

# Urinary Mucosal Cryoglobulin: A Review

Ibrahim M. S. Shnawa, Naeem R. R. Algebery

**Abstract**—The procedure for the assessment of the urinary mucosal cryoglobulin (UMCG) is being reviewed, testified and evaluated. The major features of UMCG are rather similar to that of serum cryoglobulin. Such evident similarities are forming the reality for the existence of the UMCG. There were seven characterizing criteria useable for the identification for UMCG. Upon matching them to the Irish criteria for serum cryoglobulin, some modifications are being proposed to the 16<sup>th</sup> standards that has been formulated and built as an Irish criteria. The existence of UMCG is being reported for the first time in human chronic infectious bacterial disease.

**Keywords**—Urinary, Mucosal, Cryoglobulin, Standard Immunofixation.

## I. INTRODUCTION

**C**RYOGLOBULINEMIA is a state of rise of cryoglobulin in the blood serum of the patients with chronic infectious, autoimmune, and lymphoproliferative disease [1]-[5]. Among the chronic infectious diseases whose causative agents are either facultative or obligate intracellular pathogens, secondary mixed cryoglobulinemia was associated with, like leprosy, tuberculosis [6]-[8] brucellosis [9]-[11], and typhoid [12]-[14]. Decades ago workers hold the faith that cryoglobulin is present only in the peripheral blood sera of the patients [1]-[5]. However, recently evidence obtained about the presence of mucosal globulin in the urine of tuberculous patients [6], [15]. Such cryoglobulin was showing marked mimicry to serum cryoglobulin. The current review was aimed at the assessment and evaluation of urinary mucosal cryoglobulins.

## II. PROTEINS

Proteins are either homo or heteropolymers of sum of amino acids and other organic or inorganic conjugates like metalloproteins or lipoproteins...etc. They have specific folding patterns with definite functional stereochemical structure. In the systematic sense proteins are of two major classes, the fibrous and the globular forms [16], [17].

## III. GLOBULINS

Globulins are globular glycoproteins found in peripheral blood circulation and found within the mucosa associated lymphoid tissues. In the electrophoretic analysis of the fresh mammalian sera, the **globulin** components are going faster than pre albumin and albumin globulin; however, separated into alpha, beta, and gamma globulins. Gamma globulin in all mammalian species has five isotypes, namely; IgM, IgG, IgA,

IgD, and IgE. The bases for such isotyping were the amino acid sequence, and carbohydrate contents. When the immunoglobulins are exposed to a temperature ranges, they will behaves as either/or one of the followings [16], [17].

- i- Normoglobulins
- ii- Cryoglobulins
- iii- Pyroglobulins

## IV. CRYOGLOBULINS

Cryoglobulin is an immunoglobulin variant that precipitate at 4C and dissolved at either 37 or 45C and present in mammalian sera and/or plasma. Search in literature about urinary mucosal cryoglobulin have revealed paucity in information [18]. But from the theoretical point of view, one may put down a hypothesis about the presence of a cold acting B lymphocyte clones that have the ability to synthesize and secret cryoglobulin in a mechanism similar or analogous to that of normoglobulin. Hence, urinary cryoglobulin is being proved in the following paragraphs [18]-[23].

## V. MUCOSAL COMPARTMENT OF URINARY TRACT

The urinary mucosal compartment has an associated lymphoid tissues that may act as an inductive site whereby urinary pathogen antigens in combination with MHC molecules on the surface of the antigen presenting cells activate T helper cells which in turn trigger the mucosal B lymphocytes to grow, proliferate, and expand as an antibody producing plasma cells. Secretory antibodies may be of IgG or IgA types. Collectively, mucus trapping mechanism, urine voiding mechanisms as well as secretory antibodies are the basic defense mechanisms operable in the urinary tract. Urinary mucosal cryoglobulin may be locally raised in a way similar to that mentioned mechanism [24]-[26].

