PLASMA PROTEINS IN CONGOLESE KELOIDAL INDIVIDUALS. CORRELATIONS BETWEEN IMMUNOGLOBULINS, COMPLEMENT FRACTIONS AND ANTISKIN ANTIBODIES

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Résumé

OBJECTIF. Déterminer le rôle des protéines plasmatiques (IgA, IgG, IgM, des fractions du complément C3 et C4) et des anticorps contre la peau chez des sujets congolais souffrant de chéloïdes.

SUJETS ET METHODES. 17 patients chéloïdiens (12 femmes et 5 hommes) et 15 sujets normaux (8 femmes et 7 hommes) ont été enrôlés dans l'étude. L'âge moyen de l'échantillon était de 31.72 ± 12.04 ans (30.35 ± 12.06 pour les femmes et 34.00 ± 12.17 pour les hommes). Les IgA, IgG, IgM, C3 et C4 étaient déterminés par néphélémétrie. Les anticorps contre la peau étaient mesurés par immunofluorescence indirecte. Les paramètres biologiques étaient mesurés dans le sang périphérique suivant les techniques habituelles : les méthodes de Drabkin, de Westergreen et de Biuret respectivement pour l'hémoglobine, la vitesse de sédimentation et le fibrinogène. Les valeurs de référence étaient celles rencontrées dans la population congolaise normale. L'étude de la régression logistique a permis de disposer de statistiques descriptives et des corrélations. Les données entre les sujets normaux et les sujets atteints ont été comparées quant au sexe et à l'âge.

RESULTATS. Les valeurs des immunoglobulines étudiées étaient exprimées en gr/l et se présentaient comme suit : IgA: 1.933 + 0.538 dans la population normale contre 2,1624 + 0,696 chez les chéloïdiens. IgG: 17,4933 ± 2,7009 dans la population normale contre 19,1059 + 3,5245; IgM: 1,2947 ± 0.3344 versus 1,6053 ± 0,5843 chez les chéloïdiens; C3: 1,1213 + 0,2411 dans la population normale versus 1,3547 ± 0,3216 chez les chéloïdiens ; C4: 0,2787 ± 0,063 dans la population normale versus 0,3676 ± 0,1695 chez les chéloïdiens. Les anticorps contre la peau étaient présents chez deux sujets : une personne chéloïdienne et une autre normale. Chez les patients chéloïdiens, les corrélations semblaient positives et significative entre les âges, les fractions du complément C3 et C4 et significatives mais négatives entre IgM et la fraction C3 du complément.

A âge égal, le profil des sujets chéloïdiens est significativement différent de celui des normaux (p = 0.025). Cette différence est due essentiellement aux taux d'IgG (p = 0.046). Les taux de C3 semblaient élevés chez les chéloïdiens par rapport à la population normale (p = 0,03).

Aucune autre différence n'a été trouvée entre les chéloïdiens et les sujets normaux en ce qui concerne les taux des IgA, IgM et C4.

La différence de taux moyen observée en analyse uni variée entre les sexes (F vs M : 1.59 ± 0,52 vs 1,24 ± 0,39 ; p = 0,054) s'est annulée en analyse multi variée lorsqu l'effet de l'âge a été contrôlé (1,56 ± 0,109 vs 1,30 ± 0,14 ; p = 0,159)

CONCLUSION. Ces résultats suggèrent que parmi les protéines plasmatiques étudiées, les IgG affectent la maladie chéloïdienne de façon significative.

Mots-clés : Chéloïdes, Protéines plasmatiques, Anticorps contre la peau, Corrélations

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ABSTRACT

Objective. To look for the role of plasma proteins (IgA, IgG, IgM, complement fractions C3 and C4) and antiskin antibodies in keloidal Congolese individuals.

