# "THE EXPRESSION OF IgG4 IN INTERSTITIAL CYSTITIS IN A TERTIARY CARE CENTRE- A HOSPITAL BASED PROSPECTIVE STUDY"

**By**

**REG NO: BN0122001**

# Dissertation

***Submitted to the KLE Academy of Higher Education and Research, Belagavi, Karnataka***

***In Partial Fulfilment***
***of the Requirements for the Degree of***

## DOCTOR OF MEDICINE

## IN

## PATHOLOGY

**DEPARTMENT OF PATHOLOGY**
**JAWAHARLAL NEHRU MEDICAL COLLEGE,**
**BELAGAVI, KARNATAKA**

**SEPTEMBER /OCTOBER 2025**

**KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH**

**BELAGAVI, KARNATAKA**

# *Endorsement by the HOD, Principal/Head of the Institution*

This is to certify that the dissertation entitled **"THE EXPRESSION OF IgG4 IN INTERSTITIAL CYSTITIS IN A TERTIARY CARE CENTRE- A HOSPITAL BASED PROSPECTIVE STUDY"** is a bonafide research work done by **REG NO: BN0122001.**

**DR. Vijayalaxmi Dhorigol** MD,
Professor and HOD
Department of Pathology,
J. N. Medical College,
Belagavi, Karnataka

Professor & Head
Department of Pathology
J.N. Medical College,
BELAGAVI.

Date: 21/3/25
Place: Belagavi.

**DR.(Mrs) N. S. Mahantashetti** MD (Paed).
Principal
J. N. Medical College,
Belagavi, Karnataka.

PRINCIPAL
Jawaharlal Nehru Medical College
BELAGAVI

Date: 21/3/25
Place: Belagavi.

# UNDERTAKING

I, **(REG. NO: BN0122001),** hereby declare that the information and the data mentioned in my dissertation entitled **"THE EXPRESSION OF IGG4 IN INTERSTITIAL CYSTITIS IN A TERTIARY CARE CENTRE- A HOSPITAL BASED PROSPECTIVE STUDY"** belongs to me and is original. I am aware of the definition of plagiarism as detailed below:

- An act or instance of using or closely imitating the language and thoughts of another author without authorization and the representation of that author's work as one's own, as by not crediting the original author.
- A piece of writing or other work reflecting such unauthorized use or imitation.
- The deliberate or reckless representation of another's words, thoughts or ideas as one's own without attribution in connection with submission of academic work, whether graded or otherwise.

I hereby declare that the dissertation prepared by me is original and does not involve plagiarism anywhere. In case at a later stage, it is found that I have indulged in plagiarism, then I am solely responsible for the same and the institution is at liberty to take any disciplinary action against me including cancellation of dissertation or any other penalties imposed by the university.

**Date:21-03-2025**

**Place: Belagavi** **(REG. NO: BN0122001)**

# PLAGIARISM ACCEPTANCE LETTER

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH

**JAWAHARLAL NEHRU MEDICAL COLLEGE**

(A constituent unit of KLE Academy of Higher Education & Research Deemed-to-be-University)

(Recognized by National Medical Commission, New Delhi)

**Accredited 'A+' Grade by NAAC (3rd Cycle)** **Placed in Category 'A' by MoE (GoI)**

*Nehru Nagar, Belagavi- 590 010, Karnataka, INDIA*

0831 – 2471350 0831 – 2470759 www.jnmc.edu rincipal@jnmc.edu

Ref No: MDC/PG/ Date: 18-03-2025

## "ACCEPTANCE LETTER"

The softcopy of thesis entitled: **"THE EXPRESSION OF IGG4 IN INTERSTITIAL CYSTITIS IN A TERTIARY CARE CENTRE – A HOSPITAL BASED PROSPECTIVE STUDY"** has been submitted for anti-plagiarism check through Turnitin software. The scan has been carried out and the scanned output reveals a match percentage of **02%** which is within the acceptable limits of **10%** as per the guidelines given by UGC.

**Guide.**

KAHER BELAGAVI J. N. MEDICAL COLLEGE

**Dr. (Mrs.) N.S. Mahantashetti.**
Chairperson-Antiplagiarism Committee &
Principal,
J. N. Medical College, Belagavi.

To,
**Reg. No. BN0122001**
Postgraduate Student,
2022-23 Batch,
Department of Pathology
J. N. Medical College, Belagavi.

# ETHICAL CLEARANCE LETTER

K.L.E. ACADEMY OF HIGHER EDUCATION AND RESEARCH
(Deemed – to- be- University)
Accredited 'A+' Grade by NAAC in (3rd Cycle) Placed in Category 'A' by MHRD (GoI)
**JNMC INSTITUTIONAL ETHICS COMMITTEE**
**JAWAHARLAL NEHRU MEDICAL COLLEGE,**
**NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)**
Website: http//www.jnmc.edu
E-Mail : dome@jnmc.edu
Phone: (+ 91-(0)831 Office : 2472550
Principal: 2471701
Fax No. +91 (0)831 – 2470759

**Ref No.MDC/JNMCIEC/** 140 **Date: 08/04/2023**

To,

**(REG. NO: BN0122001)**
PG Student in Pathology
J. N. Medical College,
BELAGAVI.

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"THE EXPRESSION OF IgG4 IN INTERSTITIAL CYSTITIS IN A TERTIARY CARE CENTRE- A HOSPITAL BASED PROSPECTIVE STUDY",** is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee.

**(Dr. Smita Sonoli)**
Member Secretary
JNMC Institutional Ethics Committee
J.N.Medical College, Belagavi.

**(Dr. Harsha Hegde)**
Chairman,
JNMC Institutional Ethics Committee
J.N.Medical College, Belagav

# LIST OF ABBREVATIONS USED

| S.No | Abbreviation | Expansion |
|---|---|---|
| 1. | **IC** | Interstitial Cystitis |
| 2. | **BPS** | Bladder Pain Syndrome |
| 3. | **ESSIC** | European Society for the Study of Interstitial Cystitis |
| 4. | **AUA** | American Urological Association |
| 5. | **IHC** | Immunohistochemistry |
| 6. | **IgG4** | Immunoglobulin G4 |
| 7. | **RD** | Related Disease |
| 8. | **HPF** | High-Power Field |
| 9. | **LUTS** | Lower Urinary Tract Symptoms |
| 10. | **UTI** | Urinary Tract Infection |
| 11. | **TURBT** | Transurethral Resection of Bladder Tumor |
| 12. | **CBC** | Complete Blood Count |
| 13. | **ESR** | Erythrocyte Sedimentation Rate |
| 14. | **H&E** | Hematoxylin and Eosin |
| 15. | **FFPE** | Formalin-Fixed Paraffin-Embedded |
| 16. | **DAB** | Diaminobenzidine |
| 17. | **DPX** | Dibutylphthalate Polystyrene Xylene |
| 18. | **SPSS** | Statistical Package for the Social Sciences |

| | | |
|---|---|---|
| 19. | **CI** | Confidence Interval |
| 20. | **p-value** | Probability Value |
| 21. | **PCR** | Polymerase Chain Reaction |
| 22. | **CT** | Computed Tomography |
| 23. | **MRI** | Magnetic Resonance Imaging |
| 24. | **NGF** | Nerve Growth Factor |
| 25. | **HP** | Histopathology |

# ABSTRACT

## TITLE: "THE EXPRESSION OF IgG4 IN INTERSTITIAL CYSTITIS IN A TERTIARY CARE CENTRE- A HOSPITAL BASED PROSPECTIVE STUDY"

**Background:** Interstitial cystitis (IC), also known as bladder pain syndrome (BPS), is a chronic inflammatory condition of the bladder with unclear etiology. Recent research suggests a possible link between IC and IgG4-related disease (IgG4-RD), characterized by plasma cell infiltration and fibrosis.

**Objective:** This study aims to assess IgG4 immunostaining in bladder biopsies of IC patients and correlate IgG4 expression with clinicopathological findings.

**Methods:** A two-year prospective study (January 2023–December 2024) was conducted at Jawaharlal Nehru Medical College and Dr. Prabhakar Kore Hospital and Medical Research Centre, KAHER, Belagavi. 30 bladder biopsies from clinically diagnosed Interstitial cystitis patients were analyzed histopathologically and immunohistochemically for IgG4 expression. Serum IgG4 levels were also measured. The severity of inflammation was graded, and statistical analysis was performed using SPSS.

**Results:** Among the 30 cases, IC was the most common diagnosis (50%), followed by chronic cystitis (33.3%). The majority of patients (36.7%) were in the age group of 31–50years , with a slight male predominance (53.3%). Lower urinary tract symptoms (LUTS) were the most common presentation (53.3%). IgG4 positivity in bladder tissue was observed in 56.7% of cases, with weak expression in 43.3% and intermediate expression in 13.3%. Serum IgG4 levels were elevated (>135 mg/dL) in 50% of cases. No significant correlation was found between IgG4 expression and

inflammation severity ($p = 0.290$). The study analyzed IgG4 expression in 15 IC patients, finding the highest positivity in the 31–50 age group, males, and moderate inflammation cases. While no significant correlations emerged, higher serum IgG4 levels (>135 mg/dL) showed a trend toward significance ($p = 0.06$).

**Conclusion:** The findings suggest that a subset of IC cases exhibits IgG4-mediated inflammation, supporting the potential classification of IC as an IgG4-related condition. Serum IgG4 assessment and immunohistochemical staining in bladder biopsies could refine diagnostic criteria and guide targeted treatment strategies. Further research is needed to validate these findings and explore therapeutic implications.

**Keywords:** Interstitial cystitis, bladder pain syndrome, IgG4-related disease, chronic cystitis, immunohistochemistry, plasma cell infiltration, serum IgG4 levels, inflammation grading, histopathology, immunomodulatory therapy.

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# INTRODUCTION

Interstitial cystitis (IC) or bladder pain syndrome (BPS) is defined as persistent inflammation or damage to the bladder wall that results in pain in the bladder[1,2]. The term "IC/BPS" describes a cluster of symptoms that manifests as a large range of clinical subtypes and is characterised by prolonged pain that is believed to be associated to bladder along with frequency and/or urgency of urination[3,4]. There are two types of IC seen by high pressure cystoscopy (up to 80 cmH2O): ulcerative (classic or Hunner ulcer) and non-ulcerative. While individuals with non-ulcerative IC have numerous strawberry-like petechial haemorrhages, known as glomerulations[5], people with ulcerative IC (also called Hunner ulcer) develop enormous irregular ulcers[6]. Trigone is not involved in interstitial cystitis usually[3].

American Urology Association (AUA) currently defines IC as "an unpleasant sensation (pain, pressure, and discomfort) perceived to be related to the urinary bladder, associated with lower urinary tract symptoms of six weeks duration, in the absence of infection or other identifiable cause"[1,3,7]. Urgency, increasing frequency, and nocturia are the related urine symptoms[1]. There is no known aetiology for this condition, which is more prevalent in young and middle-aged women. There is unknown pathophysiology or aetiology for IC. Numerous reasons have been presented as possible triggers of the inflammatory response, such as autoimmune, genetic vulnerability, and chronic or subclinical infection. Previously, it was believed that bacterial infection was the primary cause of the IC alterations[8,9].

Some believe the disease may develop through different pathways due to its varied symptoms, severity, and clinicopathologic features.

Inflammatory cell infiltration, mainly composed of lymphocytes and plasma cells, has been a key pathological feature of IC. In previous studies, epithelial denudation has also been commonly reported[10].

However, the importance of the pathological evaluation of IC, especially in distinguishing HIC from NHIC, has less importance in recent years[3,11]. The immune system's reaction to infectious pathogens and tolerance to allergens are both influenced by IgG4[12]. IgG4's physiological role and its ability to function as an autoantigen or antibody are not much understood. Recently, some pathologic investigators hypothesised that IC might be a component of a systemic IgG4-related illness (IgG4-RD) because individuals with IC displayed fibrosis and significant plasma cell infiltration in the afflicted bladder tissue[1,3,12].

IgG4 is involved in both the immune system's reaction to pathogenic infectious agents and tolerance to allergens. It's unclear how IgG4 functions physiologically and how it functions as an antibody or autoantigen. Several IC patients presented fibrosis and significant plasma cell infiltration in the affected bladder tissue, which suggest IC might be an IgG4RD[12].

IgG4 is an antibody that has a unique structure and function; in healthy individuals, it makes up less than 5% of total IgG. In a healthy individual, serum IgG4 concentrations may vary by more than 100 times (reference values range from 0.01 to 1.4 mg/dL), however IgG4 levels are frequently constant between individuals. This is in contrast to IgG1, 2, and 3. Complement component (CI) and Fc receptors are faintly bound by IgG4, which is not actively involved in immune activation[13,14].

A gold standard method for counting IgG4 plasma cells does not exist. Despite frequently having considerable background staining, the IgG immunostain is an essential diagnostic adjunct that performs well on paraffin-embedded tissue with distinct, strong cytoplasmic positivity[3].

# OBJECTIVES

**Primary objective :**

- To evaluate the expression of IgG4 immunostaining in urinary bladder biopsies of Interstitial cystitis

**Secondary objective:**

- To correlate IgG4 expression with clinicopathological findings in Interstitial cystitis.

# REVIEW OF LITERATURE

## EMBRYOLOGY

Between fourth to seventh weeks of development, the cloaca splits into anal canal posteriorly and the urogenital sinus anteriorly. The upper part of the urogenital sinus gives rise to bladder. There are three distinct parts of urogenital sinus: the bladder, which is the largest and uppermost section. The bladder and allantois are continuous initially, but when the allantois's lumen is destroyed, the allantois transforms into a thick fibrous cord called as urachus, or adult median umbilical ligament, which stays and joins the bladder's apex to the umbilicus[(15)]. In adults, it is termed as the median umbilical ligament. The trigone of the bladder is formed by the fusion of the lower ends of the mesonephric ducts with the posterior bladder wall. After some time, the urogenital sinus beneath the bladder receives the mesonephric ducts' opening. Due to its aetiology from the urogenital sinus and gut tube, the transitional epithelium lining the bladder is derived from endoderm. A layer of mesoderm that lies between the urogenital sinus and the primitive anal canal is known as the urorectal septum[(16)]. The perineal body will be formed by the septum's tip. The pelvic portion of the urogenital sinus, which in men gives birth to the prostatic and membranous portions of the urethra, is the next section. It is a relatively thin canal. The urogenital sinus's phallic portion is the final section. It is compressed from side to side, and this portion of the sinus will be drawn ventrally as the genital tubercle enlarges[(15)].

