Determination of some immune parameters in Glomerulonephritis patients

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Abstract:

The present study had been designed to determined some immune parameters in serum and urine of glomuolonephritis patients. Fifty persons patients and ten normal subjects were examined to detect the cryoglobuline .Twenty five persons were tested for cryoglobuline in sera and twenty five were tested in urine .CRP and Immune – fixation of Igs of this groups were tested in blood and urine .Mean serum cryocrit was (4.354) while mean urine cryocrit was (3.916).Serum cryo C-ractive protine was higher in serum than ueine and immune-fixation in this study showed mixed cryoglobulinemia of IgG-IgA-IgM.Serum results showed increase in Immno-cryoglobulins ,CRP and Igs .

Biology Classification QR 180 -189.5

Key wards:
Glomulonephritis,Immunoglobulin,Cryoglobulins

Introduction:

Glomerulonephritis is an important cause of renal failure thought to be caused by autoimmune damage to the kidney. While each type of glomerulonephritis begins with a unique initiating stimulus, subsequent common inflammatory and fibrotic events lead to a final pathway of progressive renal damage**(5).** In a review of therapy both immediate life saving treatment given when glomerulonephritis causes acute renal failure and more specific treatments designed to modify the underlying mechanisms of renal injury are considered. glomerulonephritis is an important cause of renal impairment accounting for 10%– 15% of cases of end stage renal failure in the USA **(13)**. In defining acute glomerulonephritis, we those glomerular diseases that may present with a nephritic syndrome—that is with haematuria, proteinuria, and impaired renal function together with hypertension, fluid overload, and oedema. Their pathology involves intraglomerular inflammation and cellular proliferation with secondary renal impairment over days to weeks**(10).** This definition\ excludes glomerular diseases without cell proliferation or nephritic presentations, such as minimal change disease, membranous nephropathy, and focal segmental glomerulosclerosis that can, none the less, chronically compromise renal function **(11).** In primary glomerulonephritis, disease is almost entirely restricted to the kidneys (as in IgA nephropathy or post-streptococcal glomerulonephritis) while in secondary glomerulonephritis it occurs in association with more diffuse inflammation (as in systemic lupus erythematosus or systemic vasculitis). Prompt diagnosis of glomerulonephritis is vital as patients with even mildly impaired renal function, hypertension, and urinary abnormalities may rapidly lose kidney function if not treated urgently. Although our understanding of the causes of glomerulonephritis is still at a basic level, inflammation is thought to be autoimmune mediated and involve both cellular and humoral immune systems **(6).** In each case a unique initiating stimulus (occurring by one of at least four different mechanisms) is followed by a common pathway of inflammatory and subsequently fibrotic events. Cryoglobuline is abnormal immunoglobulin s which form complexes and precipitate out of serum at low temperature and resoluble on warming In antiglomerular basement membrane disease, patients produce antibodies that react directly with the specialised basement membranes Cryoglobulinemia is caused by Igs that precipitate in the cold. Depending on the clonality of the precipitating Igs, three types of cryoglobulinemia **(4).** Although type I cryoglobulinemia consists of a single monoclonal cryoprecipitable Ig, type II and III cryoglobulins are mixed, i.e., composed of monoclonal (type II) or polyclonal (type III) Abs with rheumatoid factor activity against other Igs. In 1987, Gyotoku Cryoglobulinemia induces an immune complex-mediated glomerulonephritis that is characterized

by the presence of large immune deposits, including complement C3 and C5b-9, marked macrophage influx and mesangial cell proliferation. The precise role of complement in cryoglobulin-induced glomerulonephritis in humans remains unclear, whereas in mice there has been evidence that complement activation might be a central factor favoring glomerular inflammation, particularly by the recruitment of neutrophils **(1).** We report on an exceptional case of cryoglobulin-induced glomerulonephritis in a patient with mixed essential cryoglobulinemia type II **(7).** The clinical features included relapsing proteinuria and renal function impairment that were controlled by plasmapheresis. Complement was low in plasma and two renal biopsies at 1-year interval showed prominent immunoglobulin and complement deposits, with unusual high numbers of neutrophils. In a 1-patient clinical trial, we tested whether the monoclonal anti-C5 antibody eculizumab would be sufficient to control renal function at the time of a relapse. Although during the initial weeks renal function was stabilized, slow increase in creatinine could not be controlled by this treatment, so that plasmapheresis was reinstituted. **(12).**

Aim of study was to determined some immune parameters in serum and urine of glomuolonephritis patients .