Subjects and methods. 17 keloidal patients (12 females and 5 males), 15 non keloidal subjects (8 females and 7 males) were admitted in this study. Mean age of the whole group was 31,72 ± 12,72 years (30,35 ± 12.06 for females and 34,00 ± 12,17 for males). IgA, IgG, IgM, C3 and C4 were determined by nephelemetry. Antiskin antibodies were determined by indirect immunofluorescence. Biological parameters were measured in peripheral blood according to Drabkin, Westergreen and Biuret methods for haemoglobin, erythrocyte sedimentation rate and fibrinogen respectively. Reference values were those usually found in normal Congolese population. Logistic regression studies provided descriptive statistics, correlation and regression curves and their equation. Data were matched as to sex, age and clinical status.

Results. Mean values of Immunoglobulins expressed as g/l were as follows IgA: 1.933 + 0.538 in normal population versus 2,1624 ± 0,696 in keloidal patients. IgG: 17,4933 ± 2,7009 in normal population versus 19,1059 + 3,5245 in keloidal patients; IgM: 1,2947 ± 0.3344 in normal population versus 1,6053 ± 0,5843 in keloidal; C3: 1,1213 + 0,2411 in normal population versus 1,3547 + 0,3216 in keloidal ; C4: 0,2787 ± 0,063 in normal population versus 0,3676 ± 0,1695 in keloidal patients. Antiskin antibodies were present in 2 subjects: one keloidal and one normal.

In keloidal patients, correlations seemed positive and significant between ages, complement fractions C3 and C4 and significant but negative between IgM and complement fraction C3.

At matched age keloidal subjects profile was significantly different with normal (p = 0.025). The difference was due to IgG levels (p = 0.046).

No further difference was disclosed concerning IgA, IgM and C4 levels between keloidal and non keloidal subjects. However C3 was elevated in keloidal as compared to non keloidal population (p = 0,03). The mean IgM level observed between sexes (F versus M : 1,59 + 0,32 vs. 1,24 + 0,39 ; p = 0,054), was cancelled when age effect was controlled (1,56 + 0,109 vs. 1,30 + 0,14 ; p = 0,159).

Conclusion. These results suggest that IgG levels are altered in keloidal subjects, other parameters remaining normal.

Key-words: Keloids, Plasma proteins, Antiskin antibodies, Correlations

INTRODUCTION

The role of plasma proteins in fibroproliferative disorders such hypertrophic scars and keloid is still subject of controversies. It has been shown that plasma levels of IgG (Kazeem, 1988), IgM and complement fraction C3 (Bloch *et al*, 1984) were higher in keloidal patients than in normal individuals, whereas IgA and complement fraction C4 (Kazeem, 1988; Bloch, 1984) and C3 levels (Kazeem, 1988) were found to be lower.

Some authors found that plasma levels of IgG (Cohen *et al*, 1979), IgM, C3 and C4 (Cohen *et al*, 1979) were not altered in keloidal subjects.

It is unanimously admitted that IgG levels (Cohen *et al*, 1979), IgA, IgG and IgM levels (Kischer *et al*, 1983) are higher in keloidal tissue and hypertrophic scars suggesting attritional leakage of several plasma proteins from microvasculature in the lesions.

Congolese population is characterized by hypergammaglobulinemia (Michaux, 1966; Mbuyi *et al*, 1982) and hypercomplementemia (Mbuyi *et al*, 1980). Likewise but at lesser level this has been shown in Afro-Americans (Thuma, 1976). Although reasons for such differences remain ununderstood, these high levels may be attributed to malnutrition (Chandra, 1975; Haller *et al*, 1978), to malaria (Coombs and Coombs, 1953; Cohen *et al*, 1961; Cohen *et al*, 1969) and to poor hygiene, milieu and food conditions (Turner and Voller, 1966).