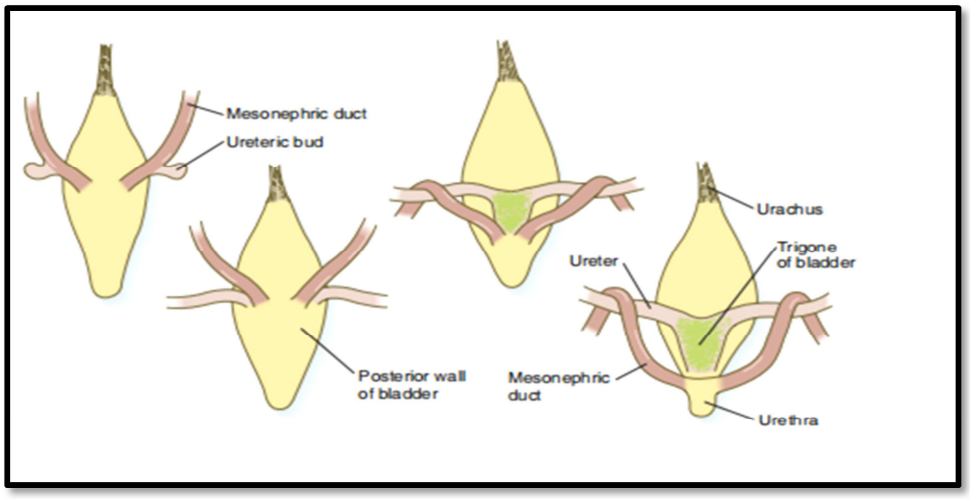



**FIGURE 1. Development of urinary bladder (Dorsal views) showing changing relationships of mesonephric ducts and the ureters. *Right,* the incorporation of portions of the walls of mesonephric ducts into the trigone of bladder[48].**

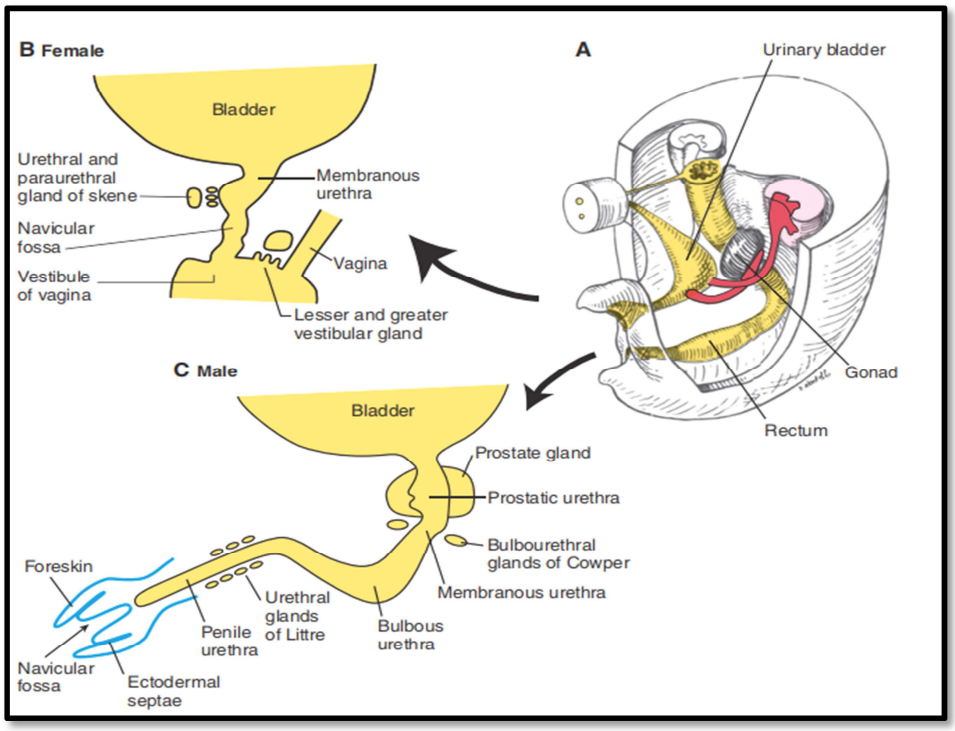



**FIGURE 2: A. Diagram of embryo, the urogenital sinus's upper component grows into the bladder (*yellow*)and its lower part into the female and male urethra. B. Female urethra. C. Male urethra[11].**

## ANATOMY OF THE URINARY BLADDER

Urinary bladder is a hollow organ with strong muscular walls and is highly distensible. It act as a temporary reservoir for urine. Its size, shape, position, and surrounding relationships change depending on its contents and the state of adjacent organs[17]. The apex of an empty bladder is pointed forward and has a tetrahedral shape. The base or fundus of the bladder is positioned posteriorly. Neck is the most stable and lowest part. The three surfaces of the bladder are: Left inferolateral, Right inferolateral, and superior. It also features four borders: anterior, posterior, and two lateral borders. When empty, the adult urinary bladder is positioned within the lesser pelvis. It lies partly above and partly behind the pubic bones, separated from them by the potential retropubic space (of Retzius). Mostly situated beneath the peritoneum, it rests anteriorly on the pubic bones and pubic symphysis. Posteriorly, it is in contact with the prostate in males and the anterior vaginal wall in females. A distended bladder is ovoid in shape and has an apex, which is directed upwards towards the umbilicus and neck that is pointing downhill. Anterior and posterior surfaces are the two[18].

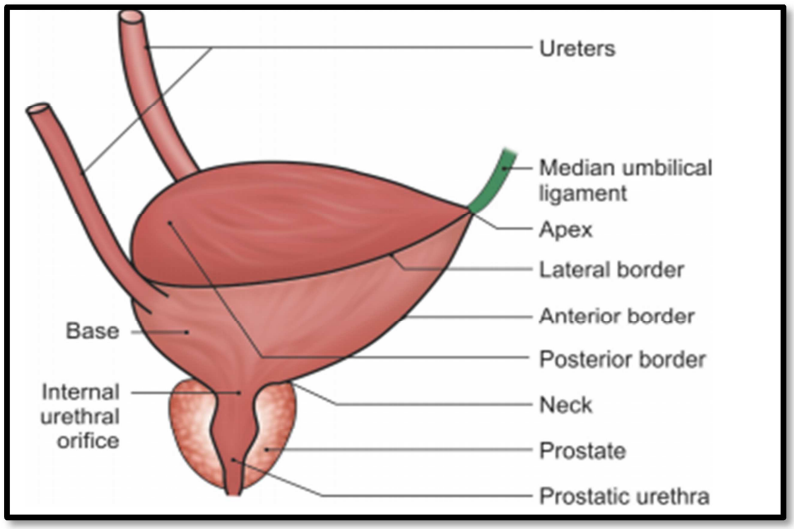



**FIGURE 3: Shape of the urinary bladder[13]**

Urinary bladder is mostly mobile within the extraperitoneal fat tissue. However, its neck is firmly anchored by the lateral bladder ligaments and the tendinous arch of the pelvic fascia. In males, stability is further provided by the puboprostatic ligament, while in females, the pubovesical ligament serves this function. In females, the bladder's posterior surface rests directly against the anterior vaginal wall. The lateral attachment of vagina to the tendinous arch of the pelvic fascia, called as paracolpium, plays an indirect yet crucial role in bladder support. The apex is connected to the umbilicus by median umbilical ligament, a remnant of the embryonic urachus. In females, base is positioned near the uterine cervix and the vagina[17].

In males, the upper portion of the bladder's base is separated from the rectum by the rectovesical pouch, which contains loops of the intestine. The lower portion is positioned near the seminal vesicles and the terminal segments of the vas deferens. A triangular area between the two vas deferens is shielded from the rectum by Denonvilliers' rectovesical fascia. Internal urethral meatus passing through the neck, which located 3 to 4 cm behind the lower section of the pubic symphysis. Smooth muscle fascicles round the preprostatic urethra and neck of bladder in males. There are no parasympathetic cholinergic nerves in the preprostatic sphincter. In females, the pelvic fascia surrounding the upper urethra is attached to the bladder neck. In infantile polpulation, bladder is positioned aa slightly higher. The internal urethral meatus aligns with superior border of pubic symphysis[18].

After puberty, it slowly moves downward to attain its adult position.The peritoneum completely covers superior surface in males, and it is adjacent to loops of the terminal ileum and sigmoid colon. In female population, peritoneum is covering most of superior surface, with the exception of a small area adjacent to posterior

surface that connects supravaginal portion of uterine cervix. Superior surface of the peritoneum extending to uterine isthmus leading to the formation of vesicouterine pouch. The inferolateral surfaces are not covered by the peritoneum. They are separated from superior surface by lateral borders and distinguished from each other anteriorly by the anterior borders. The male pubis, puboprostatic ligaments, retropubic fat, levator ani, and obturator internus are all connected to each surface[(18)].

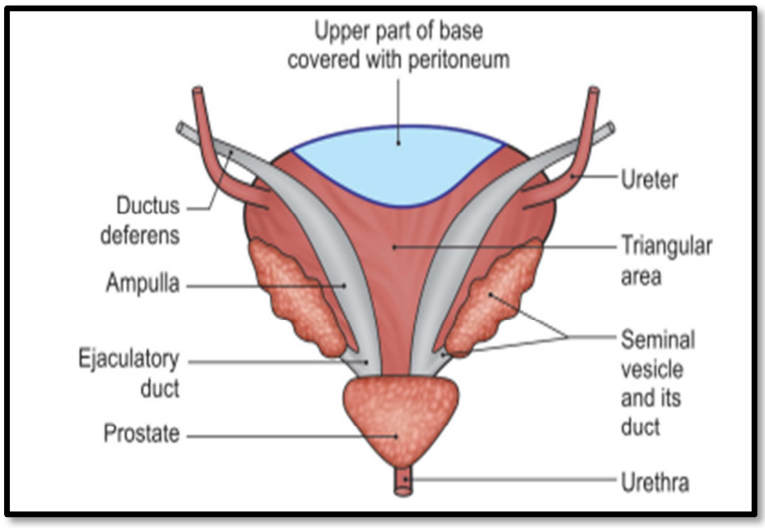



**FIGURE 4: Male urinary bladder posterior aspect with connections to the glands and genital ducts[13]**

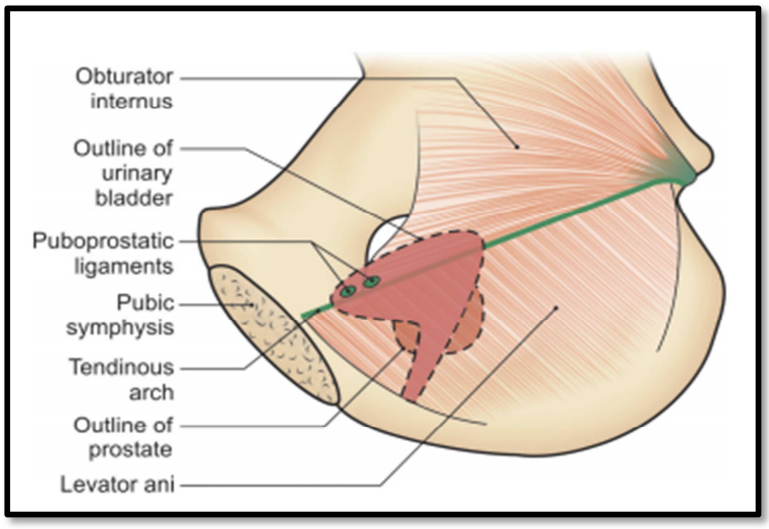



**FIGURE 5: Medial view of pelvic diaphragm and inferior part of pelvic wall[13]**

Ligaments of the urinary bladder

There are True ligaments and False ligaments;

True Ligaments are dense formations of pelvic fascia that provide support around base and neck of bladder. On upper aspect levator ani, they are continuous with the fascia. Lateral true ligament of bladder extends from its side to tendinous arch of pelvic fascia. Floor of retropubic area is made up of the ligaments on both sides. The pubovesical ligaments are bands that resemble the puboprostatic ligaments in females. They terminate at the bladder's neck. The posterior bladder ligament follows the vesical venous plexus, extending upward and backward. It connects the bladder base to the pelvic wall on both sides[(18)].

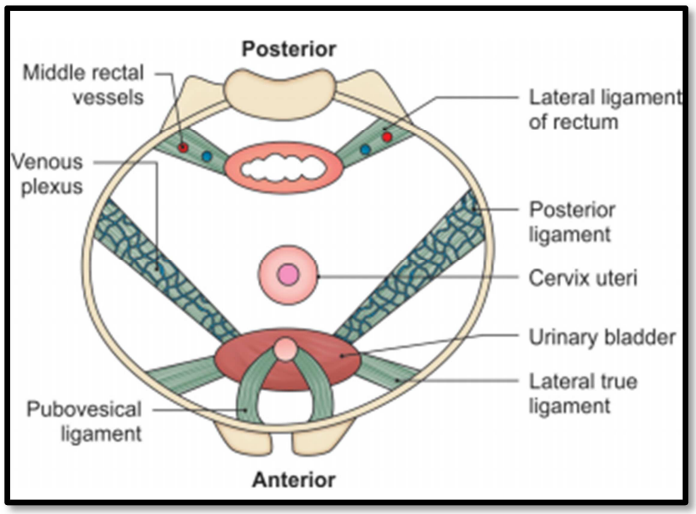



**FIGURE 6: True ligaments of the female bladder[13]**

False Ligaments are folds of peritoneum that do not offer structural support to bladder. These includes,

- **Median and Medial umbilical fold**
- **Lateral false ligament**
- **Posterior false ligament**.

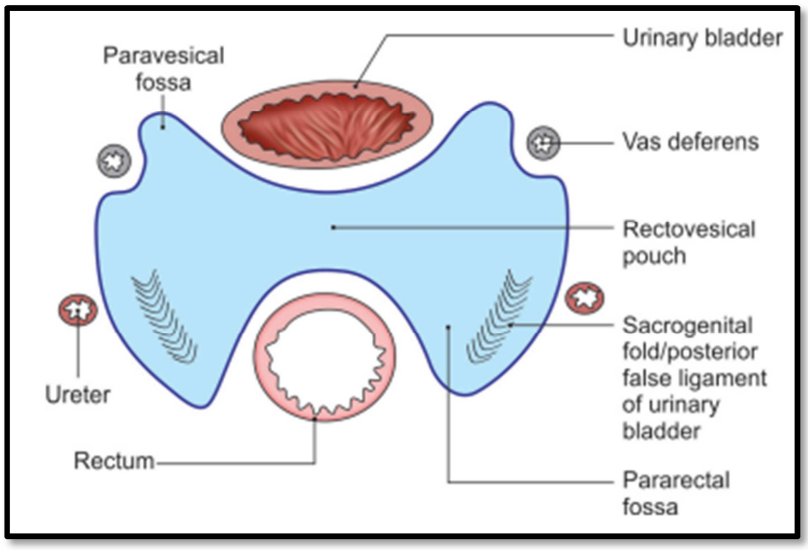



**FIGURE 7: Horizontal view of the male pelvis showing posterior false ligament of urinary bladder[13]**

Arterial Supply

1. Bladder receives its primary supply of blood from the vesical arteries (inferior and superior) which originate from internal iliac artery - anterior trunk.

2. Further blood supply comes from obturator and inferior gluteal arteries. The uterine and vaginal arteries replace the inferior vesical artery in females[(18)].

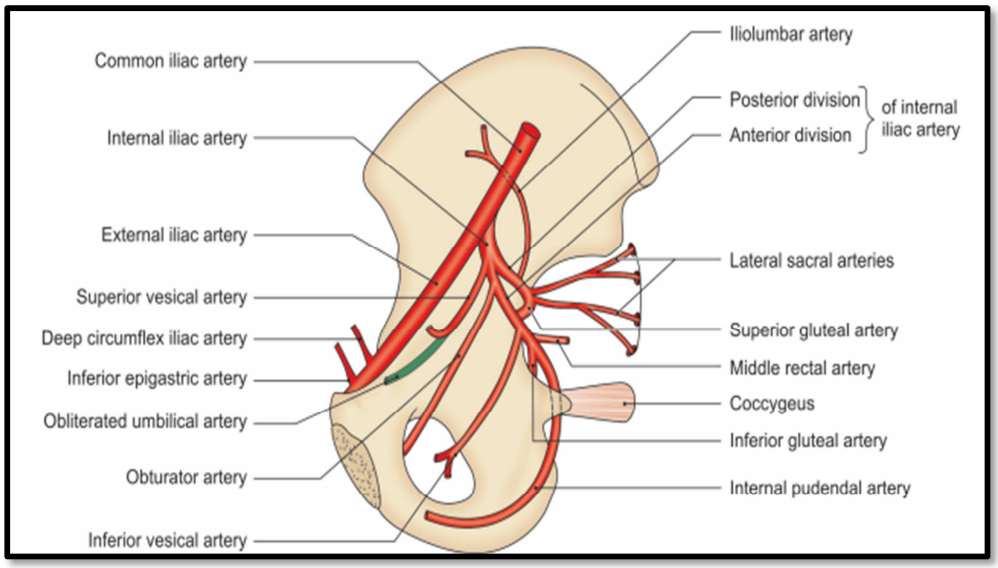



**FIGURE 8: Right internal iliac artery branches[13]**

Venous Supply

The vesical venous plexus is situated on inferolateral surfaces of bladder. Veins from the plexus drains into internal iliac veins, passing backward through the posterior bladder ligaments[18].

Lymphatic Drainage

External iliac nodes are the places where majority of the lymphatics from the bladder end. There are a few arteries that can go to lateral aortic or internal iliac nodes[18].

Nerve Supply

The vesical plexus of nerves, which comprises of fibres from inferior hypogastric plexus, supplies the bladder. Both the sympathetic & parasympathetic branches of vesical plexus contain motor(efferent) and sensory(afferent) fibres.

1. Parasympathetic efferent fibres -Also called as nervi erigentes, S2, S3, S4 which serves the detrusor muscle, but do not supply the preprostatic sphincter. Normal micturition cannot occur if they are destroyed.
2. Sympathetic efferent fibres (T11 to L2)- It is believed that the preprostatic sphincter mechanism is motorised by these fibres and inhibited by the detrusor.
3. Somatic pudendal nerve (S2–4) - It innervates the urethral wall, while voluntary sphincter urethrae is supplied by the somatic pudendal nerve (S2–4).
4. Sensory Nerves: Sensory nerves: Parasympathetic nerves carry the majority of pain sensations, however sympathetic nerves also contribute[18].

