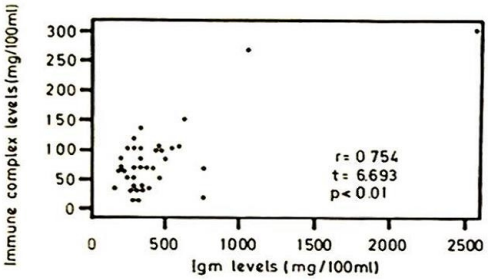


**Fig. 3:** Correlation between Immune complex and IgM levels in patients with primary liver cell carcinoma

## Discussion

The present study confirms our recent demonstration of elevated concentrations of soluble immune complexes in patients having PLCC[4]. These findings, using polyethylene glycol precipitation method correlated with the previous findings of Brown *et al.*[13] who employed both CIq and conglutinin solid phase assays. Levels of circulating immune complexes in tumour bearer serum have been determined using other assays which not only exploit the binding of these factors to CIq but also to receptors on Raji cells and all have shown elevated levels of circulating immune complexes[14-16].

Several previous investigations have indicated that measurement of blood levels of immune complexes may be of value as a prognostic indicator and particularly for monitoring tumour growth. This was originally suggested in studies on serum "blocking factors" which are known to interfere with the *in vitro* cytotoxicity of sensitized lymphoid cells for cultured tumour cells, since tumour — specific immune complexes are known to be involved in these effects[17,18].

Jennete and Feldman[19], using Raji cell radioimmunoassay reported that the concentrations of immune complexes were related to tumour growth in the early stages of growth. Follow up studies by Hoffken *et al*[20] demonstrated that employing CIq binding method of immune complexes was useful in monitoring the growth of experimental animal tumours. It is however difficult to evaluate to what extent (using the currently available methods) levels of immune complexes can be used to accurately measure the degree of tumour growth. A number of factors may affect the level of immune complexes in the sera of tumour bearing animals. These include

(i) rate of production and release of antigen from the tumour. This depends on the metabolic turn over and shedding of the antigen from the tumour as well as the degree of tumour necrosis and cell death;

(ii) the interaction of antibody with membrane antigens at the tumour cell surface may promote the release of immune complexes[21] and

(iii) the rate of elimination from the body by phagocytosis and deposition in renal glomeruli and other tissues[22,23].

Reports on the immunoglobulin levels in primary liver cell carcinoma have been conflicting. Our findings of elevated IgG and IgM levels confirm previous findings in patients in Thailand by Hitanant *et al*[24], and in South Africa negro patients[25]. In Singapore Chew *et al*[26] observed that the IgG and IgA levels were elevated 65 and 70 per cent respectively whereas the IgM levels were reported low. Studies by Fahey[27] demonstrated increased IgG level, normal IgA and low IgM levels in six cases of PLCC. Primack *et al*[28] observed no increase in serum immunoglobulin levels whilst Hirayama *et al*[29] reported raised levels. Recent studies by Ayoola and Olubuyide[30] showed that the immune complexes of these patients are formed with antibodies of the IgG and IgM classes. Our findings of elevated IgG and IgM concentrations and their close correlation with the soluble immune complex levels in the blood would indicate that, in this liver disease considerabe increased quantities of IgG and IgM antibodies are being produced, some of which are being complexed with the specific antigen.

The mechanism that induces increases in serum immunoglobulins and immune complexes in this disorder is not understood but can be speculated. It is possible that damaged liver cells unmasked liver injury releases antigen which stimulates antibody (immunoglobulin) synthesis[31]. The antibody combines with the antigen causing the formation of

immune complexes. For example, presence of serum antibody, antigen and immune complexes hae been reported in experimental animals during the growth of hepatoma tumours[32]. In addition, an association between elevated serum immunoglobulin levels and presence of Hb-Ag have been reported in the serum of patients with PLCC[28]. Our study shows the same correlation coefficient between soluble immune complexes on the one hand and IgG ($r$ = 0.332) and IgA (r = 0.334) on the other hand. This findings suggests that same fractions of IgG and IgA are involved in complexes in PLCC. Further studies are required to confirm this speculation.

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