Some authors suggest that keloid might be an autoimmune disease, because antibodies against fibroblasts have been isolated from peripheral blood of some patients (De Limpens and Cormane, 1982). Yet, it is suggested that cell-mediated, major complex histo-compatibility complex-class II restricted immune response play an important role in the development of hypertrophic scars and keloids (Santucci *et al*, 2001). At least, it has been shown that keloids are more frequent in black skinned population, the incidence varying between continents and human races (Moreno *et al*, 1986; Brown and Pierce, 1976; Owulasanmi, 1974; Yagi *et al*, 1979; Ramakrishnan, 1974). Considering this frequency of keloids in black Africans, we initiated the present study in order to evaluate whether or not plasma proteins (immunoglobulins, complement fractions and antiskin antibodies) may be affected by keloids.

Therefore, IgA, IgG, IgM, C3, C4 and antiskin antibodies were assayed in peripheral blood of keloidal patients and compared to normal values in the non keloidal congolese population.

MATERIAL AND METHODS

Subjects

Thirty two subjects aged 12 to 58 years (mean 31.72 + 12.04) were included in the study. Seventeen (5 males and 12 females) were keloidal and aged 12 to 55 years (mean 34.9 + 12.3). Control group was composed with 15 normal subjects (7 males and 8 females) aged 13 to 44 years (mean 28.06 ± 7.8). The two groups were matched for sex and age.

Material

Blood samples were obtained by venopuncture and allowed to stay at room temperature until clotting. Serum was obtained by centrifugation at 3000 rpm during 15 minutes and then kept at -20°C until transportation in an isotherm bag to the Laboratory of Immunology (Prof Bossuyt), University Hospital, Leuven, Belgium.

Methods

IgA, IgG, IgM, complement fractions C3 and C4 were assayed by laser nephelemetry (Immage, Beckman-Coulter, Brea, CA). Antiskin antibodies were determined by indirect immunofluorescence using esophagus as substrate (Immco, Buffalo NY). Other parameters were measured in peripheral blood according to usual methods: haemoglobin according to Drabkin, erythrocyte sedimentation rate according to Westergreen and fibrinogen according to Biuret or Podmore.

Statistics

Data were recorded on SPSS 10.1 for Windows. Statistical analysis (parametric

and non-parametric) was done for the whole group and for the subgroup of females. Logistic regression studies provided descriptive statistics and correlations (determination coefficient *r* and Pearson® correlation coefficients). The Student *t* test was used for comparison of the means.

RESULTS

A. Immunoglobulins and complement fractions. Raw data expressed as g/l are shown in table 1 for normal population and on table 2 for keloidal patients with the corresponding age and sex.

Mean age was 31,72 + 12,04 years (30,35 + 12,06 years in women and 34,00 + 12,17 years in males. There was no significant difference between males and females regarding age (table 3).

Mean levels of immunoglobulins assayed were as follows. IgA: 1,9033 ± 0,5738 in normal population versus 2,1624 + 0,6966 in keloidal patients; IgG: 17,4933 ± 2,7009 in normal population vs. 19,1059 + 3,5245 in keloidal; IgM: 1,2947 ± 0,3344 in normal population vs.1,6053 + 0,5843 in keloidal; C3: 1,1213 ± 0,2411 in normal population vs.1,3547 + 0,3216 in keloidal and finally C4: 0,2787 ± 0,063 in normal population vs. 0,3676 ± 0,1695 in keloidal.

Descriptive statistics are given in table. They are done for sex and clinical status, i.e. keloidal or normal (table 4).

With ANOVA, IgM seemed higher in female than in men (table 5). The p (0,054) approached but did not reach the meaning threshold.

Considering clinical status, C3 seemed higher in keloidal female than in normal (p = 0,029) (table 6).

Correlations seemed significant at 0,05, level between IgA and age, IgG and age, IgM and C3, C4 and C3 and at 0,01 level between C3 and age,C3 and IgA (table7)
Logistic regression (table 8) showed that the main significant factor was clinical status (p = 0,025) for IgG, but not sex (p = 0,056). Intercept showed a background sound that had to be searched for.

B. Biological parameters are summarized in table 10. Even for long term keloids, haemoglobin, leucocytes and fibrinogen were in a normal range. Only erythrocyte sedimentation rate was higher in infected keloidal patients than in normal population or in non infected keloids.