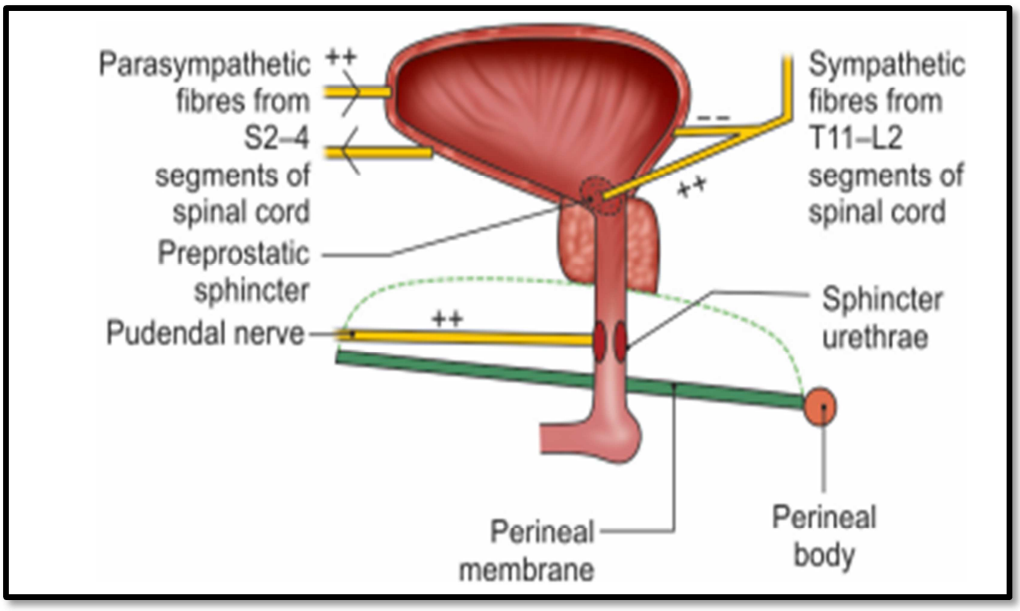



**FIGURE 9: Urinary bladder nerve supply[13]**

## HISTOLOGY OF URINARY BLADDER

Urinary bladder wall is composed of thick muscle layers. Apart from its thickness, it is similar to the lower third of the ureter. It contains of three loosely arranged smooth muscle layers and elastic fibers, which contract during urination.

The three layers are:

- Inner longitudinal,
- Middle circular, and
- Outer longitudinal layers[(19)]

The three layers are arranged into smooth muscle bundles that anastomose, with interstitial connective tissue situated in between. The three separate muscle layers cannot be distinguished, and the muscular bundles are divided into different planes. The serosal connective tissue combines with the interstitial connective tissue. Mesothelium is the outermost layer that covers the connective tissue of serosa.

Superior surface of bladder is lined with serosa, while its inferior surface is coated in adventitia, a connective tissue that combines with the connective tissue of nearby tissues. Many mucosal folds that are present in the mucosa of an empty bladder disappear when the bladder distends. The transitional epithelium consist six cell layers and is thicker than the ureter. Compared to the ureters, the lamina propria beneath the epithelium is broader. There are more elastic fibres in the deeper zone's loose connective tissue. Numerous blood vessels of varying sizes are present in the lamina propria between smooth muscle bundles and in the serosa[20].

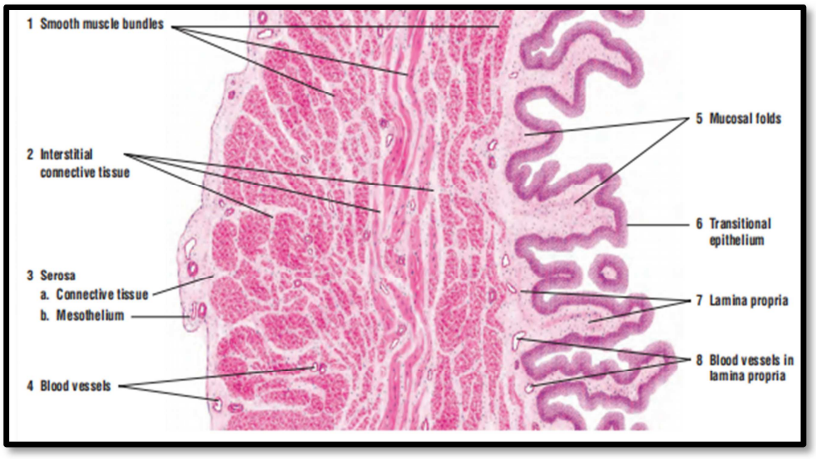



**FIGURE 10:Transverse section of Urinary bladder wall[15]**

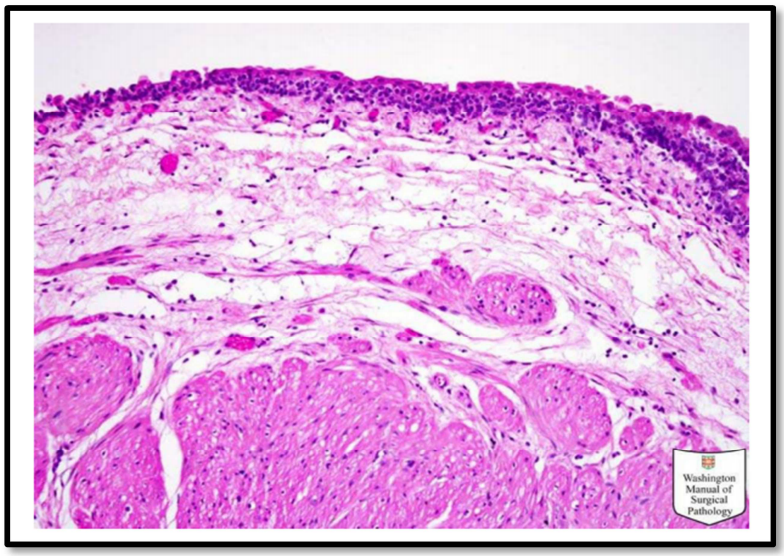



**FIGURE 11: Normal urinary bladder urothelium[19]**

The transitional epithelium, also known as the urothelium, is unique to the urinary system and is found only in the conducting tubes. The stratified epithelium is made up of three to six cell layers, with the number of layers increasing with the degree of epithelial distention upon fixation. When compared to the intermediate layers' more columnar cells, which have their nuclei oriented at right angles to basement membrane, basal layer's cells are compact and cuboidal in shape[19].

The transitional epithelium's surface cells have a dome-like appearance and are low cuboidal, or columnar and are able to keep the epithelium impermeable to urine even when it is fully stretched. The umbrella cells (Um) are big and spherical, with plenty of eosinophilic cytoplasm and circular nuclei. Few superficial cells could have two nuclei and be binucleate[19].

The superficial cytoplasm is fuzzy, unclear, and more highly pigmented than the rest of the cytoplasm, and the surface outline has a distinctive scalloped appearance[19]. The superficial cells of the epithelium have a prominent outer plasma membrane. The basal cells in the epithelium are more columnar, while the deeper cells are spherical[20].

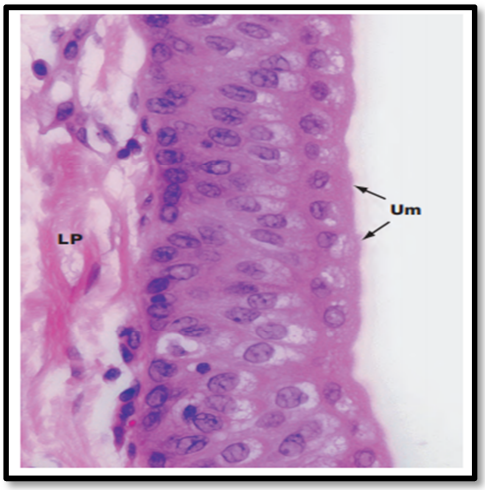



**FIGURE 12: Transitional epithelium of Urinary Bladder[14]**

Fine connective tissue fibres, a large number of fibroblasts, and blood vessels, including venules and arterioles, are all found in the subepithelial lamina propria (LP). Three separate muscle layers make up the muscularis, which is evident as smooth muscle bundles divided in longitudinal and transverse planes. The transitional epithelium undergoes a morphological change when the bladder fills with fluid[(20)].

A larger bladder seems to have fewer cell layers, squamous surface cells, and a transitional epithelium that is only around three layers deep. This is a result of the surface cells flattening to make room for the growing surface area. The stretched condition may cause the transitional epithelium to resemble stratified squamous epithelium in other body parts. Observe that the basement membrane is not folded, and the folds in the bladder wall vanish. Venules and arterioles are found in the connective tissue beneath, just like in an empty bladder. Smooth muscle is located beneath the connective tissue[(20)].

## INFLAMMATORY LESIONS OF BLADDER

### Cystitis

The majority of clinical signs and symptoms are caused by bladder diseases, especially inflammation (cystitis). Typically, these conditions cause more disability than death[21]. Cystitis is the most common inflammatory bladder condition, and it can present in both acute and chronic forms. Cystitis can be caused by infections by bacteria, viruses, fungi, or protozoa, but it can also result from calculus, other local trauma, radiation, or chemotherapy[22]. Bladder function is directly impacted by bladder inflammation, or cystitis. Both infectious and non-infectious aetiologies can cause it. Bacterial infections can be caused by Gram-positive bacteria, which like Enterococcus faecalis, Staphylococcus saprophyticus and *group B streptococci*,as well as Gram-negative bacteria such as Proteus, Klebsiella, Citrobacter, Enterobacter and Pseudomonas species[8,23]. Young women who are of reproductive age and elderly age groups of both sexes are especially prone to cystitis[21].

### Infectious cystitis

The most common cause of both acute and chronic cystitis is bacterial infection[24]. However, the most frequent cause of infectious cystitis is Escherichia coli[8,23]. In females and in cases of intermittent urinary blockage or stasis, the incidence is greater.

Although it is not recommended to do a biopsy when an infection exists, it can be done to rule out neoplasia in situations of chronic cystitis. Histopathologic features include a varied degree of lamina propria oedema and a nonspecific acute and/or

chronic inflammatory infiltration, often accompanied by lymphoid clusters or follicles[24].

*Bacterial cystitis*-

It is most frequently observed in women due to their shorter urethra and is commonly caused by coliform bacteria. Various factors contribute to its development, including systemic conditions like diabetes, chronic kidney disease, and immunosuppression. Structural abnormalities of the genitourinary tract, such as exstrophy, urethral malformations, fistulae, and diverticula, along with bladder stones, urinary stasis, and alkaline urine, also increase susceptibility[25].

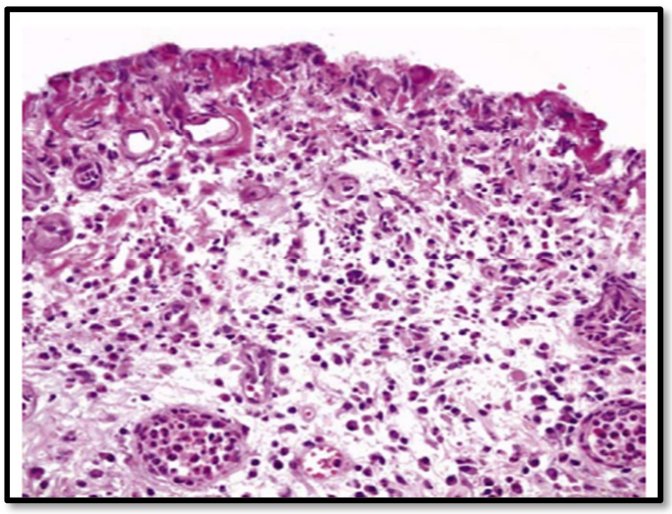


**FIGURE 13: Acute Bacterial cystitis[25]**

*Tuberculous cystitis*-

In almost all cases, mycobacterium tuberculosis causes tuberculous cystitis, which typically occurs after renal TB and occasionally as a primary infection[25]. Histologically, it is composed of caseating granulomas with Langerhans giant cells,

primarily in the lamina propria, and frequently with ulceration of the mucosa. When acid-fast bacilli are present, they should be visible with specific stains such as ZN stain[22].

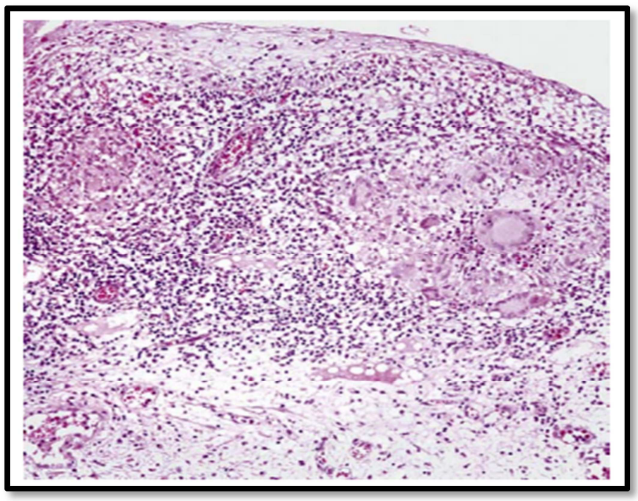


**FIGURE 14: Tuberculous cystitis[25]**

*Viral cystitis*-

Following a bone marrow or kidney transplant, immunocompromised patients typically experience viral cystitis. Adenovirus types 11 and 21, papovavirus, and infrequently herpes simplex type 2, herpes zoster, or cytomegalovirus are the most common causative agents[25].

*Fungal cystitis*-

Candida albicans is the primary cause of fungal cystitis, which is infrequently caused by Aspergillus species or other fungus. Women, debilitated patients, patients with diabetes, or patients receiving antibiotic therapy. Histologically, hyphae and yeast forms cause ulceration and inflammation in the lamina propria[25].

*Schistosomal cystitis*-

It is prevelant in the Nile Valley and subSaharan Africa but is rarely seen in other countries. Infestation with Schistosoma haematobium is typically the cause, with Schistosoma mansoni occurring infrequently. The eggs are placed in the veins of the muscularis propria of the bladder, where they degenerate and open up[(22)].

Microscopically, when schistosomal eggs are in active stages, there is a lot of eosinophils and a severe granulomatous inflammation. As the disease progresses, fibrosis, atrophy, calcified and destroyed schistosomal eggs, intestinal and keratinising squamous metaplasia, and decreased inflammation are noted[(25)].

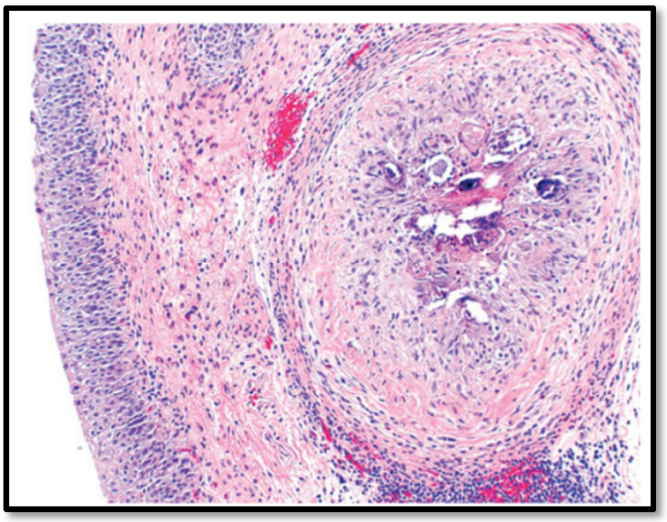


**FIGURE 15: Schistosomal cystitis[25]**

**Polypoid cystitis**

It is a broad-based polypoid or finger-like papillary structure is formed by an exophytic reactive lesion. It can be caused by any inflammatory insult to the mucosa of the bladder. More common in people who have catheters in their bodies and frequently seen in bladder fistula patients[(25)]. It mostly appears on the dome and posterior wall of the bladder and histologically it is characterised by a congested,

persistently It mostly appears on the bladder's dome and posterior wall, and histologically it is characterised by a congested, persistently inflammatory, and markedly oedematous stroma on the surface of a normal or mildly hyperplastic urothelium[22].

**Eosinophilic cystitis**

Bladder inflammation that can be idiopathic but is also linked to a number of systemic triggers, including food allergies, asthma, allergic gastroenteritis, topical insults from chemicals, parasites, systemic medications, local trauma, and previous transurethral resection or catheterisation[25–27].

It clinically presenting episodes of increased frequency of urination, dysuria, along with gross haematuria[22,27,28]. Histologically, the lamina propria is oedematous, and there is mixed inflammatory infiltrate with eosinophils being predominant[25].

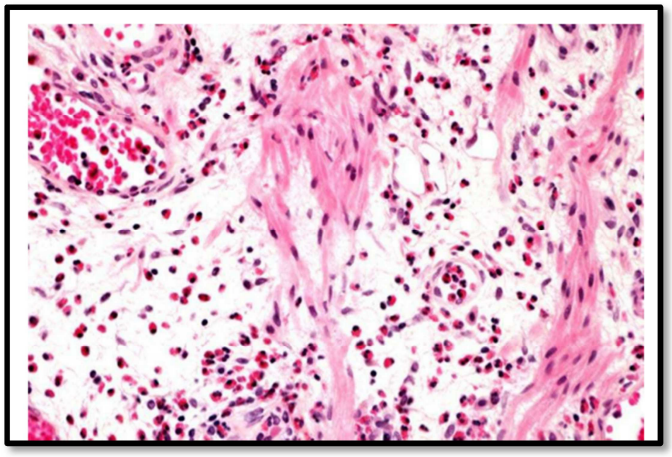


**FIGURE 16: Eosinophilic cystitis[24]**

**Hemorrhagic cystitis**

Cyclophosphamide medication seldom causes this side effect, which causes widespread ulceration and bleeding that, in extreme cases, might require cystectomy.