Material and methods:

1-Patients and Controls :

Fifty patients and ten controls were elected .They shows chronic disease of glomulonephrytis from 25 patients we took urine samples and from another 25 patient we took blood sample about 5 ml amounts were collected clotted and sera separated**(13)** and kept at -18C◦.

2-Cryopriecipitation programmer for blood:

Blood from each patient were collected into plan tube about 5 ml and samples were transported to the laboratory at 40 C° 9the sample temperature must not to be allowed to fall below 37 C° (**13**).Samples were allowed to clot at least 37 C° and centrifuged at 37 C° for 5 minutes at 1500. After that 2 ml of serum stored at 4C° in Hb tubes Samples were observed daily for up to 7 days to detect and precipitates the cryoglobulin (cryocrit**) (7).**

3-Cryopriecipitation programmer for Urine:

Urine from each patients were collected about 3 ml after it filtered by Wattman papers .3 ml from P.A.G(polyethylene glycol) /6000 were added to the samples at 73 C° and centrifuged at 37C° for 20 minutes at 4000. After that 2ml of samples were stored at 4C° in Hb tubes. Samples were observed daily for up to 5 days to detect and precipitates the cryoglobulin (cryocrit percent) **(2).**

4- Serum and Urine cryoglobulin Responses :

The Cannella *etal* 2012 standard ,suggest by Lynch 2006 were followed in detection of cryoglobulin .The essential criteria were presentation at 4C° ,dissolved at 37C° and representation at 4C° **(8).**

-Immunofixation :

Immunodiffusion plate by using radial (From LAT.s.r.L-Milano,15/F).

6-Count of CRP:

By using CRP-Latex kit (From spinrect ,S.A..ctra santa colma 7E-17176 SANT) **(6)**

Results:

Table NO.(1) Cryoglobulin in the Serum and Urine of glomulonephritis patients :

Age	Females		Age	Males	
	Serum cryocrit	Urine cryocrit		Serum cryocrit	Urine cryocrit
54	4	3	32	6	4
47	7	5	47	5	3
65	4	3	60	6	4
35	5	4	29	6	3
60	6	4	21	8	7
70	3	2	30	6	5
27	4	4	75	3	2
71	3	3	47	5	4
70	4	3	35	5	4
60	4	3	45	4	3
40	3	3	32	5	4
25	4	4	23	4	3
40	3	3	77	2	1
60	3	3	35	6	4
23	7	6	30	6	5
22	3	3	68	3	2
60	2	2	64	5	4
25	5	2	49	6	2
46	3	2	70	2	1
77	4	2	60	6	3
29	4	3	55	4	2
19	8	2	64	4	2
			60	3	2
			92	2	1
			25	6	2
			37	7	2
			28	7	3
			77	2	1

Table No.(2) : Statistic feature of Cryocrite percent in Serum and Urine :

	Mean	Median	Range	S.D	S.E
Serum cryocrite	4.354	4.0	8.0	1.617	0.233
Number of patients	25				
Urine cryocrite	2.916	3.5	3.0	1.164	0.336
Number of patients	25				

S.D:Standard Deviation S.E: Standard Error

\Table No.(3) : C- Reactive protein (CRP) in Serum of glomulonephritis patients

NO.	Age	Titer	Concentration
1-	74	2	12
2-	29	8	48
3-	30	16	69
4-	35	8	48
5-	60	8	48
6-	23	4	24
7-	60	8	48
8-	40	4	24
9-	70	4	24
10-	45	8	48
11-	60	8	48
12-	25	2	12
13-	25	4	24
14-	65	2	12
15-	60	2	12

Table No.(4) : Statistic feature of C- Reactive protein (CRP) in Serum and Urine:

		Mean	Median	Range
CRP Serum	Titer	6.4	4	14
	Concentration	35.2	24	48
	Number of patients	15		
CRP Urine	Titer	1.875	2	3
	Concentration	11.25	12	18
	Number of patients	8		

Table No.(5) : C- Reactive protein (CRP) in Urine of glomulonephritis patients

NO.	Age	Titer	Concentration
1-	29	1	6
2-	27	2	12
3-	32	2	12
4-	22	2	12
5-	45	1	6
6-	77	4	24
7-	37	1	6
8-	60	2	12

Table No.(6) : Statistic feature for Concentration of (IgM-IgG-IgA) in Serum and Urine value by Mg/ml:

		Mean	Median	Range
Serum	IgM	1.506	1.696	0.579
	IgG	13.746	13.418	16.503
	IgA	4.465	4.944	8.949
Urine	IgM	1.233	1.406	1.471
	IgG	11.907	11.208	8.251
	IgA	2.121	2.334	3.076

Discussion:

Glomerulonephritis is an important feature of a number of human diseases, including cryoglobulinemia, systemic lupus erythematosus, serum sickness, and bacterial endocarditis **(2).** It is characterized by the formation and/or deposition of Ag-Ab complexes in the glomerulus, followed by an influx of inflammatory leukocytes **(6).** The mechanisms inducing the glomerular inflammation and the subsequent tubulointerstitial injury are not well understood.

Deciding on the treatment is a complex issue that should take numerous variables into account, including the patient's age, general state of health,

likelihood of response, and medical conditions that may decrease life expectancy or contraindicate the use of immunosuppressive**(7).**

Clinical features such as nephritic syndrome with renal function impairment, and subnephrotic or nephrotic proteinuria with active urine sediment are valuable indicators of renal disease severity **(11, 14).** In addition, renal biopsy can be used not only to establish the diagnosis, but also to make therapeutic decisions since it may contribute to identifying the patients who would most benefit from antiviral or immunosuppressive therapy. Specific histological findings suggesting a renal flare include prominent glomerular monocyte infiltration, intraluminal thrombi, large crescents, and vasculitis**(9).**

Cryocrit percent in serum higher than in urine (Table 2) ,.this give us clear picture about the systemic and hum oral immune responses that happened through the infection**(4)** .As well as the cryocrit percent was parallel in blood and in urine give evidence about the range of systemic and humoral cryoresponses **(14).** are differ in CRP concentration in blood and urine .This differences is caused by increasing the circulation proteins in plasma **(12)**.As well as there are different models for secreting the CPR in urine and blood depending on the kind of Ag that induce the humoral and systemic immune response which specific for antigen that expressed about it by cryoglobulins**(9)** mean of IgM-IgG-IgA concentration were higher in serum more than urine .This is similar with **(5)** from immunofixation .There are mixed cryoglobuuline responses noted as IgM-IgG-IgA and this is accordance with **(1)**.

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تحديد بعض المقاييس المناعية لمرضى خمج الكبيبات الكلوية

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الخلاصة:

صممت الدراسة الحالية للتحري من اجل تحديد بعض المقاييس المناعية في مصل وادرار مرضى التهاب الكبيبات الكلوية . 25 شخص مصاب و10 اسوياء فحصوا من اجل التحري عن الكلوبيولينات الباردة . في مصل 25 شخص منهم تم دراسة الكلوبيولين الباردو25 شخص في الادرار .وتم دراسة بروتينات الطور الحاد وتثبيت نوع الكلوبيولينات المناعية الباردة في المصل والادرار لمجاميع الدراسة .وكان الوسط الحسابي لنسبة الكرايوكرت في المصل كانت (4,354) بينما في الادرار كانت (2,916).وكانت نسبة كل من الكرايوكرت وبروتينات الطور الحاد وتراكيز الكلوبيوليسنات المناعية اعلى في المصل منه في الادرار .واظهر تثبيت نوع الكلوبيولين ان الكلوبيولين من النوع المختلط IgM,IgG,IgA.

كلمات مفتاحية:خمج الكبيبات الكلوية,الكلوبيولينات المناعية,الكلوبيولين المناعي البارد