C. Antiskin antibodies
Antiskin antibodies were present in only 2 cases: a man aged 55 years and a girl aged 19 years.

DISCUSSION

The aim of this study was to evaluate the role of immunoglobulins and complement fractions in keloidal patients. Our results disclosed a statistical significant difference for IgG. Indeed, it is shown that at matched age, keloidal subjects profile discloses a significant difference with normal (p = 0.025). Considering parameter by parameter, this difference is due to IgG levels. (p = 0.046). Other parameters did not show significant difference within the two groups. Linear projection demonstrated mean values (± standard deviation) of IgG within the 2 groups adjusted for 31.72 years (keloidal versus normal: 19.7 + 0.85 versus 17.2 + 0.8 g/l).

The difference between the mean levels of IgM (F versus M : 1,59 + 0,32 vs. 1,24 + 0,39 ; p = 0,054), was cancelled when age effect was controlled (1,56 + 0,109 vs. 1,30 + 0,14 ; p = 0,159).

Many attempts have been made in order to describe immunologic profile of keloidal patients. In Nigeria, Kazeem (1988) noted that IgG was higher, C3 and C4 lower in keloidal patients than in normal population. In the United States, Bloch and co-workers (1984) showed that IgM and C3 were in higher level and C4 in lower concentration in keloidal patients than in normal population. These authors, as Yagi (1979) supported that factors implicated in keloidal tissues might influence immunologic parameters. Cohen *et al*, (1979) did not find differences between normal subjects and keloidal in their immunoglobulins and complement fractions content.

Our results suggest that hypergammaglobulinemia and hypercomplementemia encountered have direct impact on keloidal

high incidence in our populations. Indeed, there is a wide variability in immunoglobulins and complement fractions between human races on the different continents. Even in normal state, unfavourable hygiene, milieu and nutritional conditions seem to play a great role in the antigenic stimulation (Michaux, 1966; Neumann *et al*, 1973). Individual variations exist as well: IgA concentration for example may vary from 1 to 10 (Bachmann et Laurell, 1965). Our results demonstrated in normal control a significant increase in IgM in accordance with Michaux (1966). Moreover, the latter demonstrated for IgG minimal and not significant differences between men and women. In another study on Bantu in Kinshasa, Mbuyi *et al* (1980) showed that IgA and IgG concentrations were higher in men in a significant manner. They also showed that C3, C4 and C5 rates did not depend on sex or age, the only significant factor being the period of sampling. IgG levels are elevated in many chronic conditions, including *S mansoni* and *S haematobium* (Madwar, 1978), myeloma, and hepatic chronic disease.