A similar pattern may also be produced by an adenovirus infection in immunocompromised people[24].

**Follicular cystitis**

The presence of lymphoid follicles with germinal centre formation is a characteristic of inflammatory cystitis. Compared to adults, children are more frequently afflicted.

It might be connected to an ongoing urinary tract infection or urothelial cancer.It present with symptoms like as urgency, frequency, haematuria, and dysuria. On evaluation for bladder cancer, it might be found by coincidence. Cystoscopically, it might show a nodular white grey appearance on an erythematous mucosa background. Histologically, the lamina propria present with numerous of lymphoid follicles. There are prominent germinal centres which associated with acute and chronic inflammation. Reactive changes may be seen in the urothelial lining[25,29].

**Cystitis cystica and cystitis glandularis**

These are proliferative or reactive changes in von Brunn nests, leading to cystic dilation (cystitis cystica) or glandular metaplasia (cystitis glandularis). These conditions are common, often associated with chronic cystitis or mucosal irritation[25,30]. Clinically, they may present as papillary or polypoid masses but are often incidental findings on cystoscopy. Histologically, they feature urothelial nests in the superficial lamina propria with cystic dilation, sometimes lined by glandular cells. They exhibit a non-infiltrative growth pattern and may show intestinal metaplasia, extravasated mucin, and occasional degenerative atypia[25].

**Interstitial cystitis**

*Definition*

The term **"interstitial cystitis"** was first mentioned in 1876 in the third edition of Samuel D. Gross's book, later revised and edited by his son, Samuel W. Gross[31]. IC/BPS is defined by ongoing pelvic pain, pelvic pressure, or discomfort believed to be associated to the bladder, along with urinary symptoms such as increased frequency or an urge to void, in the absence of other underlying conditions[32].

The American Urology Association (AUA) currently defined IC as "an unpleasant sensation (pain, pressure, and discomfort) perceived to be related to the urinary bladder, associated with lower urinary tract symptoms of six weeks duration, in the absence of infection or other identifiable cause". Urgency, increasing frequency, and nocturia are the related urine symptoms[1,7]. IC/BPS describes a symptom syndrome complex that manifests as a large range of clinical subtypes[33,34]. IC is a syndrome defined by irritative symptoms and bladder pain that lasts longer than six months[8,35]. The condition is also referred to as Hunner ulcer because of the persistent ulceration. The lesion may occur anywhere in the bladder[36]. IC is another uncommon and poorly known inflammatory condition that may have an autoimmune cause[22]. It is challenging to recommend appropriate treatment plans when IC is not well understood. To alleviate symptoms, several treatments are frequently applied empirically or in combination[1]. BPS/IC patients bladders have minimal to no inflammatory changes. The epithelium is usually in good condition. Typically, BPS/IC bladder biopsy specimens are identical to normal bladder biopsy findings[32,37]. The two subtypes of IC are currently recognised as (i) IC/BPS with

Hunner lesions, referred to as ESSIC BPS type 3, and (ii) IC/BPS without Hunner lesions, which known as ESSIC BPS types 1 and 2[33,38].

**TABLE 1: Clinical differences between IC/BPS with and without Hunner lesions[33,38]**

| | **IC/BPS with Hunner lesions** | **IC/BPS without Hunner lesions** |
|---|---|---|
| *Age of diagnosis* | Older patients | Younger patients |
| *Degree of symptoms* | Bladder-centric | Bladder-beyond |
| *Severity of symptoms* | Severe | Less Severe |
| *Capacity of bladder* | Small | Large |
| *Comorbid Conditions* | Less | More |

*Epidemiology*

Usually affects females in their middle years, with a 10:1 female-to-male ratio[25]. And has no known aetiology, and is actually a diagnostic of exclusion[8]. First-degree relatives are more likely to experience this. Greater percentage of concordance between monozygotic and dizygotic twins[39]. Adolescents and children could have IC. Compared to the general population, bladder issues in children are ten times more common in patients with IC[8,40].

*Pathophysiology*

IC/BPS's pathophysiology is unknown. Neurogenic inflammation, extrabladder illnesses, neural hyperactivity, or functional deficiencies of the urothelial barrier may be the primary factors contributing to the pathophysiology of IC/BPS[32]. Regardless of the cause, chronic inflammation results from an noxious stimulus that

lasts longer. A vicious, self-reinforcing cycle of chronic inflammation and recurring damage to the bladder epithelium is the outcome, which is caused by a series of interconnected events[8,41].

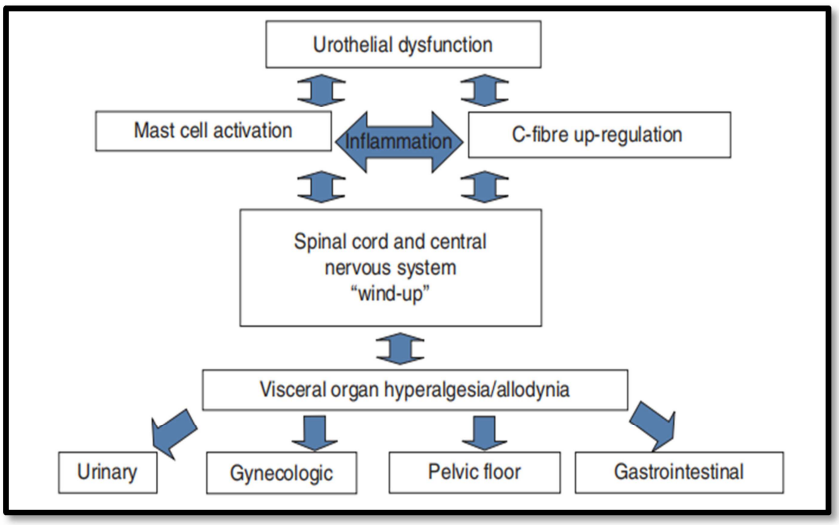



**FIGURE 17 . Integrated pathophysiology of Interstitial cystitis Sonal.G et al[40]**

*Clinical Presentation*

Pain is a hallmark symptom for both men and women, including pressure or discomfort[39,42]. Followed by urinary frequency, dysuria, and gross haematuria, is the most often reported symptom. Incontinence, urgency, gastrointestinal problems, urinary retention, and microscopic haematuria are some of the less common symptoms[25]. Women might experience discomfort in the lower abdomen, back, rectum, vulva, vagina, and urethra. Pain can become more intense during menstruation. Men may also have pain that is referred to their lower back, rectum, and genitalia. Dietary, environmental, and emotional stress can affect the intensity of symptoms, which can have varying remissions and exacerbations[43]. Physical examination findings include levator pain and spasm, suprapubic tenderness, and bladder base tenderness on pelvic examination[32].

*Diagnosis*

Clinically diagnosed based on bladder discomfort, at least one other clinical urine symptom, and the exclusion of related conditions[42].

To make the diagnosis, a cystoscopy is necessary. It can show a distensible bladder with distinctive pinpoint petechial haemorrhages and one or more ulcerations, which can occur anywhere in the bladder but are not necessary for the diagnosis[22]. Thickening of the wall is the primary finding on computed tomography or ultrasonography[25]. Many diagnostic biomarkers are under consideration, such as serum or urinary NGF, 30,74 serum or urinary pro-inflammatory cytokines, or chemokines[3,32,44]. Interstitial cystitis can only be diagnosed when all other possible reasons have been evaluated and repeated urine cultures for viruses, bacteria, and fungus have come up negative[22]. If the patient has undiagnosed microhematuria and/or a history of smoking, urine cytology may be performed[7]. Histologically, there is inflammation of the submucosa, ulceration, and a degenerated urothelium. Nonulcerative findings (punctuate haemorrhages of mucosa, multiple mucosal ruptures) and ulcerative findings (ulcers of wedge shaped with granulation tissue, necrosis and fibrin) can be separated[25]. There should be a lot of mast cells visible in the muscularis propria as well as in the lamina propria, along with a generalised inflammatory cell infiltration. There could be superficial muscularis propria or patchy fibrosis of the lamina propria[22]. European Society for the Study of Interstitial Cystitis has outlined supportive histologic criteria, which include: Intrafascicular fibrosis, detrusor mastocytosis (N28 mast cells/mm2), formation of granulation tissue and inflammatory cell infiltration[1,34].

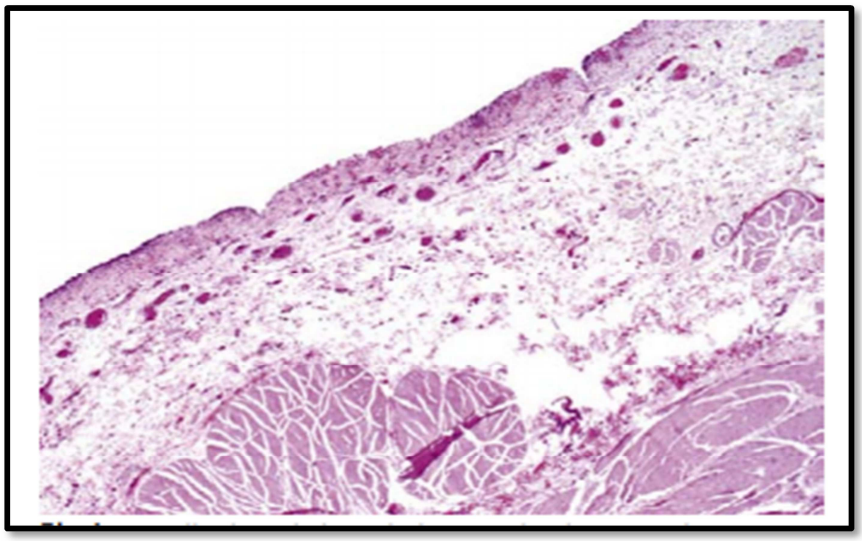


**FIGURE 18: Interstitial cystitis- Urothelium is focally denuded with edematous lamina propria[25]**

*Treatment*

The 2011 guidelines established by the American Urological Association (updated in 2015) classify interstitial cystitis treatment into six lines. First-line includes OTC supplements (quercetin, phenazopyridine) and patient education. Second-line involves physical therapy for muscle contractures and multimodal pain management. Third-line includes pain control, transurethral lesion destruction, hydrodistention, and triamcinolone for Hunner lesions. Fourth-line recommends neuromodulation for non-responders. Fifth-line involves intradetrusor onabotulinum toxin A and oral cyclosporine A when other treatments fail. Sixth-line includes bladder augmentation, diversion, or cystectomy as a last resort[(7)].

## IGG4 - IMMUNOHISTOCHEMISTRY MARKER

IgG4 is a unique antibody in both structure and function, accounting for less than 5% of total IgG in healthy individuals[(13,14)]. Complement component (CI) and Fc receptors are faintly bound by IgG4, which is not actively involved in immune

activation. The immune system's reaction to infectious pathogens and tolerance to allergens are both influenced by IgG4. IgG4's physiological role and its ability to function as an autoantigen or antibody are not much understood[13].

This antibody is designed for use on frozen tissue sections, cell preparations, and formalin-fixed paraffin-embedded tissues (FFPE) in immunohistochemical and immunofluorescence applications. The synthetic peptide that corresponds to residues in the hinge region of human IgG4 is the immunogen for Igg4. IgG1, IgG2, and IgG3 do not cross-react with it[45]. Although blood IgG4 levels and tissue samples on pathology are suggestive of the condition, they cannot be used to diagnose it. The classic features include storiform fibrosis, obliterative phlebitis (partial or total obliteration of a vein), and extensive lymphoplasmacytic infiltration[46].

**TABLE 2: Details of IgG4 IHC Antibody[45]**

| | |
|---|---|
| **Antibody** | Rabbit Monoclonal |
| **Isotype** | IgG |
| **Reactivity** | Paraffin, Frozen |
| **Localisation** | Cytoplasmic staining |
| **Species Reactivity** | Human |
| **Control** | Tonsil,Spleen |

Anti-IgG4 is a rabbit monoclonal antibody is extracted from cell culture supernatant that has been dialysed, concentrated, filter sterilised, and diluted in a pH 7.5 buffer that contains sodium azide and BSA as preservatives. A gold standard

method for counting IgG4 plasma cells does not exist. Despite frequently having considerable background staining, the IgG immunostain is an essential diagnostic adjunct that performs well on paraffin-embedded tissue with distinct, strong cytoplasmic positivity[45].

IgG4-related disease is extremely likely to be diagnosed if at least two of these symptoms are present along with the infiltration of IgG4-positive plasma cells; the cutoff value for the number of IgG4-positive plasma cells is determined for each organ. The tissue contains diffusely distributed IgG4-positive plasma cells, even in people with normal serum IgG4 levels[46].

Significant numbers of IgG4-positive plasma cells in affected organs are another characteristic pathogenic hallmark. The quantity of these cells varies by organ, though. Given that needle biopsy samples are significantly smaller than tissue resection samples, the quantity of IgG4-positive cells may be influenced by tissue size[47].

**TABLE 3: Validated diagnostic guidelines for the Japanese population, based on the Japanese Health Ministry[47] and adapted from Umehara H. et al.**

| **Clinical Guidelines** | **Localized or diffuse inflammation affecting in one or multiple organs** |
|---|---|
| *Serologic Criteria* | IgG4 levels exceeding 135 mg/dL (1.35 g/dL) |
| *Histopathologic Criteria* | Extensive lymphocytic infiltration, presence of plasma cells, and tissue fibrosis with an IgG4:IgG ratio exceeding 40%, along with IgG4 ≥ 10 plasma cells per HPF. |

A study conducted by Suzanne Crumley MD, et.a1[(1)]. revealed a notable rise in IgG4-positive plasma cells, with an IgG4/IgG ratio above 0.5 and more than 30 IgG4 plasma cells per high-power field. IC cases shows IgG4-positive plasma cells, is statistically significant difference when compared to IgG4-negative cases. Patients with IgG4 positivity were generally older, exhibited more severe inflammation, and had a lower bladder capacity than those who were IgG4-negative. The findings suggest that a subset of IC patients may have an IgG4-related condition. Further research, including serum IgG4 measurement, is required to better understand this association.

Another study conducted by Yoshiyuki Akiyam,et.a1[(10)] concluded that while IC without Hunner lesions is a non-inflammatory disease with limited histological signs, IC with Hunner is a unique inflammatory disease marked by pancystitis, frequent clonal growth of invading B lymphocytes, and epithelial damage. Absolutely distinct clinical entities are connected by the general phrase "IC/BPS." The accurate subtyping and clinical management of IC/BPS are thus significantly influenced by pathological assessment. It is also necessary to create language that distinguishes between IC with Hunner lesions and IC without Hunner lesions.

Another study by Montaño-Roca B, E, et a1[(13)]. concluded that the current literature review provides nephrologists, urologists, and pathologists with essential components that can help with the early diagnosis of this newly discovered and emerging urological condition, outlining its mechanism, significant clinical information, and effective treatment.

Another study by Kim, et al[(3)] concluded that IC/BPS remains **a** diagnosis of exclusion**,** with initial evaluation based on symptoms and ruling out similar

conditions**.** And 60% of IC patients showed IgG4 positivity in both serum IgG4 levels and the IgG4-to-IgG ratio. This finding supporting the hypothesis that IC could be a part of IgG4-RD, potentially offering new insights into its pathogenesis. Cystoscopy and biopsy aid in confirmation and classification. Advances in pathophysiology and urinary biomarkers have contributed to evolving diagnostic criteria.

Study by S Grover et al[8]. concluded that inflammation impacts bladder function, with acute cases (e.g., UTI) causing temporary irritation and symptoms that resolve once the trigger is removed. Chronic inflammation**,** however, leads to recurrent bladder injury, fibrosis, and persistent symptoms, as seen in IC**.** Treatment focuses on multimodal therapy, while surgery is reserved for severe, refractory cases with fibrosis.

Another study by Berry et al[2] concluded that Less than half of the women who met the high-specificity criteria for BPS/IC sought medical care for pelvic pain and urinary symptoms and consulted a urologist or were diagnosed. BPS/IC is underdiagnosed and frequently overlooked, as shown by the 10% diagnosis rate. For BPS/IC, there was no definitive test or standardised diagnostic procedure available at the time of the study.

# MATERIALS AND METHODS

**SOURCE OF DATA:**

Materials for study consisted of all bladder biopsies of the urinary bladder received in the department of pathology from outpatients and patients admitted in the Departments of Urology, KLE's Dr. Prabhakar Kore Hospital and Research Centre, KAHER, Belagavi. Specimens obtained from these patients were received in the Histopathology laboratory at Jawaharlal Nehru Medical College and KLE's Dr. Prabhakar Kore Hospital and Research Centre, KAHER, Belagavi, Karnataka. The present study was a prospective study that was carried out in the Department of Pathology over a span of two years from January 2023 to December 2024.