## VI. PRINCIPLES

The scraps made from the mucus surface lining the internal organs are freed from leukocytes, epithelial cells, cellular remnants, as well as acellular organic or inorganic debris through centrifugation. Free supernatants collected and treated with suitable protein precipitant. The precipitated protein will be washed twice with cold sterile saline (partially pure). These preparations are reconstituted with sterile normal saline and loaded in the cryocrit tubes, then vertically assembled in special made racks and incubated at 4C in refrigerator with a daily check up approach from the first till the seventh day for the presence of the precipitates [27]. The color and texture of the precipitate as well as the time onset for the first appearance were noted [27]-[30].

Ibrahim M. S. Shnawa and Naeem R. R. Algebery are with the Babylon University, College Of Science, Department of Biology.Hilla.P.O.B.4/Hilla, Iraq (e-mail: ishnawa@yahoo.com).

## VII. ASSESSMENT CRITERIA

*A-Criteria*

The cryoprotein preparations consist of several protein entities such as cryoglobulin, cryo complement fractions, cryo acute phase proteins, cryo rheumatoid factor and cryo fibrinogens [19]-[21]. Thus, there were inclusion and/or exclusion criteria for the identification of cryoglobulins. These criteria should be followed in sequence [21], [28] as;

- i- The onset and the optimal time of the appearance of cryoprecipitate.
- ii- Physical texture of the precipitate
- iii- Dissolving time and temperature
- iv- Determination of concentration
- v- Separation
- vi- Detection of specific microbial cold antibodies
- vii- Immunofixation
- viii- Test for cryo RF, cryo complement and cryo acute phase proteins[6].

*B-Procedure*

- 1- Collect 7 mls of a clean catch fresh voided urine samples into sterile plain tube (hold at 39C).
- 2- Filter through Whatman number one filter paper and fennel to separate inorganic debris at 37C.
- 3- Centrifuge at 1500g for five min, collect supernatant into sterile plain tube, and discard the deposit.
- 4- A volume of the supernatant (step 3) is mixed with equal volume of polyethylene glycol 6000.6% and incubate at 4C for one hour (May be extended into an overnight period for those cases of low protein-urea).
- 5- Centrifuge at 1500g for 10min, discard supernatant, and keep deposit. Wash the deposit with cold saline then dissolve it in 0.5% sterile formal normal saline (Urinary mucosal globulin, UMG)
- 6- Load 2 mls of the UMG in a cryocrit tube at an upright position and incubate at 4C for 1-7days. Fix up the descriptions of the texture and the appearance time.
- 7- Incubate the precipitate at 37C and 45C to note the dissolving temperature and time.
- 8- Compare the UMCG characters; cryocrit %, concentration, texture and the reversible precipitation with the characters specific for serum cryoglobulin of the same patient, Table I.

## VIII. MAJOR IMMUNE FEATURES

The designation for the separated precipitates in the first run was UMG and in the second run was UMCG. The UMCG immune features were as follows: 1- Proteins by the virtue of positivity with biuret reagent 2- Reversible precipitation at 4C and dissolving at 37 or 45C, 3- Forms colloid of pale yellow color, 4- May and may not associated with; complement, rheumatoid factor and or acute phase protein C, 5- May bear pathogen specific cold antibodies and 6-Associated with facultative and obliged intracellular pathogenic viral, bacterial and protozoal infections. These features are the evidence for reality of UMCG [6], [31], [32], Table I.