REFERENCES

1. Bachmann R, Laurell CB. Study on serum γ-1A-globulin level. Healthy subjects and hypergammaglobulinemic patients. *Scand Clin Lab Invest* 1965; **17**:39-45.
2. Bloch FF, Holl MG, Benson MJ, Slay Solomon. General Immune reactivity in keloid Patients. *Plast Reconstr Surg* 1984; **73**: 448-451.
3. Chandra RK. Serum complement fraction and immunoglobulins in malnutrition. *Arch Dis Chilhd* 1975; **50**: 225-229.
4. Cohen IK, Mc Coy BJ, Mokanakumar T, Diegelman RF. Immunoglobulins, complement and histocompatibility antigens study in keloid patients. *Plast Reconstr Surg* 1979; **63**: 689-695.
5. Cohen S, McGregor IAA, Carrington S. Gammaglobulin and acquired immunity to human malaria. Nature1961; 193:733-736.
6. Cohen S, Bulcher GA, Grandall RB. Action of malaria antibody *in vitro*. *Nature* 1969; **22**:368-371.
7. Coombs AM, Coombs RRA. The conglutination Phenomenon. IX. The production of immunoconglutinin in rabbits. *J Hyg* 1953; **51**: 509-512.
8. De Limpens AMP, Cormane RH. Studies on the immunologic aspects of keloids. *Arch Dermatol Res* 1982; **274**: 259-266.
9. Dow J, Lindsay G, Morrison J. Biochemistry: Molecules, Cells and the Body. Addison Wesley, Ed. 1996: 483-484.
10. Halle L, Zubler PH, Lambert PH. Plasma levels of complement components and complement haemolytic activity in protein-energy malnutrition. *Clin exp Immunol* 1978; **34**: 248-252.
11. Kischer CW, Shetlar MR, Shetlar CL, Chvapil M. immunoglobulins in hypertrophic scars and keloids. *Plast Reconstr Surg* 1983; **71**: 821-825.
12. Madwar JW, O'Shea JM, Skelton JA, Soothill JF. Complement components and immunoglobulins in patients with schistosomiasis. *Clin exp Immunol* 1978; **34**: 354-358.
13. Mbuyi-Muamba JM, Tshiani A, Nyomba G, Muyembe T, Bloemmen F, Stevens E. Etude du taux normal du complément dans une population de Kinshasa, Zaïre. Influence du sexe, de l'âge et des périodes de prélèvement des sérums. *Ann Soc Belge Med trop* 1980 ; **60** :387-393.
14. Mbuyi-Muamba JM, Tshiani A, Nyomba G, Muyembe T, Bloemmen F, Stevens E. Nephelometric determination of normal serum immunoglobulin levels in adult Bantu from Zaire. *Ann Soc Belge Med trop* 1982 ; **62** :253-258.
15. Michaux JL. Les immunoglobulines des Bantu à l'état normal et pathologique. Thèse. *Ann Soc Belge Med trop* 1966 ; **46** : 483-674.
16. Neumann CG, Glen JL, Stiehm ER, Swenseid ME, Herbert J, Amman AJ, Jacob M. Immunologic responses in malnourished children. *Am J Clin Nut* 1975; **28**: 89-104.
17. Santucci M, Borgognoni L, Reali UM, Gabbiani G. Keloids and hypertrophic scars of Caucasians with distinctive morphologic and immunophenotypic profiles. *Virchows Arch* 2001; **438**: 457- 463.
18. Smith CJ, Payne VM, Scott SM, Luterman A. Immunoglobulin E level and anticollagen antibodies in patients with postburn hypertrophic scars. *J Burn Care Rehabil* 1997; **18**: 411-416.
19. Thuma RS. Determination of normal levels of 12 serum proteins by single radial diffusion. I. Influence of race and sex. Meloy Laboratories Inc, Biological products division, 6715 Electronic Drive, Springfield, 1976.
20. Turner MW, Voller A. Studies on immunoglobulins of Nigerians. Part I. The immunoglobulin level of a Nigerian population. *J trop Med Hyg* 1966; **69**: 99-104.
21. Yagi KI, Dafalla AA, Osman AA. Does an immune reaction to sebum in wound cause keloid scar? Beneficial effect of desentization. *Br J Plast Surg* 1979; **32**:223-225.

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ANNEXES

Table 1. Immunoglobulins levels and complement fractions C3 and C4 in normal subjects

Patient n°	Age (years)	Sex	IgA	IgG	IgM	C3	C4
1	30	M	1.69	16.3	0.84	1.19	0.27
2	44	M	1.79	13.8	1.21	1.03	0.26
3	29	M	1.1	12.3	0.95	1.16	0.29
4	30	M	1.72	19.4	1.05	1.12	0.31
5	33	M	3.26	16.9	0.86	1.7	0.37
6	13	M	2.55	19.9	1.03	0.86	0.3
7	24	M	1.72	14.4	1.48	1.12	0.31
8	38	F	1.24	18.4	1.28	1.24	0.31
9	32	F	2.42	16.8	1.05	1.56	0.38
10	28	F	1.74	20.8	1.75	0.94	0.18
11	25	F	1.75	17.3	1.59	1.15	0.15
12	29	F	2.31	18.9	1.79	1.05	0.22
13	29	F	2.00	16.2	1.6	0.95	0.23
14	20	F	2.15	21.6	1.22	0.88	0.28
15	17	F	1.12	19.8	1.72	0.87	0.32