**INCLUSION CRITERIA:**

All bladder biopsy from patients clinically diagnosed with interstitial cystitis.

**EXCLUSION CRITERIA:**

All inadequate, benign, malignant and metastatic lesions of bladder.

**ETHICAL CLEARANCE:**

The ethical clearance was acquired from the Institutional Ethics Committee, JNMC, Belagavi before the commencement of study.

**STUDY DESIGN:** A hospital based two year prospective cross sectional study

**STUDY PERIOD:** January 2023 to December 2024-Two year study

**SAMPLE SIZE: 30**

**SAMPLING TECHNIQUE:** Universal sampling

**DATA COLLECTION PROCEDURE:**

For cross sectional study period (January 2023 to December 2024) all bladder biopsies received in the department was followed up. Patient details were systematically recorded from the proforma provided by the clinician, including age, sex, and presenting symptoms. Investigations such as CBC, ESR, and serum IgG4 levels were documented. The biopsy samples underwent IHC staining for IgG4. All bladder biopsy specimens received in the pathology department at Jawaharlal Nehru Medical College and KLE's Dr. Prabhakar Kore Hospital and Research Centre, KAHER, Belagavi, were fixed in 10% formalin for 24 hours. Gross examination was performed, and findings were documented. After fixation, tissue samples were embedded in paraffin and processed using an automated tissue processor. Sections of 3-4 microns were cut with a microtome, stained with Hematoxylin and Eosin, and examined under a microscope.

**Histological evaluation:**

The slides were evaluated by a pathologist and reporting was done. The diagnosis of interstitial cystitis (IC) was established through H&E staining after excluding other conditions with similar clinical features. Histological criteria for IC included increased inflammatory infiltrate mainly of lymphoplasmacytes, presence of mast cells, and lamina propria fibrosis, while ensuring other conditions were ruled out.

The severity of inflammation was assessed as follows:

**TABLE 4: Grading of Inflammation[1]**

| Grade | Severity | Histopathological description |
|---|---|---|
| **1+** | **Mild** | Sparse inflammatory cells present without lymphoid aggregates |
| **2+** | **Moderate** | Dense inflammation in less than 50% of the tissue or a single lymphoid aggregate |
| **3+** | **Severe** | Dense infiltration affecting over 50% of the tissue or at least two lymphoid aggregates |

**Immunohistochemical analysis:**

IgG4 IHC staining was manually performed , 3-micron tissue sectioning, followed by baking at 37°C overnight and 60°C for 1 hour. Deparaffinization is done using xylene (10 min × 2) and absolute alcohol (10 min × 2), followed by water rinsing. Antigen retrieval is performed using Tris-EDTA buffer (pH 8.0) in a pressure cooker (3 whistles), then cooled for 1 hour. Endogenous peroxidase blocking is done with 3% hydrogen peroxide (8–10 min). The Anti-IgG4 antibody is applied and incubated for 45–60 min, followed by washing. The detection process involves antibody amplifier (15 min), polymer HRP (25–30 min), and DAB chromogen (10 min). Finally, counterstaining with hematoxylin (2 min), blueing (1 min), xylene clearing, and DPX mounting.

**Immunohistochemical staining evaluation:**

Brown color in the cytoplasm of the plasma cell is considered as positive immunoexpression.

The degree of biomarker expression can be determined by observing the percentage of plasma cells that have been stained with the biomarker. Scoring was based on the number of IgG4 positive plasma cells high power field(hpf)[1];

Score 0: No IgG4 positive plasma cells detected per hpf

Score 1: Presence of 1-5 IgG4 positive plasma cells per hpf

Score 2: Presence of 5-30 IgG4 positive plasma cells per hpf

Score 3: Presence of >30 IgG4 positive plasma cells per hpf

**STATISTICAL ANALYSIS:**

Analysis using descriptive and inferential statistics was carried out in the present study. Data was analyzed using The SPSS Software version 26 and Microsoft word and Excel were used to generate Graphs, tables etc. Mean, & Percentages of descriptive statistics were calculated. The relationship between expression of markers and other clinicopathological variables was analyzed using chi-square test and Fisher's exact test.

The Probability (P) value < 0.05 was considered significant.

# RESULTS

From January 2023 to December 2024, Thirty cases of bladder biopsies were analysed. Among these, majority cases were 27 bladder biopsies , 02 cases of TURBT specimens and 01 case of cystostomy.

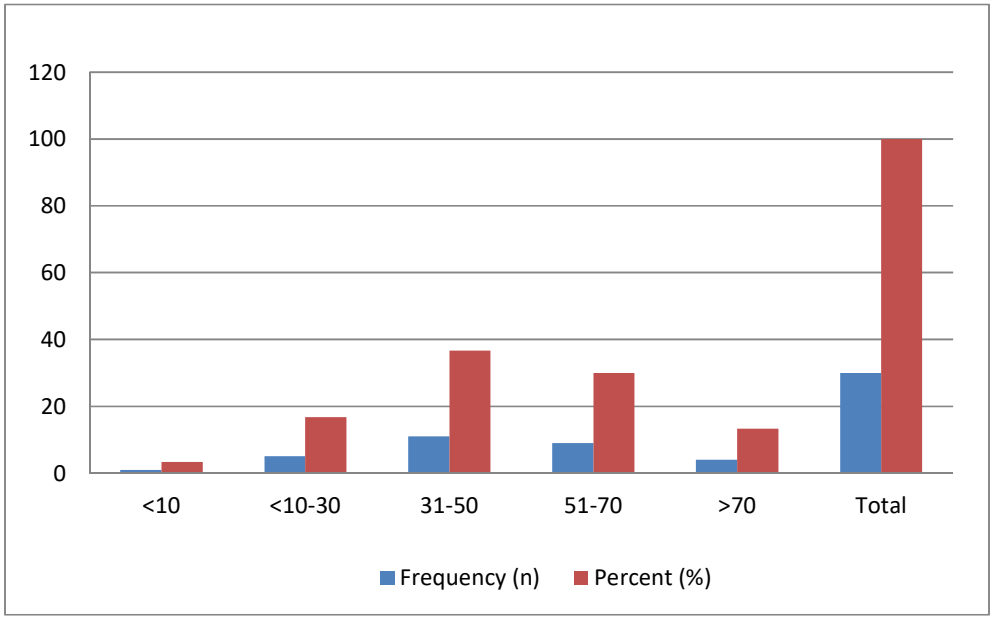
The pre-biopsy total serum IgG4 levels were obtained.

The data obtained from this study was compiled and tabulated. It was then statistically analyzed.

**TABLE 5: Distribution of Cases according to Age**

| AGE | Frequency (n) | Percent (%) |
|---|---|---|
| <10 | 1 | 3.3 |
| 10-30 | 5 | 16.7 |
| 31-50 | 11 | 36.7 |
| 51-70 | 9 | 30 |
| >70 | 4 | 13.3 |
| **Total** | **30** | **100** |

**FIGURE 19: Distribution of Cases according to Age**

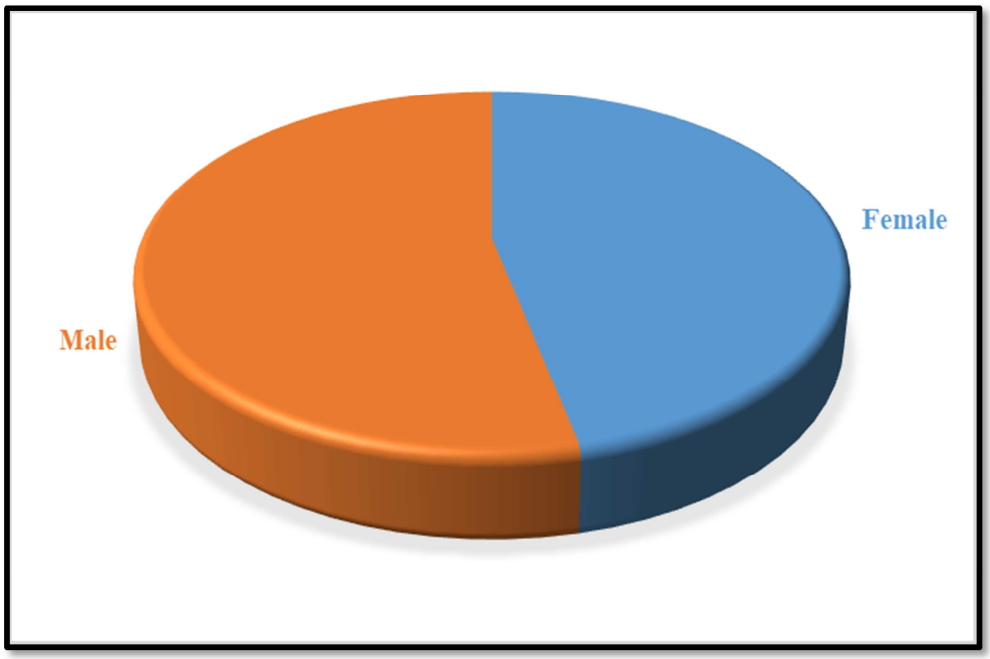



The age distribution for 30 individuals the patient ranged from 5-88 years with majority of the cases in the age group of 31-50 years (11 cases,36.7%) followed by 51-70 years (9cases, 30%). Out of 30 cases youngest age was 5 years and eldest was 88 years (Table 5 and Figure19).

**TABLE 6: Distribution of Cases according to Gender**

| GENDER | Frequency (n) | Percent (%) |
|---|---|---|
| Male | 16 | 53.3 |
| Female | 14 | 46.7 |
| **Total** | **30** | **100** |

**FIGURE 20: Distribution of Cases according to Gender**

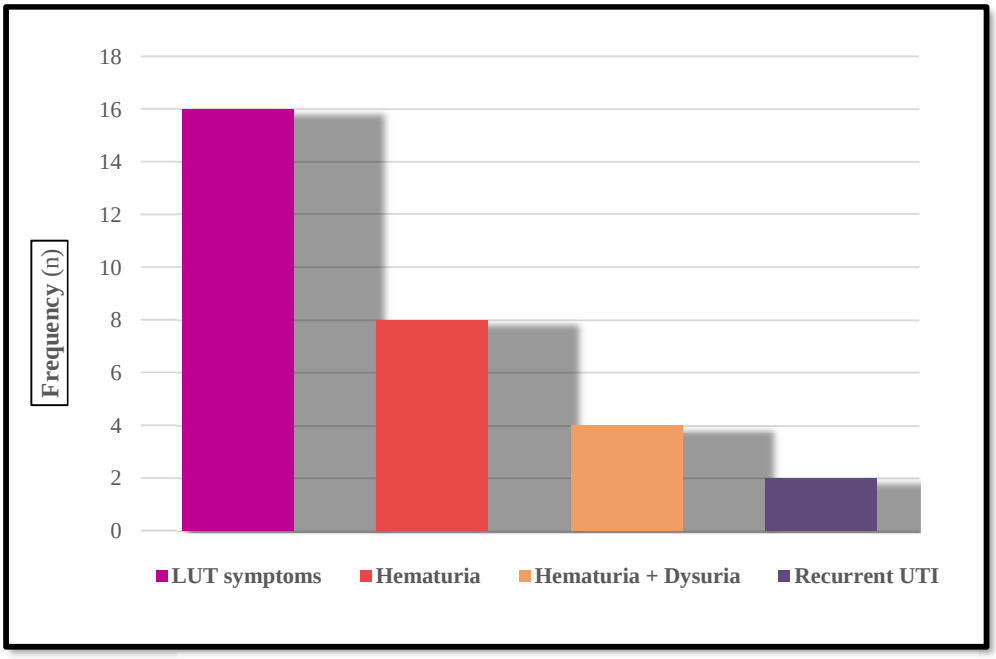



In 30 cases, 16 males (53.3%) and 14 females (46.7%), with a slight male majority (Table 6 and Figure 20).

**TABLE 7: Distribution of Cases according to Clinical History**

| CLINICAL HISTORY | Frequency (n) | Percent (%) |
|---|---|---|
| LUT symptoms | 16 | 53.3 |
| Hematuria | 8 | 26.7 |
| Hematuria + Dysuria | 4 | 13.3 |
| Recurrent UTI | 2 | 6.7 |
| **Total** | **30** | **100** |

**FIGURE 21: Distribution of Cases according to Clinical History**

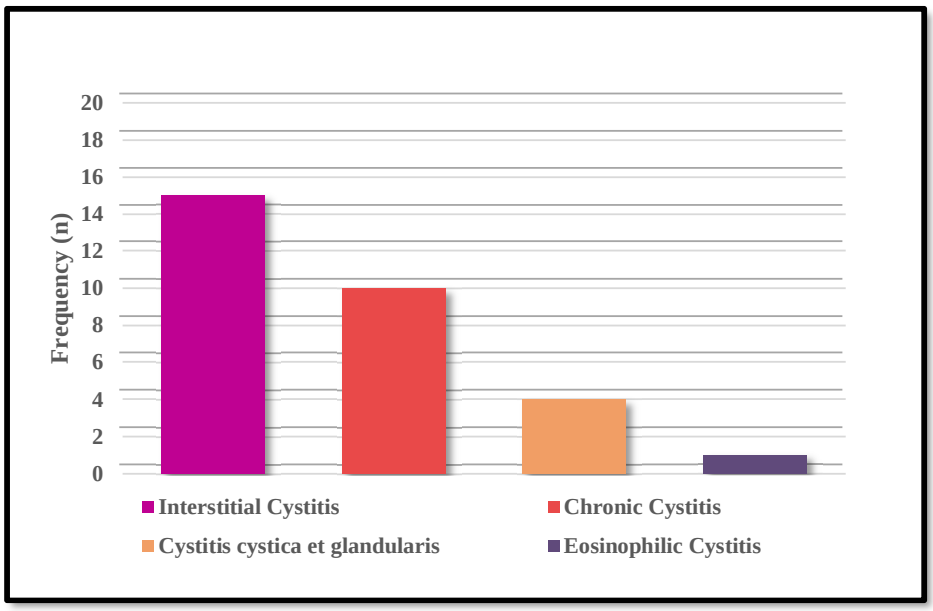



Out of 30 cases, most common presentation in the participants was lower urinary tract (LUT) symptoms reported in 16 cases(53.3%), followed by hematuria in 8 cases(26.7%) (Table 7 and Figure 21).

**TABLE 8: Distribution of Cases according to Histopathological Diagnosis**

| HP DIAGNOSIS | Frequency(n) | Percent(%) |
|---|---|---|
| Interstitial Cystitis | 15 | 50 |
| Chronic Cystitis | 10 | 33.3 |
| Cystitis cystica et glandularis | 4 | 13.4 |
| Eosinophilic Cystitis | 1 | 3.3 |
| **Total** | 30 | 100 |

**FIGURE 22: Distribution of Cases according to Histopathological Diagnosis**

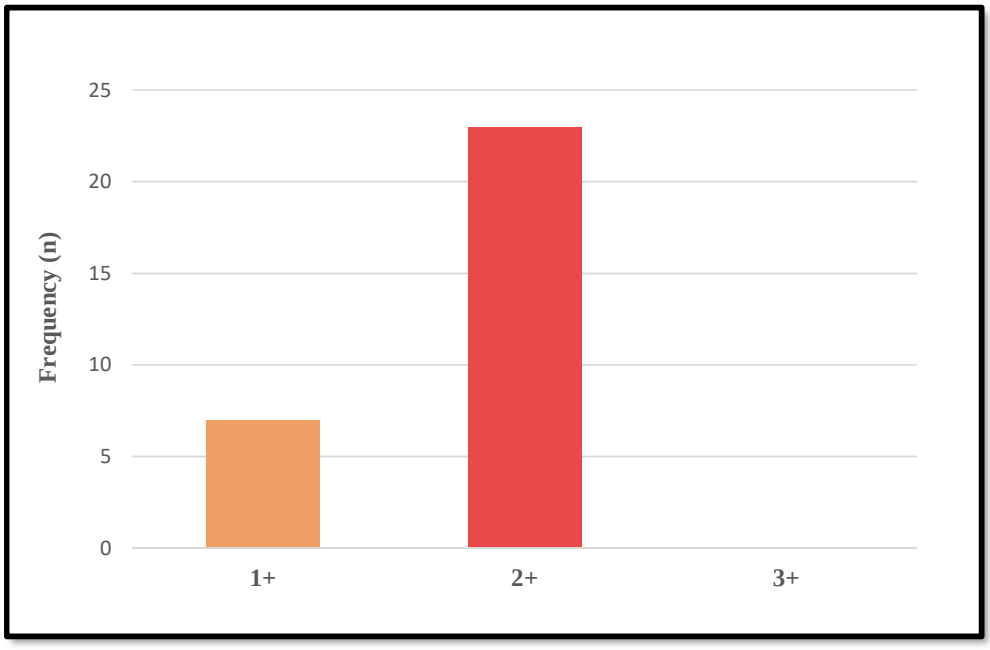



Among 30 cases, 15 cases(50%) were diagnosed as Interstitial cystitis followed by 10 cases(33.3%) of chronic cystitis. Cystitis cystica et glandularis was observed in 4 cases(13.4%), while eosinophilic cystitis is the least common, reported in 1 case(3.3% ) (Table 8 and Figure 22).

