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STUDIES ON COLD INSOLUBLE GLOBULIN IN DERMATOLOGICAL PATIENTS

II. *Its Immunochemical Quantitation in Citrated Plasma in a Group of Unselected Dermatological Inpatients*

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Abstract. Cold insoluble globulin (CIG) is the main non-clottable protein of heparin precipitable fraction from dermatological patients. CIG is a glycoprotein with a molecular weight of about 440 000. It is found on cell membranes on fibroblasts and is a normal protein of human plasma and serum. Levels of CIG in citrated plasma are sex- and age-dependent, but the origin and its physiological significance is unknown. In plasma of 294 dermatological patients, levels of CIG were elevated in eczema contactum allergicum (males and females 30-49 years), psoriatic arthritis (females 50 years and above) and were depressed in dermatitis herpetiformis (males 30–49 years).

Key words: Cold insoluble globulin; Healthy adults; Sex; Age; Dermatological patients

After injections of bacterial endotoxin into rabbits, producing a generalized Shwartzman reaction, the appearance of a cryoprecipitate in heparinized plasma (which was not found in serum) was reported by Thomas et al. in 1954 and named 'heparin precipitable fraction' (21). This cryofraction can also be found in normal persons, but is increased in certain diseases, most of them with an inflammatory component, and often combined with arthritis or tissue necrosis (4, 20). In dermatology, increased amounts of heparin precipitable fraction have been described in psoriatic arthritis, Reiter's disease and in other diseases (5, 18). Studies on heparin precipitable fraction from dermatological patients, mostly with psoriatic arthritis, have demonstrated the presence of a thrombin-clottable portion identified as normal or insignificantly changed fibrinogen (8), and a non-clottable part containing one major protein identified as cold insoluble globulin (CIG) (6).

CIG was first described by Morrison et al. in 1948 and isolated from pooled citrated plasma as Cohn fraction I (14). The protein has been further characterized chiefly by Mosesson et al. (2, 15, 16, 17). Clinical studies on CIG in plasma have demonstrated a surprisingly constant concentration of CIG in different disease states (1, 10, 11), an increase of CIG in plasma being reported only in recurrent cholestasis of pregnancy (3).

We have previously investigated CIG in heparin precipitable fraction from dermatological patients and its concentration in citrated plasma from dermatological patients demonstrating high levels of heparin precipitable fraction (6, 8). The aim of the present study was to investigate the concentration in plasma in an unselected group of dermatological patients as compared with a group of healthy adults.

MATERIALS

Citrated plasma

Blood samples were drawn from fasting donors from the antecubital vein under stasis and collected with the Vacutainer system technique obtaining 5 ml citrated blood containing 10% of 3.8% sol. sodium citrate and 0.2 mg/ml potassium sorbate as antimycoticum. This was centrifuged at 2 400 *g* at 20°C, and the separated plasma used directly or stored at −25°C for some days.

Reference group

Ten healthy adults (mean age 33, range 19–60 years), 6 women (mean age 28.6, range 19-44 years) and 4 men (mean age 39.6, range 29–60 years), served as a reference group for levels of CIG in citrated plasma. Equal amounts of citrated plasma from these persons were pooled into

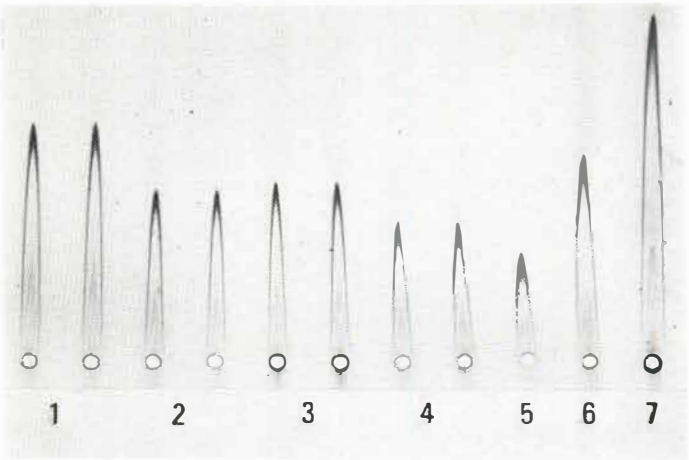

Fig. 1. Electroimmunoassay for quantitita-tion of cold insoluble globulin (CIG) in citrated plasma in 1% agarose containing 0.1% absorbed antiserum against CIG. 1–4: Patient plasmas (20% dilutions) with two paralels from each plasma. 5–7: Reference plasma dilutions containing 10, 25 and 50 U/ml of CIG. The main peak of the "rock-ets" represents CIG, the small peak factor VIII related antigen (7).

one plasma pool serving as reference sample for all runs. For the present study, one unit (U) of CIG was defined as one-hundredth of the amount of CIG present in 1 ml of this citrated plasma, i.e. 1 ml of the reference plasma contained 100 U CIG.