The results were expressed as g/l (tables 1 and 2)

Table 2. Immunoglobulins levels and complement fractions C3 and C4 in keloidal patients

Patient n°	Age (years)	Sex	IgA	IgG	IgM	C3	C4
1	43	M	2.46	16.1	1.38	1.48	0.37
2	43	M	2.09	23.3	1.5	1.64	0.35
3	54	M	2.78	16.9	1.21	1.09	0.17
4	46	M	2.02	22.9	1.1	1.56	0.43
5	19	M	1.97	22.7	2.28	1.33	0.234
6	45	F	2.42	17.6	2.07	1.22	0.32
7	23	F	1.54	26.1	1.76	1.01	0.22
8	16	F	1.43	16.1	1.58	0.97	0.20
9	22	F	1.73	14.5	1.22	1.3	0.25
10	46	F	4.15	21.7	0.72	2.18	0.58
11	29	F	2.50	19	1.44	1.18	0.29
12	55	F	1.86	17.1	1.57	1.5	0.37
13	27	F	1.67	23.6	3.29	0.76	0.11
14	52	F	3.06	18.5	2.05	1.61	0.65
15	22	F	1.59	15.7	1.117	1.38	0.21
16	12	F	2.04	17.5	1.64	1.29	0.39
17	40	F	1.45	15.5	1.36	1.51	0.44

Table 3. Comparison between Ages, IgA, IgG, IgM, C3, C4 and SEX

SEX		AGE	IgA	IgG	IgM	C3	C4
F	Mean	30,35	2,0085	18,6350	1,5910	1,2285	0,3385
	N	20	20	20	20	20	20
	SD	12,06	0,6943	2,9258	0,5212	0,3335	0,1650
M	Mean	34,00	2,0950	17,8750	1,2408	1,2733	0,3050
	N	12	12	12	12	12	12
	SD	12,17	0,5794	3,7492	0,3950	0,2654	6,974E-02
Total	Mean	31,72	2,0409	18,3500	1,4597	1,2453	0,3259
	N	32	32	32	32	32	32
	SD	12,04	0,6453	3,2209	0,5015	0,3060	0,1367

Table 4. Descriptive statistics

	SEX	CLIN	MEAN	SD	N
IgA	F	1	1,8413	,4744	8
		2	2,1200	,8096	12
		Total	2,0085	,6943	20
	M	1	1,9743	,7033	7
		2	2,2640	,3467	5
		Total	2,0950	,5794	12
	Total	1	1,9033	,5738	15
		2	2,1624	,6966	17
		Total	2,0409	,6453	32
IgG	F	1	18,7250	1,9263	8
		2	18,5750	3,5235	12
		Total	18,6350	2,9258	20
	M	1	16,0857	2,8910	7
		2	20,3800	3,5598	5
		Total	17,8750	3,7492	12
	Total	1	17,4933	2,7009	15
		2	19,1059	3,5245	17
		Total	18,3500	3,2209	32
IgM	F	1	1,5000	,2784	8
		2	1,6517	,6402	12
		Total	1,5910	,5212	20
	M	1	1,0600	,2238	7
		2	1,4940	,4655	5
		Total	1,2408	,3950	12
	Total	1	1.2947	,3344	15
		2	1,6053	,5443	17
		Total	1,4597	,5015	32
C3	F	1	1,0800	,2337	8
		2	1,3275	,3613	12
		Total	1,2285	,3335	20
	M	1	1,1686	,2589	7
		2	1.4200	,2171	5
		Total	1,2733	,2654	12
	Total	1	1,1213	,2411	15
		2	1,3457	,3216	17
		Total	1,2453	,3060	32
C4	F	1	,2588	7,736E-02	8
		2	,3917	,1884	12
		Total	,3385	,1650	20
	M	1	,3014	3,579E-2	7
		2	,3100	,1068	5
		Total	,3050	6,974E-2	12
	Total	1	,2787	6,346E-2	15
		2	,3676	,1695	17
		Total	,3259	,1367	32