**TABLE 9: Distribution of cases according to Grading of Inflammation**

| GRADE | Frequency(n) | Percent(%) |
|---|---|---|
| **1+** | 7 | 23.3 |
| **2+** | 23 | 76.7 |
| **3+** | 0 | 0 |
| **Total** | 30 | 100 |

**FIGURE 23: Distribution of cases according to Grading of Inflammation**

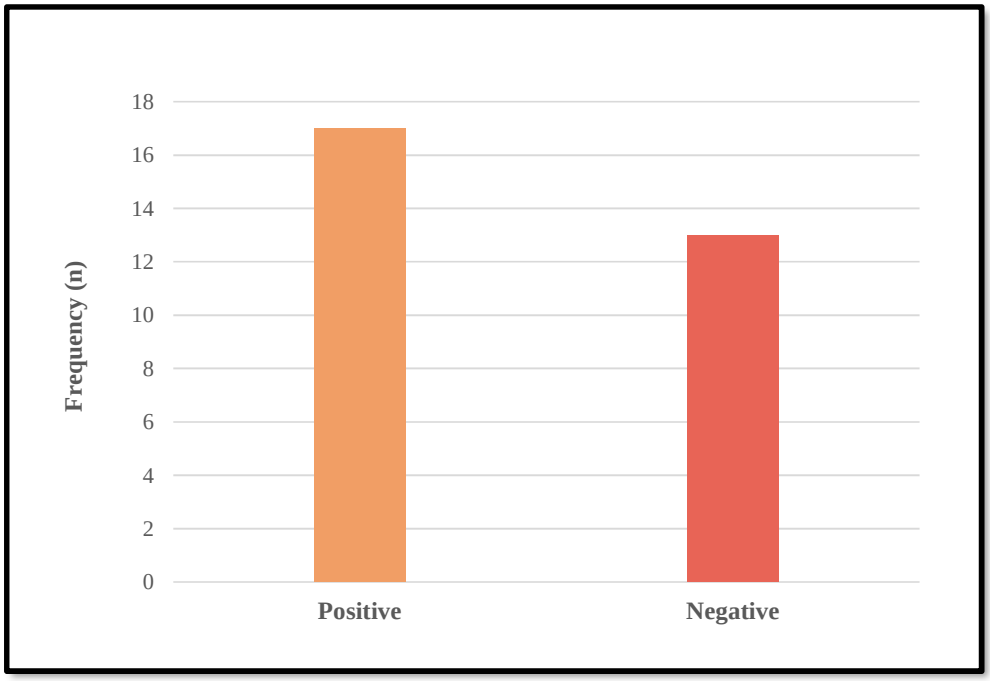



Among 30 cases, 23 cases (76.7%) were of Grade 2+ (Moderate inflammation) and 7 cases (23.3%) were of Grade 1+ (Mild inflammation). In our study none of the cases showed grade 3+(Severe inflammation). (Table 9 and Figure 23).

**TABLE 10: Distribution of IgG4 Expression in plasma cells**

| IgG4 EXPRESSION STATUS | Frequency(n) | Percent(%) |
|---|---|---|
| Positive | 17 | 56.7 |
| Negative | 13 | 43.3 |
| **Total** | 30 | 100 |

**FIGURE 24: Distribution of IGG4 Expression in plasma cells**

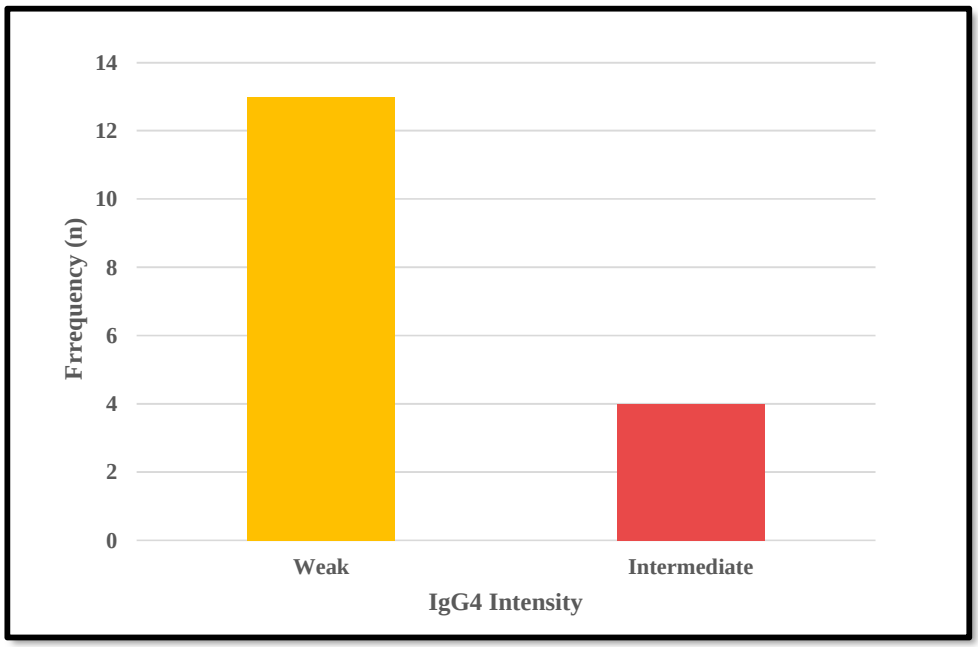



Among 30 cases, 17 cases(56.7%) showed positive expression of IgG4 in plasma cells and 13 cases(43.3%) were showed negative expression of IgG4 in plasma cells. (Table 10 and Figure 24)

**TABLE 11: Distribution of IgG4 Intensity in IgG4 Positive cases**

| IgG4 INTENSITY | Frequency(n) | Percent(%) |
|---|---|---|
| Weak | 13 | 43.3 |
| Intermediate | 4 | 13.3 |
| Strong | 0 | 0 |
| **Total** | **17** | **56.7** |

**FIGURE 25: Distribution of IGG4 Intensity in IgG4 Positive cases**

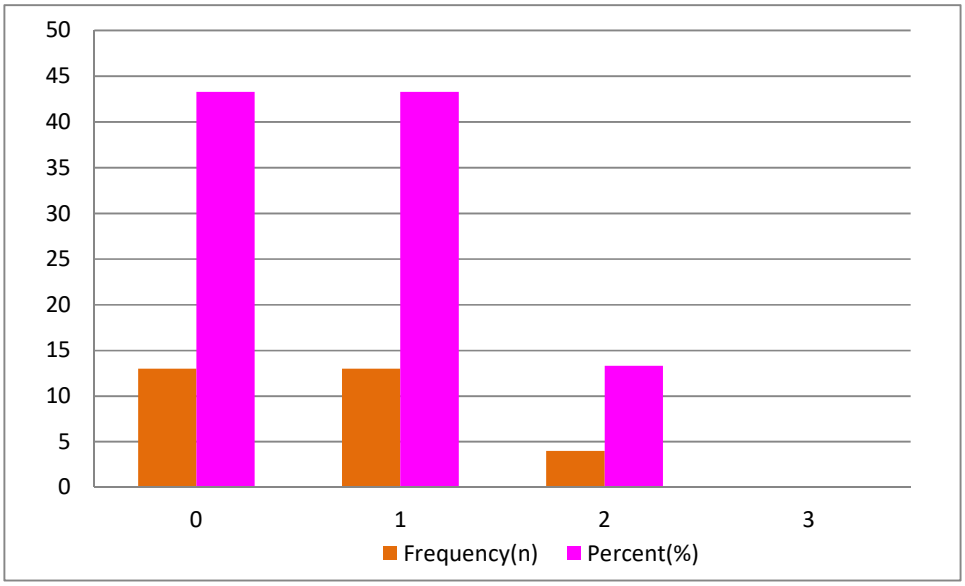



Among 17 IgG4-positive cases, weak positivity is observed in 13 cases(43.3%), while intermediate positivity observed in 4 cases(13.3%). In our study none of the cases showed strong positivity. (Table 11 and Figure 25)

**TABLE 12: Distribution of IgG4 score**

| IgG4 SCORE | Frequency(n) | Percent(%) |
|---|---|---|
| **0** | 13 | 43.3 |
| **1** | 13 | 43.3 |
| **2** | 4 | 13.3 |
| **3** | 0 | 0 |
| **Total** | **30** | **100** |

**FIGURE 26: Distribution of IgG4 score**




Among 30 cases, 13 cases (43.3%) showed a score of 0, 13 cases (43.3%) showed a score of 1, and 4 cases (13.3%) showed a score of 2. (Table 12 and Figure 26).

**TABLE 13: Distribution of Serum IgG4 Levels**

| | **N** | |
|---|---|---|
| **SERUM IGG4 LEVEL** (mg/dL) | **<135**(Normal) | 15 (50%) |
| | **>135**(High) | 15 (50%) |

Among 30 cases, 15 cases (50%) had normal IgG4 levels (<135 mg/dL) while the other 15 cases (50%) had Increased IgG4 levels (>135 mg/dL). Highest was 158mg/dL. (Table 13)

**TABLE 14: Comparison of Serum IgG4 Levels Among Histopathological Diagnosis**

| SERUM IGG4 LEVEL (mg/dL) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **HP DIAGNOSIS** | **N** | **Mean** | **Std. Deviation** | **Std. Error** | **95% Confidence Interval for Mean** | | **Minimum** | **Maximum** | **Pvalue** |
| | | | | | **Lower Bound** | **Upper Bound** | | | |
| Eosinophilic Cystitis | 1 | 156 | | | | | 156 | 156 | 0.04* |
| Interstitial Cystitis | 15 | 132.6 | 16.762 | 4.328 | 123.32 | 141.88 | 96 | 158 | |
| Chronic Cystitis | 10 | 126.3 | 18.88 | 5.97 | 112.79 | 139.81 | 86 | 148 | |
| Cystitis cystica et glandularis | 4 | 105.33 | 19.655 | 11.348 | 56.51 | 154.16 | 93 | 128 | |
| **Total** | **30** | **130.06** | **13.824** | **5.412** | **119.92** | **134.81** | **86** | **158** | |

**p<0.05***

Serum IgG4 levels vary significantly across HP diagnoses (**p = 0.04**) (Table 14). Interstitial cystitis has the highest IgG4 level **(**158 mg/dL**),** while chronic cystitis has the lowest **(**86 mg/dL**).** The mean IgG4 level is 132.6 mg/dL (range: 96–158 mg/dL) in interstitial cystitis**,** 126.3 mg/dL (range: 86–148 mg/dL) in chronic cystitis**,** and 105.33 mg/dL (range: 93–128 mg/dL) in cystitis cystica glandularis**.** The overall mean IgG4 level is 130.06 mg/dL with a **s**tandard deviation of 13.824**,** highlighting variability in IgG4 expression across different diagnoses (Table 14).

**TABLE 15: Correlation Between Grading of Inflammation and IgG4 Intensity among positive cases**

| GRADE | IgG4 INTENSITY | | | Total | P-value |
|---|---|---|---|---|---|
| | Weak | Intermediate | Strong | | |
| **Mild** | 3 | 0 | 0 | 3 | 0.290 |
| **Moderate** | 10 | 4 | 0 | 14 | |
| **Severe** | 0 | 0 | 0 | 0 | |
| **Total** | **13** | **4** | **0** | **17** | |

Among IgG4-positive cases (n=17), 14 cases had moderate inflammation (Grade 2+), with 10 showing weak IgG4 positivity and 4 showing intermediate positivity to plasma cells. All 3 cases of mild inflammation(Grade 1+) exhibited weak IgG4 positivity, while no cases of severe inflammation were observed. The p-value (**0.290**) indicates no significant association between IgG4 intensity and grading. Most IgG4-positive cases showed weak positivity in plasma cells(Table 15).

**TABLE 16: Correlation Between IgG4 Score and Grading of Inflammation**

| GRADE | SCORE | | | | Total | P-value |
|---|---|---|---|---|---|---|
| | IgG4 Score 0 | IgG4 Score 1 | IgG4 Score 2 | IgG4 Score 3 | | |
| **Mild** | 4 | 3 | 0 | 0 | 7 | 0.445 |
| **Moderate** | 9 | 10 | 4 | 0 | 23 | |
| **Severe** | 0 | 0 | 0 | 0 | 0 | |
| **Total** | **13** | **13** | **4** | **0** | **30** | |

Among 30 cases, Score 0 and 1 are most common in mild and moderate inflammation. Score 2 (4 cases) appears only in moderate inflammation. In our study no severe inflammation cases were recorded. The p-value (**0.445**) indicates no significant association between IgG4 score and grading of inflammation(Table 16)

**TABLE l7: Clinicopathologic correlation in 15 patients with Interstitial cystitis**

| | | IgG4 EXPRESSION STATUS | | Total | P-value |
|---|---|---|---|---|---|
| | | **Positive** | **Negative** | | |
| **AGE** | 10-30 | 2 | 0 | 2 | 0.290 |
| | 31-50 | 6 | 0 | 6 | |
| | 51-70 | 4 | 1 | 5 | |
| | >70 | 1 | 1 | 2 | |
| **Total** | | **13** | **2** | **15** | |
| **GENDER** | Female | 6 | 0 | 6 | 0.215 |
| | Male | 7 | 2 | 9 | |
| **Total** | | **13** | **2** | **15** | |
| **GRADING OF INFLAMMATION** | Mild | 3 | 1 | 4 | 0.423 |
| | Moderate | 10 | 1 | 11 | |
| | Severe | 0 | 0 | 0 | |
| **Total** | | **13** | **2** | **15** | |
| **SERUM IgG4 LEVEL** | <135mg/dL | 10 | 2 | 6 | 0.06 |
| | >135mg/dL | 3 | 0 | 9 | |
| **Total** | | **13** | **2** | **15** | |

Among 15 cases of interstitial cystitis, IgG4 positivity was most common in the 31–50 age group (6 cases) (**p = 0.290**, not significant). Males had slightly higher IgG4 positivity (7 cases) compared to females (6 cases) (**p = 0.215**, not significant). IgG4 positivity was highest in cases with moderate inflammation (10 cases), followed by mild inflammation (3 cases), with no severe cases observed (**p = 0.423**, not significant). IgG4 positivity was found in 10 cases with normal serum IgG4 levels (<135 mg/dL) and in 3 cases with high serum IgG4 levels (>135 mg/dL)(Table 17)

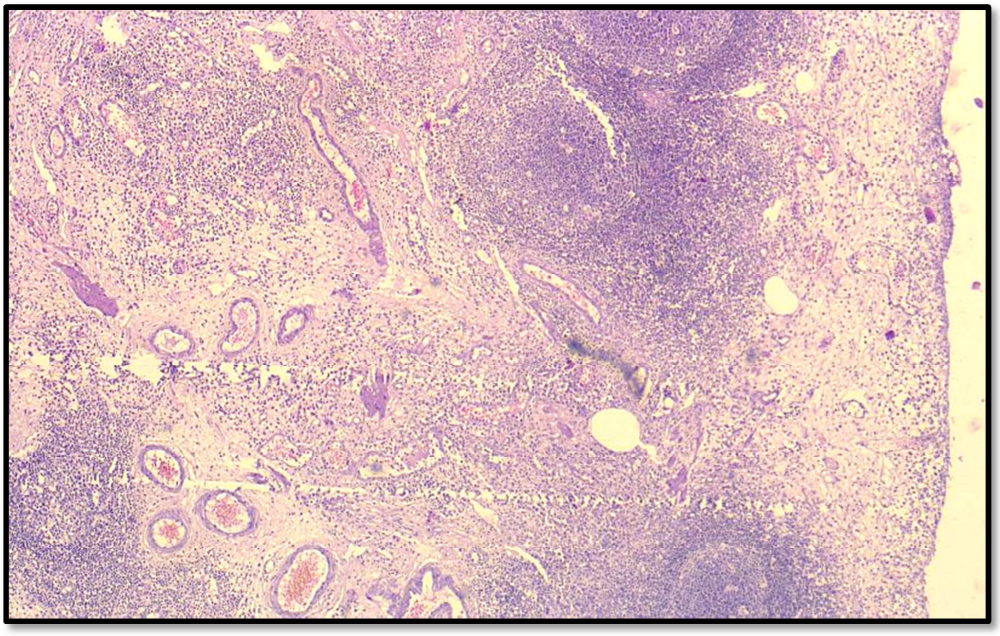


**FIGURE 27 : Interstitial cystitis : showing denuded urothelium, lymphoid aggregrates in lamina propria (H&E; 4X)**

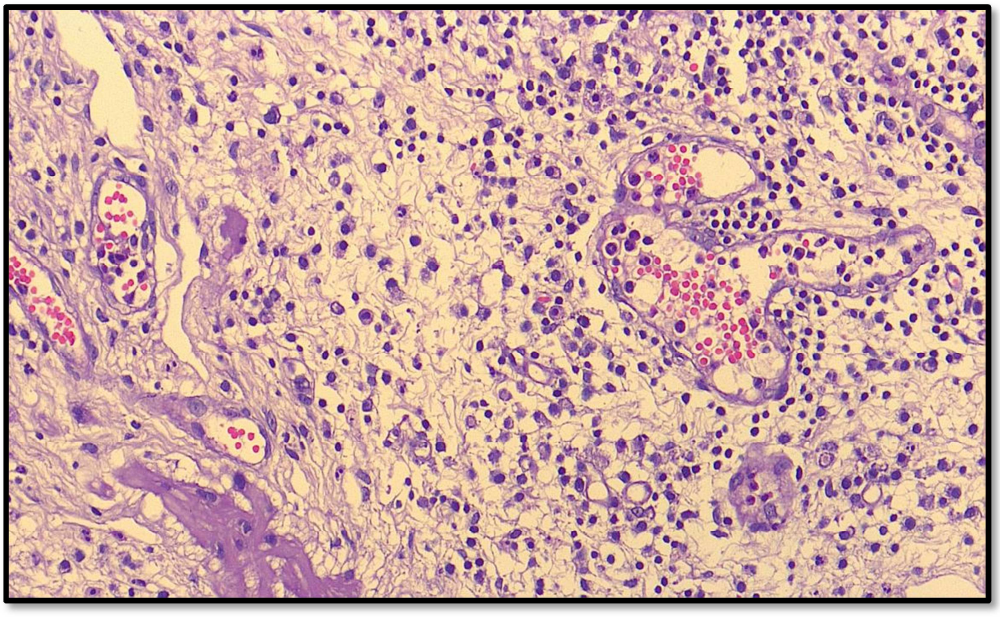


**FIGURE 28 : Interstitial cystitis : Lamina propria is infiltrated with chronic inflammatory infiltrates mainly of lymphoplasmacytes (H&E;20X)**