TABLE I  
PATIENT CHARACTERISTICS (A) AND THE MAJOR IMMUNE FEATURES OF URINE CRYOGLOBULIN (B1) AND SERUM CRYOGLOBULIN (B2)

test	Range	Urine(B1)	Serum/Blood(B2)
A) Patients' general Characteristics			
i- Age range;	12-65 years.		
ii- Sex;	Both sexes		
iii- Male /female ratios;	1.5/1		
iv- Diagnosis;	Pulmonary tuberculosis.		
v- Therapy;	Newly diagnosed, during anti tuberculus therapeutic regimen		
vi- Skin DTH, tuberculin;	38/50 positive, 12/50 negative.		
B) Major immune features			
ESR	45 -125 mm	-	65mm
LIF	45-84%	-	67%
CRP	2 – 32	13/50	37/50
RF	8-64	1/50	15/50
Tuberculin DTH	10-30 mm	-	32/50 positive
Tuberculin DTH anergy	-	-	18/50 anergic
Cryoglobulin responses			
Onset time of appearance	7 days	1-3days	1-4days
Texture	Consistency & color	Colloid, pale yellow	Gelatinous, Crystalline, flocculent
Cryocrit %	2.5-9.5	7.5	8.5
Chemical nature	Biuret positive	Protein	Protein
Optimal Precipitation Temp	>37-4C	4C	4C
Optimal dissolving tem	37-45C	37,45C	37,45C
Optimal Dissolving time	1-2 1/2hr	1/2-1 1/2	1/2-2 1/2hr
Mycobaterium tuberculosis specific antibodies (Cryo)	There is specific cryoresponse	18/50	33/50
Immunofixation	Mancini Lurel radial diffusion	?	IgM-IgG-IgA

## IX. MODULATIONS

The 16<sup>th</sup> standards [28] specific for determinations serum cryoglobulin response were found to be amenable for a proper modulation in order to be functional for urinary mucosal cryoglobulins determinations. Thus the current review provides a proposal for standards suitable for the detection of the UMCG modulated from that of serum [28].

## X. CONCLUSIONS

It was observed that there were notable cryoglobulin responses, both at the mucosal compartment (Urinary tract in this case) and systemic circulation of the patients with pulmonary human tuberculosis. Though the majority of workers they don't even mentioned UMCG, still one immunologist in his Atlas of immunology 2010 [15] believes that cryoglobulins are found in different body fluids including urine.

## XI. UMCG PROPOSED DETERMINATION STANDARDS

## Standard 1

All laboratories must have a specific protocol for collection and analysis of samples for mucosal cryoglobulin determination.

## Standard 2

Laboratory staff must directly supervise the (Urine) sample collection and sample transport to the laboratory.

## Standard 3

Sample bottles for (UMCG) determination should be maintained at 40C prior to (Urine collection) and (separation)

## Standard 4

(Seven mls of clean catch midstream urine sample should be filtered and mixed with an equal volume of the protein precipitant for separation of the urine mucosal globulin.)

## Standard 5

Samples must be transported to the laboratory at 40C and not fall below 37C.

## Standard 6

**Urine samples not arriving in the laboratory at 37C must be rejected and testing repeated.**

## Standard 7

Samples should be freed from the infiltrated cells and the cellular debris)

## Standard 8

Samples should be centrifuged at 37C for five minutes.

## Standard 9

The (Mucosal globulin) should be made in 3x aliquots of at least 2mls in conically ended plastic or glass tubes are recommended.

## Standard 10

(Two aliquots of urine globulin should be stored at 4C and one aliquot should be stored at 37C.)

## Standard 11

Samples should be observed daily for up to seven days then only after seven days should sample be reported as undetected.

## Standard 12

Any precipitates or gels must be resubelised by warming to 37 and/or 45C.

## Standard 13

All positive cryoglobulins must be quantified using standard laboratory total protein methods of appropriate sensitivity and typed using standard immunofixation techniques.

## Standard 14

Cryoglobulins should be washed at least 5 times with 10 volumes of ice cold normal or phosphate buffered saline, prior to quantification and typing.

## Standard 15

For all positive type II and III cryoglobulins the requesting physician should be contacted regarding the determination of patients' hepatitis C virus status.

## Standard 16

Each laboratory should establish a central (UMG cryoglobulin) register to be kept permanently.

The 16<sup>th</sup> standards of Lynch 2006 [28] were adopted and modified for use in urine samples. The sites were modification is instilled given between parenthesis.

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