F = female; M = male; 1 = normal population; 2 = keloidal patients

Table 5. ANOVA

			Sums of squares	Degree of freedom	Means of squares	F	p
AGE*SEX	Intergroup	Combined	99.919	1	99.919	,682	0,415
	Intraclass		4392.550	30	146.418		
	Total		4492.469	31			
IgA*SEX	Inter group	Combined	.056	1	.056	,131	0,720
	Intraclass		12.851	30	.428		
	Total		12.907	31			
IgG*SEX	Inter group	Combined	4.332	1	4.332	,410	0,527
	Intraclass		317.268	30	10.576		
	Total		321.600	31			
IgM*SEX	Inter group	Combined	,920	1	.920	4,011	**0,054**
	Intraclass		6,878	30	.229		
	Total		7,797	31			
C3*SEX	Intergroup	Combined	,015	1	.015	,157	0,695
	Intraclass		2,888	30	.096		
	Total		2,903	31			
C4*SEX	Intergroup	Combined	0,008	1	.008	,442	0,511
	Intraclass		,571	30	.019		
	Total		,579	31			

Table 6. Tableau ANOVA AGE, IgA, IgG, IgM, C3, C4*CLIN

			F	p
AGE*CLIN	Total	Combined	2.745	0,108
IGA*CLIN	Total	Combined	1,296	0,264
IGG*CLIN	Total	Combined	2.066	0,161
IGM*CLIN	Total	Combined	3,282	0,80
C3*CLIN	Total	Combined	5.274	**0,029**
C4*CLIN	Total	Combined	3.668	0,065

Table 7. Correlations between age, Immunoglobulins and complement fractions C3 and C4

		Age	IgA	IgG	IgM	C3	C4
Age	Pearson	1.000	**.398***	-.062	-.105	**.536****	.322
	Sig.(bilateral)		.024	.734	.568	.002	.072
	N		32	32	32	32	32
IgA	Pearson	**.398***	1.000	.167	-.201	**.583***	.345
	Sig.(bilateral)	.024	.	.361	.270	.000	.053
	N	32	32	32	32	323	32
IgG	Pearson	-.062	.167	1.000	**.382***	-.009	.257
	Sig.(bilateral)	.734	.361	.	.031	.960	.156
	N	32	32	32	32	32	32
IgM	Pearson	-.105	-.201	**.382***	1.000	-.373	**.386***
	Sig.(bilateral)	.568	.270	.031	.	.036	.029
	N	32	32	32	32	32	32
C3	Pearson	**.536****	**.583****	-.009	**-.373***	1.000	.386
	Sig.(bilateral)	.002	.000	.960	.036	.	.029
	N	32	32	32	32	32	32
C4	Pearson	.322	.345	.257	.331	**.386***	1.000
	Sig.(bilateral)	.072	.053	.156	.064	.029	.
	N	32	32	32	32	32	32

*: the correlation is significant at 0.05 level (bilateral); **: the correlation is significant at 0.01 level (bilateral).

Table 8. Logistic regression

Effect		Value	E	df	de error	p
Intercept	Pillai	,926	57,356[a]	5,000	23,000	,000
	Lambda Wilks	,074	57,356[a]	5,000	23,000	,000
	Hotelling	12,469	57,356[a]	5,000	23,000	,000
	Roy	12,469	57,356[a]	5,000	23,000	,000
AGE	Pillai	,316	2,130[a]	5,000	23,000	,098
	Lambda Wilks	,684	2,130[a]	5,000	23,000	,098
	Hotelling	,463	2,130[a]	5,000	23,000	,098
	Roy	,463	2,130[a]	5,000	23,000	,098
SEX	Pillai	,087	,436[a]	5,000	23,000	,819
	Lambda Wilks	,913	,436[a]	5,000	23,000	,819
	Hotelling	,095	,436[a]	5,000	23,000	,819
	Roy	,095	,436[a]	5,000	23,000	,819
CLIN	Pillai	,409	3,186[a]	5,000	23,000	**,025**
	Lambda Wilks	,591	3,186[a]	5,000	23,000	**,025**
	Hotelling	,693	3,186[a]	5,000	23,000	**,025**
	Roy	,693	3,186[a]	5,000	23,000	**,025**
SEX*CLIN	Pillai	,357	2,559[a]	5,000	23,000	**,056**
	Lambda Wilks	,643	2,559[a]	5,000	23,000	**,056**
	Hotelling	,556	2,559[a]	5,000	23,000	**,056**
	Roy	,556	2,559[a]	5,000	23,000	**,056**