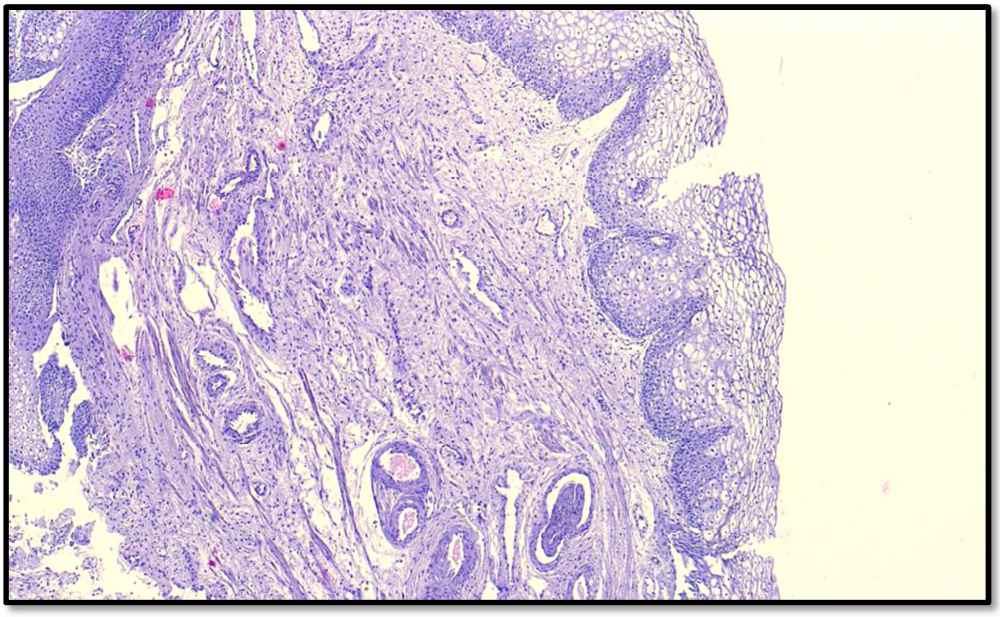


**FIGURE 29 : Interstitial cystitis: Showing reactive urothelium, lamina propria is edematous with moderate inflammatory infiltrates (H&E; 4X)**

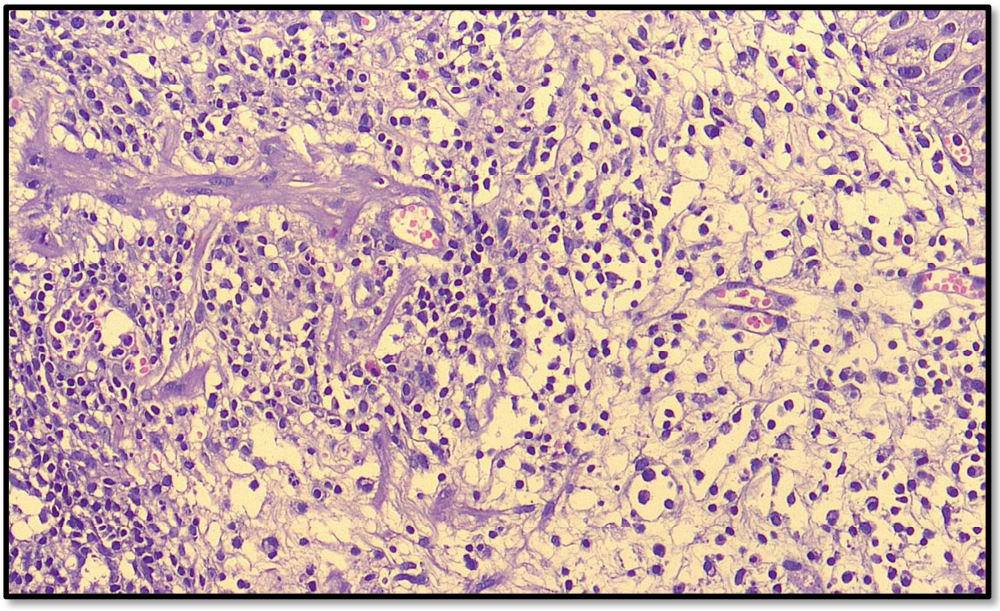


**FIGURE 30 : Interstitial cystitis : Lamina propria is infiltrated with chronic inflammatory infiltrates mainly of lymphocytes (H&E; 20X)**

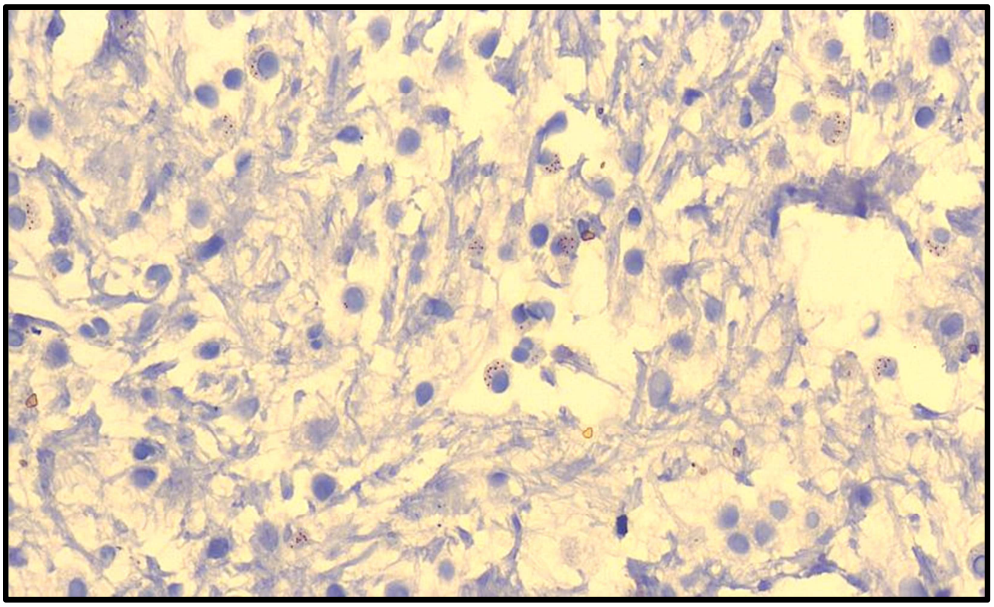


**FIGURE 31 : Showing IgG4 positivity in 7-8 plasma cells/hpf (IHC 40X)**

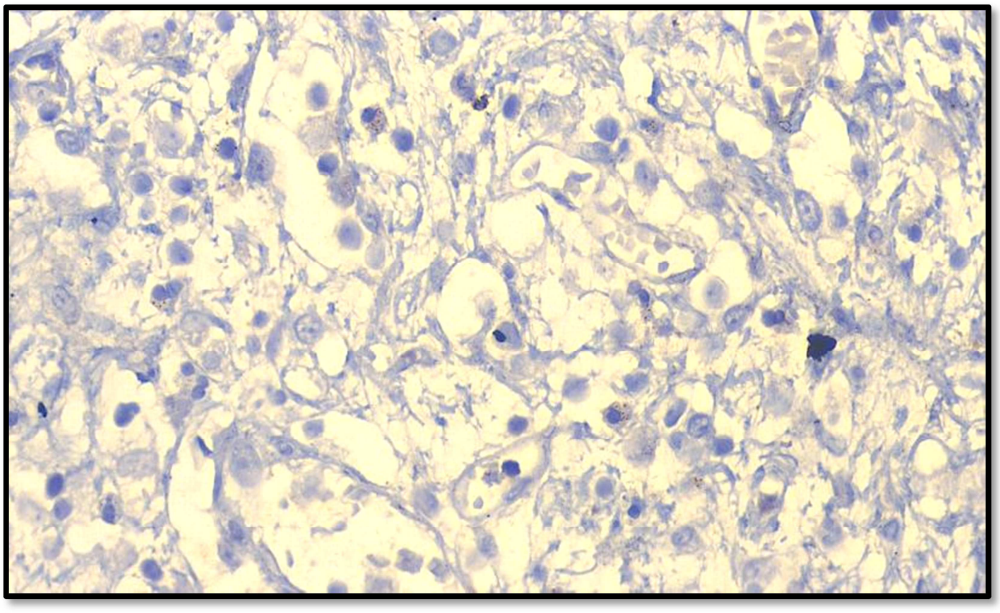


**FIGURE 32 : Weak IgG4 positivity in plasma cells(2-3/hpf) (IHC 40X)**

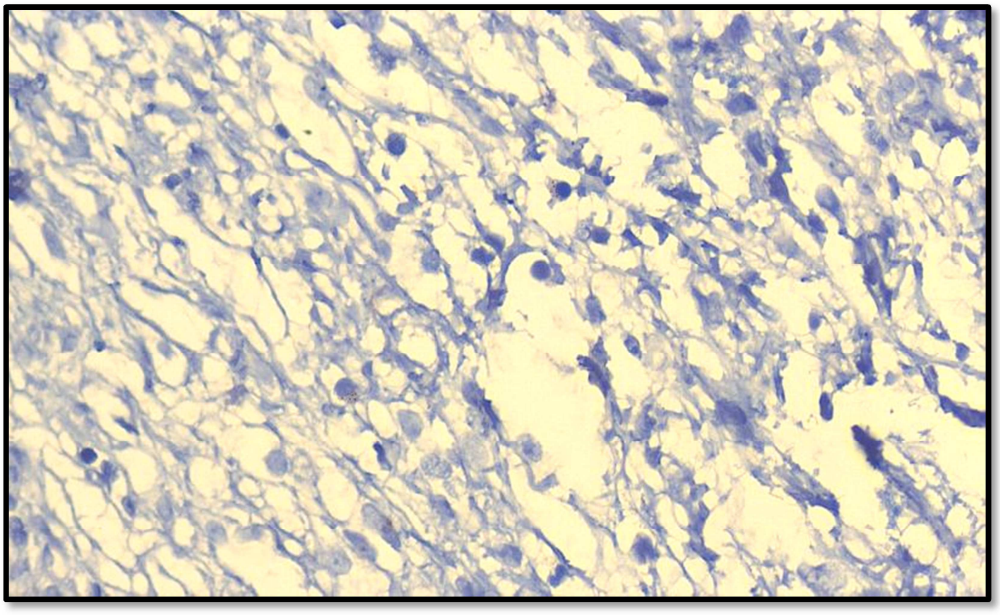


**FIGURE 33 : Weak IgG4 positivity in plasma cells(1-2/hpf) (IHC 40X)**

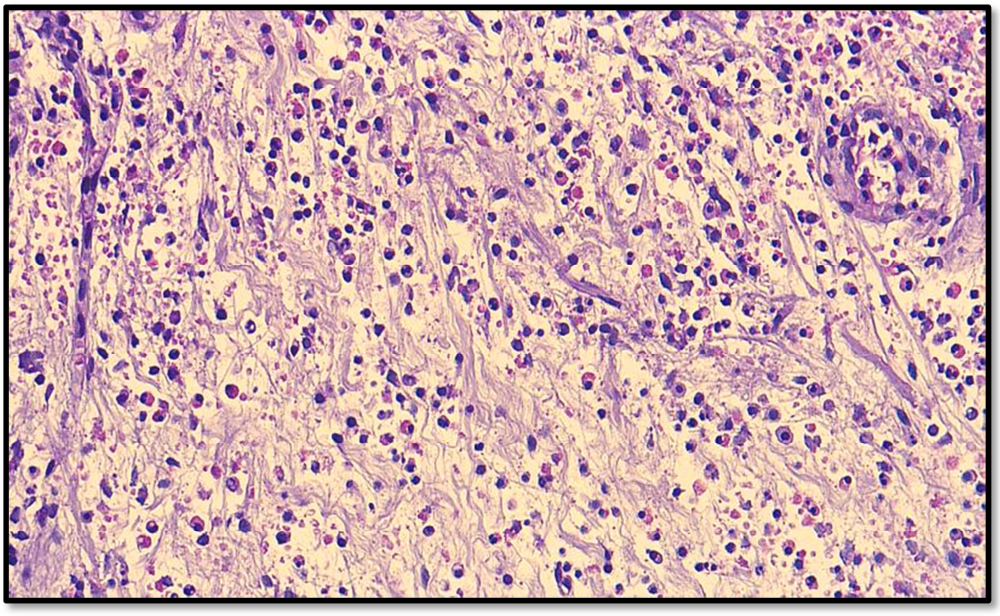


**FIGURE 34 : Eosinophilic Cystitis: Showing edematous lamina propria with diffuse mixed inflammatory infiltrates predominantly eosinophils- (H&E; 20X)**

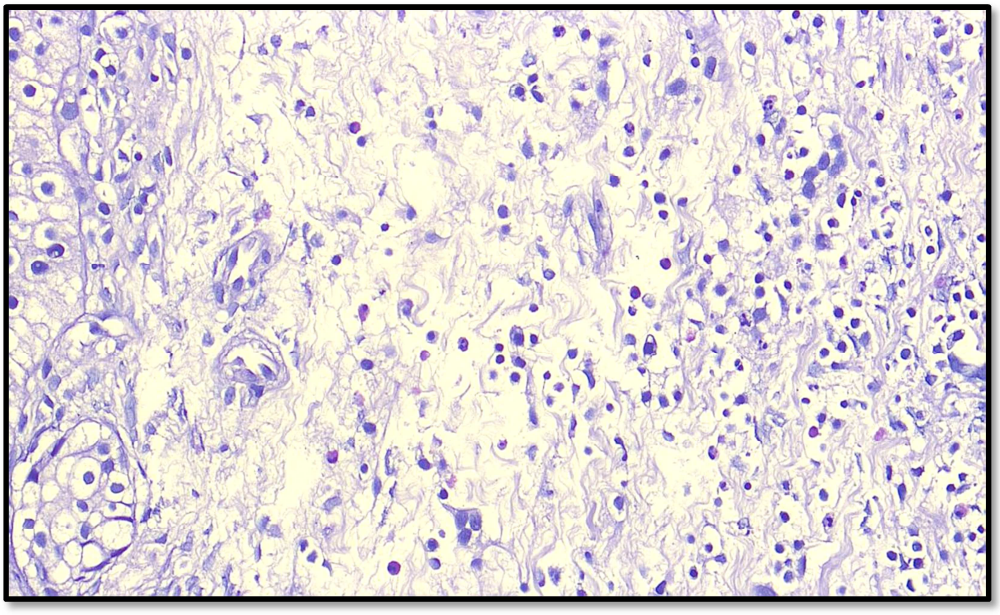


**FIGURE 35 : Chronic Cystitis- Showing edematous lamina propria having chronic inflammatory infilrates predominently lymphocytes-(H&E; 10X)**