Control group

Sixty-seven healthy adults (mean age 37.8, range 16-66 years) serving as a control group and consisting of 40 women (mean age 36.7, range 16–66) and 27 men (mean age 39,5, range 19–64 years) were examined for levels of CIG in citrated plasma. For the evaluation of CIG in different age groups, the following subgroups were constitutcd: "50 years and above", "30–49 years" and "below 30 years". The results of statistical analyses of CIG concentrations in different sex- and age-groups of this control material have been reported elsewhere (7) and these data have been used in the present study for statistical analysis (Student's *t*-test).

Patients

Twohundred-and-ninetyfour in-patients with various skin diseases, attending the Department of Dermatology, Rikshospitalet, Oslo (151 males and 143 females) were examined for concentrations of CIG in citrated plasma.

METHODS

Quantitation of cold insoluble globulin (CIG) in citrated plasma

The production of antiserum against CIG is described elsewhere (6). Quantitation was performed with an electroimmuno assay according to Laurell (12) and Weeke (23). In each run, single applications of 3 dilutions of reference plasma were run (10, 25 and 50 U/ml) together with patient plasma in double applications of 20/100 dilutions. All dilutions were done with electrophoresis barbital buffer (pH 8.6 and ionic strength 0.02). Five μl of each sample were applied at low voltage (2 V/cm) from a double constriction pipette, in holes of 2.5 mm in diameter, and run in 1 mm thick 1% agarose plates mostly at 2 V/cm for 18 hours at 20°C with 0.1% absorbed antiserum in the gel. A few runs were performed at 4 V/cm at 18°C for 4 hours and with 0.2% absorbed antiserum in the gel. After electrophoresis the gels were pressed, dried and stained (23). The amount of CIG in plasma was calculated using the linear correlation between the rocket heights and the CIG concentrations of the reference plasma dilutions (7) (Fig. 1), with a computerized calculation program based on the method of the least squares.

Table I. *Cold insoluble globulin (CIG) in citrated plasma in a control group of healthy adults*

	All			Women			Men		
		CIG (U/ml)			CIG (U/ml)			GIG (U/ml)	
Age groups	No	Mean	S.D.	No	Mean	S.D.	No	Mean	S.D.
All	67	115	29.7	40	108.8	26.8	27	124.6	31.9
≥50 years	14	131.8	28.8	8	131.6	22.8	6	132	37.9
30-49 years	32	118	28.2	17	106.8	22.5	15	129.9	29.7
<30 years	21	100	26.7	15	98.6	27.2	6	103.8	26.7

Table II. *Cold insoluble globulin (CIG) in citrated plasma of 294 dermatological patients*

	Total no.	Males					Females				
		No.	Age (y.) Mean	CIG (U/ml) Mean	S.D.	Range	No.	Age (y.) Mean	CIG (U/ml) Mean	S.D.	Range
Psoriasis vulgaris	79	43	48.5	121.8	28.6	70–188	36	43.7	119.7	25.7	75–168
Psoriatic arthritis	14	5	50.4	127	59.7	74–220	9	50.5	145	37.4	85–200
Psoriasis pustulosa	3	0					3	58.3	98	23	74–120
Dermatitis herpetiformis	24	13	50.8	104.9	39	75–173	11	42.6	104.1	24.8	66–121
Mycosis fungoides	17	14	62.1	148.3	31.4	100–216	3	55.3	168.7	42	136–216
Urticaria chronica	26	13	34.7	128	25	95–177	13	33.7	105.9	21.8	74–144
Eczema atopicum	22	10	22.3	114.7	19.5	78–153	12	26.6	98.2	17.3	61–120
Eczema contactum allergicum	22	10	52.7	155.4	44.2	100–235	12	44.9	141.5	33.6	74–235
Eczema (non-classifiable)	10	6	54.7	105	38	60–174	4	55.5	135.7	24	104–155
Actinic reticuloid	5	5	64.6	142.4	27.4	120–190	0				
Lupus erythematosus (discoid)	6	2	51	149	5.7	145–153	4	37.8	134.5	48.3	80–195
Lupus erythematosus (systemic)	2	1	26	140			1	59	173		
Sclerodermia	4	0					4	44.8	106.5	13.9	94–126
Dermatomyositis	1	1	44	222			0				
Miscellaneous	59	28	50.3	121.3	34.7	53–197	31	46.8	127.1	30.6	83–214

RESULTS

The levels of CIG in citrated plasma in the control group of healthy adults are listed in Table I, showing an increase in males compared with females ($p<0.059$) but with the difference only in the age group 30–49 years ($p<0.02$). In males, levels of CIG were increased in the group 30–49 years, compared with the group below 30 years ($p<0.1$). In females, the group of 50 years and above had increased levels compared with both lower age groups ($p<0.01$).