a: exact statistics
b PLAN: INTERCEPT+AGE+SEX+CLIN+SEX*CLIN

Table 9. Inter subjects effects Tests
It shown (table 9) that age is the confusing factor

SOURCE	Dependent variable	Sum of squares(type III)	Df	Mean of the squares	F	p
CORRECTED MODEL	IgA	2,195[a]	4	0,549	1,383	0,266
	IgG	68,717[b]	4	17,179	1,834	0,151
	IgM	1,798[c]	4	0,450	2,023	0,119
	C3	1,022[d]	4	0,256	3,669	0,016
	C4	0,143[e]	4	3,568E-02	2,207	0,095
INTERCEPT	IgA	6,414	1	6,414	16,166	0,000
	IgG	1317,161	1	1317,161	140,631	0,000
	IgM	9,071	1	9,071	40,826	0,000
	C3	2,453	1	2,453	35,214	0,000
	C4	0,129	1	0,129	7,991	0,000
AGE	IgA	1,521	1	1,521	3,835	0,061
	IgG	10,491	1	10,491	1,120	0,299
	IgM	0,219	1	0,219	0,984	0,330
	C3	**0,529**	**1**	0,529	**7,590**	**0,010**
	C4	4,928E-02	1	4,928E-02	3,049	0,092
SEX	IgA	8,823E-03	1	8,823E-03	0,022	0,883
	IgG	0,150	1	0,150	0,016	0,900
	IgM	0,467	1	0,467	2,100	0,159
	C3	6,196E-03	1	6,196E-03	0,089	0,768
	C4	9,972E-03	1	9,972E-03	0,617	0,439
CLIN	IgA	8,205E-02	1	8,205E02	0,207	0,653
	IgG	**40,475**	**1**	40,475	**4,321**	**0,047**
	IgM	0,815	1	0,815	3,666	0,066
	C3	0,141	1	0,141	2,025	0,166
	C4	1,020E-02	1	1,020E-02	0,631	0,434
SEX*CLIN	IgA	2,772E-02	1	2,772E-02	0,070	0,794
	IgG	**40,920**	**1**	40,920	**4,369**	**0,046**
	IgM	0,198	1	0,198	0,891	0,354
	C3	1,031E-02	1	1,031E-02	0,148	0,703
	C4	3,931E-02	1	3,931E-02	2,432	0,131

a. R^2 = 0,170 (adjusted R^2 = 0,047); b. R^2 = 0,214 (adjusted R^2 = 0, 097); c. R^2 = 0,231 (adjusted R^2 = 0,117)
d. R^2 = 0,352 (adjusted R^2 = 0,256); e. R2 = 0,246 (adjusted R^2 = 0,135)

Table 10. Biological parameters

Parameter	Non infected Keloids (n = 16)	Infected keloids (n = 16)	p
Haemoglobin (gr %)	13.24 ± 2.07	11.96 ± 2.6	Ns
Leucocytes (/mm³)	6201 ± 1778	6516 ± 1105	Ns
ESR (mm/h)	6.3 ± 4.5	43.85 ± 18.36	<0.01
Fibrinogen	299.17 ± 93.2	311.2 ± 101.88	Ns

n = number of cases
ESR = erythrocyte sedimentation rate
Ns = not significant