CIG levels in citrated plasma of dermatological patients (Table II) were compared with the control material, but as differences related to sex and age subgroups were observed in the control material, this was taken into consideration in statistical analysis, and the patient material was therefore split into the same subgroups (sex and age) as the control material and compared (Table III), demonstrating different results when the patient groups were compared with and without consideration of age subgroups in the control material

Table III. *Statistical analysis of the amount of cold insoluble globulin in citrated plasma in different dermatological diseases compared with corresponding sex and age-groups of a control material of healthy adults*

	All age groups		Men (years)			Women (years)		
	Men	Women	<30	30–49	≥50	<30	30–49	≥50
Psoriasis vulgaris	n.s.	$p<0.1$	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Psoriatic arthritis	n.s.	$p<0.02$			n.s.			$p<0.05$
Dermatitis herpetiformis	$p<0.05$	n.s.		$p<0.1$	n.s.		n.s.	n.s.
Mycosis fungoides	$p<0.05$	$p<0.01$			n.s.			
Urticaria chronica	n.s.	n.s.	n.s.	n.s.		n.s.	n.s.	
Eczema atopicum	n.s.	n.s.	n.s.			n.s.		
Eczema contactum allergicum	$p<0.05$	$p<0.01$		$p<0.02$	n.s.	n.s.	$p<0.01$	n.s.
Eczema (non-classifiable)	n.s.	$p<0.1$			n.s.			n.s.
Actinic reticuloid	n.s.				n.s.			
Miscellaneous	n.s.	$p<0.01$	n.s.	n.s.	n.s.	n.s.	$p<0.1$	n.s.

(Table III). When the same sex and age subgroups of the control and the patient materials were compared, increased levels of CIG were found in eczema contactum allergicum in the age group 30-49 years (males $p<0.02$, females $p<0.01$). In the same age group (30–49 years), in males, lower levels of CIG were found in dermatitis herpetiformis ($p<0.1$) and higher levels of CIG in females in miscellaneous ($p<0.1$). In the age group of 50 years and above, females showed increased levels of CIG in psoriatic arthritis ($p<0.05$).

DISCUSSION

Heparin precipitable fraction (HPF) as obtained from dermatological patients consists of about 60% of thrombin-clottable protein, and one major non-clottable protein identified as cold insoluble globulin (CIG) (6, 8). CIG is present in normal human serum and plasma, but the physiological significance and the origin of this protein is unknown (6, 15, 17). In serum, its concentration is dependent upon the clotting temperature of the blood (19). CIG is a glycoprotein, its molecular weight is about 440000 daltons and it consists of at least two polypeptide chains bound together with disulfide bridges (6, 15, 17). In gel electrophoresis, it moves between the $alpha_2$- and the $beta_1$-globulins (13).

CIG has been identified on fibroblast surfaces in cell cultures as a "fibroblast surface antigen", and its presence has also been demonstrated on glial cells (19). By transformation of fibroblasts by Rous sarcoma virus, CIG disappears from the cell surface (22). When fibrinogen is clotted by thrombin in the presence of CIG at 37°C, CIG remains in the clot supernatant, but at low temperatures it is somehow incorporated into the clot (19). This can also be demonstrated by thrombin-clotting of HPF at 37°C and 4°C (6, 8).

The cause of the HPF phenomenon and the presence of CIG in HPF is obscure. An increase of CIG in blood is not responsible for this plasma cryoprecipitate as investigated in citrated plasma from dermatological patients with increased amounts of HPF (7). CIG values in plasma in healthy humans demonstrate that the values in newborns are much lower than in adults (9) where its concentration in citrated plasma is found to be sex- and age-dependent (7).

Only a few clinical studies on CIG have been performed. These indicate that CIG is not an acute-phase reactant, and shows no significant variation in its plasma concentrations in acute myocardial infarction (11), after surgical trauma (1) or in cirrhosis of the liver in a quiescent phase (10). The only disease in which an increase in CIG has hitherto been reported is recurrent cholestasis of pregnancy where CIG showed a close correlation to an increase in serum gamma-glutamyl transpeptidase activity (3).

Because of the relatively large number of young females in the reference group, CIG levels in this group in the present study were relatively low compared with the control group, in accordance with the relation of CIG levels to sex and age. In another study (9), the standard deviation of CIG in healthy adults was 20–30%, corresponding well with the standard deviation of 29.7 U/ml of the present control group. In dermatological patients in the present study, a significant increase in CIG levels was found in eczema contactum allergicum (males and females 30–49 years) psoriatic arthritis (females 50 years and above) and depressed levels in dermatitis herpetiformis (males 30–49 years). In a few cases of systemic lupus erythematosus and in one case of dermatomyositis, elevated levels of CIG were demonstrated, but the number of patients studied was too small for statistical analysis.

The significance of these findings is at present difficult to evaluate, the source and the physiological role of CIG being unknown. Immunofluorescent studies on CIG in tissue sections in dermatological diseases may be conclusive, and are presently being performed. The results of such studies will be reported later.

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