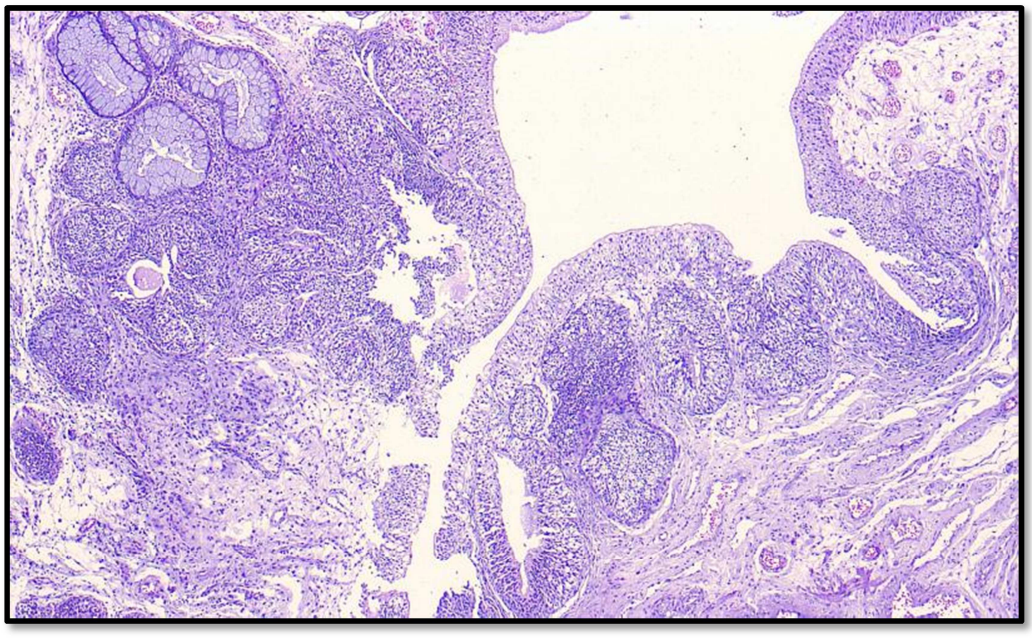


**FIGURE 36 : Cystitis Cystitica Et Glandularis: Dipping of transitional epithelium into the lamina propria with cystically dilated lumen- (H&E; 4X)**

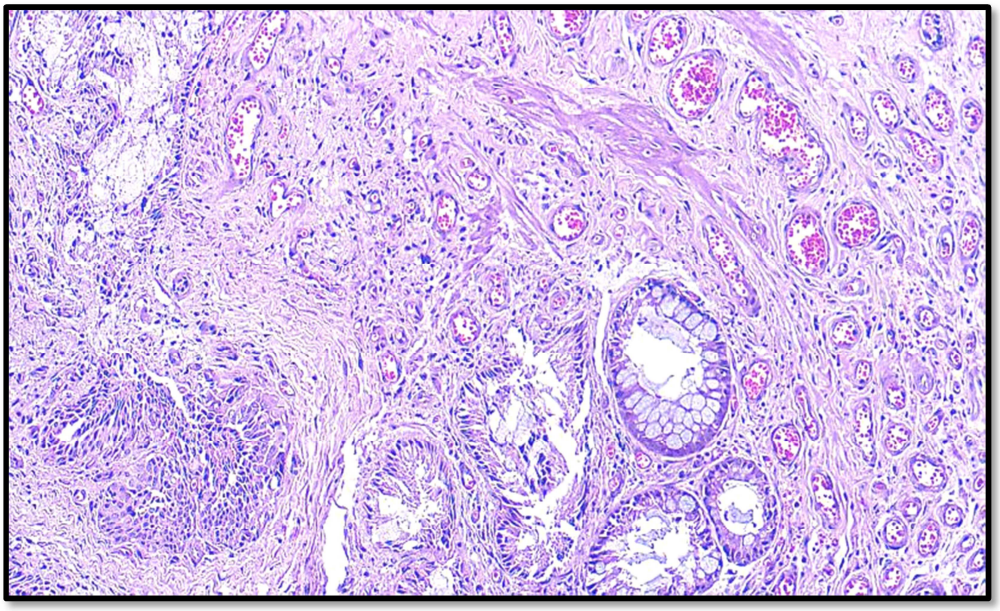


**FIGURE 37 : Cystitis Cystitica Et Glandularis: Lamina propria shows cystically dilated lumen with glandular lining cells- (H&E; 20X)**

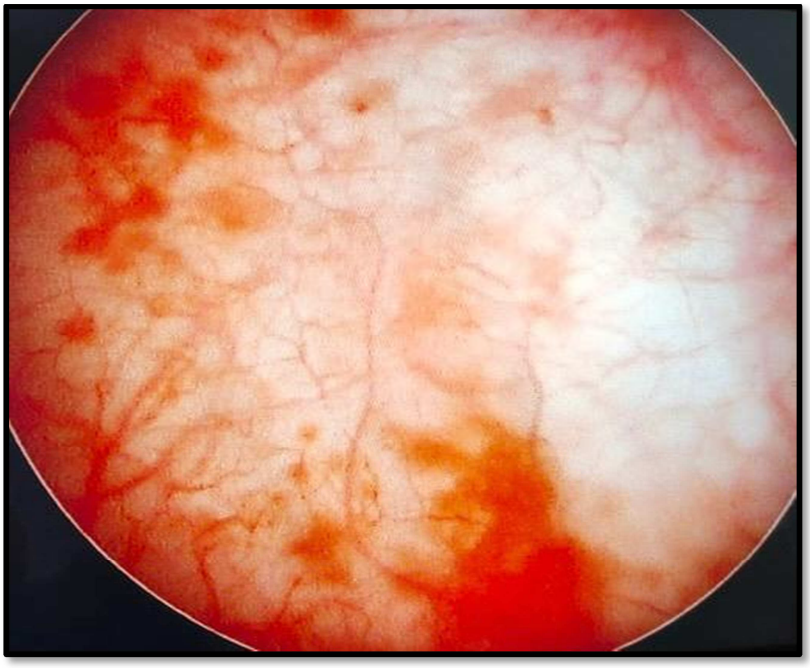


**FIGURE 38 : Cystoscopic Image of bladder showing Glomerulations in Multiple Quadrants**

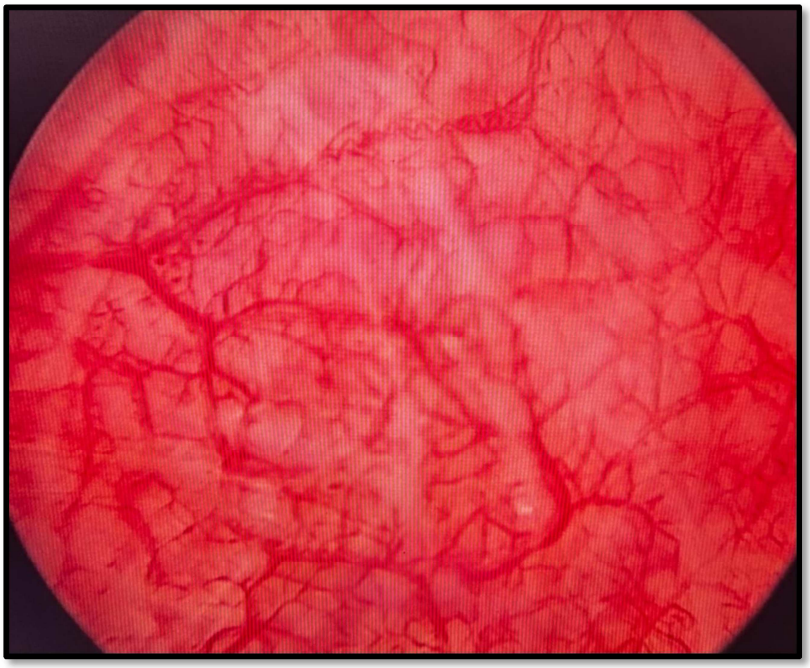


**FIGURE 39 : Cystoscopic Image of bladder showing Glomerulations with Prominent Capillaries**

# DISCUSSION

Interstitial cystitis (IC) is a long-term inflammatory disorder of the bladder. It manifests as urinary urgency, increased frequency, and pelvic pain without any underlying infection. The role of IgG4-related disease (IgG4-RD) in IC is a growing focus of research. IgG4-RD is a systemic fibro-inflammatory disorder marked by increased serum IgG4 levels and infiltration of tissues by IgG4-positive plasma cells.

In the study conducted by Crumley et al. (2013), IgG4-positive plasma cells were found in 9.1% of IC cases. Their study identified a strong correlation between IgG4 expression and disease severity, particularly in cases exhibiting severe inflammation and reduced bladder capacity. According to Crumley et al. (2013), IgG4-positive cases were more frequent in older individuals (mean age 60 years). In the present study, IgG4 positivity was most common in the age group of 31–50years(40%), followed by 51–70 years (26.7%). Crumley et al. also observed a higher proportion of IgG4-positive cases in males. Similarly, in the present study, a slight male predominance was noted (7 males vs. 6 females in IgG4-positive cases). However, interstitial cystitis remains more common in females overall. The authors proposed that IgG4-related IC might represent a distinct disease subset, emphasizing that these patients tend to be older and present with chronic bladder dysfunction. Unlike the present study, where 56.7% of cases showed IgG4 positivity, Crumley et al. found a much lower percentage, but their IgG4-positive cases exhibited more severe histopathological changes, such as dense lymphoplasmacytic infiltration and advanced fibrosis. This contrast suggests that IgG4-related IC may have varying degrees of severity, with some cases exhibiting mild inflammation while others progressing to extensive tissue damage.

In the review conducted by Montaño-Roca et al. (2020) on urologic manifestations of IgG4-related disease**,** highlighted that IgG4-RD frequently affects the urinary system, leading to tumor-like inflammatory lesions, fibrosis, and chronic lower urinary tract symptoms**.** Their study emphasized that IgG4-RD can mimic other urologic conditions, including IC**,** making histopathological and immunohistochemical evaluations crucial for differentiation. Unlike Montaño-Roca et al., who focused on mass-forming lesions in IgG4-RD**,** present study found no tumor-like lesions but demonstrated significant IgG4 plasma cell infiltration**,** further reinforcing that IgG4-related IC may be a localized inflammatory condition rather than a systemic manifestation**.**

A comprehensive meta-analysis conducted by Hao et al. (2016) evaluated the diagnostic value of serum IgG4 in IgG4-related disease**.** Their findings revealed that serum IgG4 >135 mg/dL had a sensitivity of 87.2% and a specificity of 82.6% for diagnosing IgG4-RD, suggested that raising the threshold to >270 mg/dL improved specificity but significantly reduced sensitivity**.** In comparison, present study found that 50% of IC cases had serum IgG4 >135 mg/dL, but none reached levels above 270 mg/dL**,** indicating that IgG4-related IC may exhibit only moderate systemic IgG4 elevation rather than complete IgG4-RD characteristics**.**

In the study conducted by Kim et al. (2016)**, the** diagnostic criteria for IC/BPS were evaluated**,** highlighting that histopathological findings do not always correlate with clinical severity**.** Unlike present study, where IgG4 positivity was assessed as a diagnostic marker**,** Kim et al. did not consider IgG4 involvement**,** focusing more on bladder wall pathology and inflammatory markers**.** This suggests that IgG4 testing may provide additional diagnostic insights in IC cases**.**

Grover et al. examined inflammation as a central mechanism in IC**,** noting that persistent inflammation leads to fibrosis and bladder dysfunction**.** Their findings indicated that IC may be an immune-mediated disorder**,** which aligns with present study's identification of IgG4 as a potential inflammatory contributor. However, unlike Grover et al., who found advanced fibrosis in chronic IC**,** present study did not report severe fibrotic changes in IgG4-positive cases**,** suggesting a less aggressive disease course in IgG4-associated IC.

# SUMMARY

The present study was done over a period of two years from January 2023 to December 2024 in the Department of Pathology, Jawaharlal Nehru Medical College and KLE's Dr Prabhakar Kore Hospital and Medical Research Centre, KAHER, Belagavi.

30 bladder biopsies were analyzed from patients who presented to the Department of Urology with complaints of dysuria, increased frequency, urgency, hematuria, and recurrent urinary tract infections (UTIs). These patients underwent cystoscopy, and biopsy specimens were sent to Department of Pathology, Jawaharlal Nehru Medical College and Hitech Laboratory KLE's Dr Prabhakar Kore Hospital and Medical Research Centre, KAHER, Belagavi.

The primary objective was to assess IgG4 expression and serum IgG4 levels in interstitial cystitis (IC) and related bladder conditions. The majority of cases (90%) involved bladder biopsies, with two TURBT specimens and one cystostomy case.

- The study population ranged from 5 to 88 years, with the majority of cases (36.7%) falling within the 31–50 age group. This indicates that interstitial cystitis (IC) and other chronic inflammatory bladder conditions predominantly affect middle-aged individuals.

- A slight male predominance was observed, with 53.3% of cases being male and 46.7% female.

- Lower urinary tract symptoms (LUTS), were the most frequent presenting complaint, reported in 53.3% of cases. Hematuria was the second most common symptom (26.7%), followed by recurrent urinary tract infections (UTIs). This

aligns with the typical clinical spectrum of IC and other chronic inflammatory bladder disorders.

- Interstitial cystitis was the most common diagnosis (50%), followed by chronic cystitis (33.3%), cystitis cystica glandularis (13.4%) and 1 case of eosinophilic cystitis. The findings highlight the clinical significance of IC and suggest that bladder inflammation exists on a spectrum, potentially driven by immune mechanisms.

- The majority of cases (76.7%) showed moderate inflammation (Grade 2+), while 23.3% had mild inflammation (Grade 1+). No cases had severe inflammation (Grade 3+), unlike other studies reporting fibrosis in IgG4-positive IC cases.

- IgG4 positivity was detected in 56.7% of cases, with weak expression in 43.3% and intermediate expression in 13.3%.

- Serum IgG4 was elevated (>135 mg/dL) in 50% of cases, with the highest recorded level at 158 mg/dL.

- No significant correlation was found between IgG4 intensity and inflammation severity ($p = 0.290$).

- This study provides strong evidence that a subset of IC cases is associated with IgG4-mediated inflammation.

- 56.7% of cases showed IgG4 positivity in bladder tissue, suggesting a potential role of IgG4-driven pathology in IC.

- 50% of cases had serum IgG4 >135 mg/dL, meeting the diagnostic threshold for IgG4-RD but remaining lower than levels seen in systemic IgG4-RD.

- This study analyzed IgG4 expression in 15 interstitial cystitis patients based on age, gender, inflammation severity, and serum IgG4 levels. IgG4 positivity was most common in the age group of 31-50 years , with a slight male predominance. Moderate inflammation had the highest IgG4 positivity, but no significant correlation was found. Patients with higher serum IgG4 levels (>135 mg/dL) were more frequently IgG4-positive, showing a trend toward significance ($p=0.06$). Overall, while IgG4 expression varied across groups, no strong statistical associations were observed, except for a possible link with serum IgG4 levels.

# CONCLUSION

- This study highlights an IgG4-related subset of interstitial cystitis, with IgG4 positivity in 56.7% of cases, suggesting a distinct inflammatory mechanism from non-IgG4-mediated chronic inflammation.

- Present study shows 50% of cases have increased serum IgG4 (>135 mg/dL), which is in accordance with IgG4-RD criteria but lower than systemic IgG4-RD values. This suggests that the inflammatory process is localised rather than systemic.

- Lack of severe fibrosis differentiates IgG4-positive IC from chronic IC, indicating an earlier stage or alternative trend that could affect prognosis and treatment.

- The results of the current study highlight the significance of including IgG4 testing in IC diagnostic procedures, both by immunohistochemical staining in bladder biopsies and serum IgG4 assessment, aiding targeted immunomodulatory therapy for IgG4-driven inflammation.

- This study supports IgG4-related IC as a distinct entity, emphasizing the need for further research to enhance diagnosis, classification, and personalized treatment.

Overall, this study highlights the importance of IgG4 assessment in Interstitial cystitis**,** which could help refine diagnostic criteria and lead **to** more personalized treatment approaches for IC patients exhibiting IgG4-related pathology.

# LIMITATIONS

- Present study analyzed only 30 cases, which may limit the generalizability of the findings.
- This study does not assess the progression of IgG4-related IC over time or the impact of treatment.
- A comparison with non-IC patients or systemic IgG4-RD cases could strengthen the conclusions.
- The grading of inflammation was subjective, and interobserver variability may have influenced the results.
- While 50% of cases had elevated serum IgG4, levels did not always correlate with histopathological findings.
- Present study did not assess bladder function or symptom severity in relation to IgG4 expression.

# BIBLIOGRAPHY


1. Crumley S, Ge Y, Zhou H, Shen SS, Ro JY. Interstitial cystitis: another IgG4–related inflammatory disease? Annals of Diagnostic Pathology. 2013 Oct;17(5):403–7.
2. Berry SH, Elliott MN, Suttorp M, Bogart LM, Stoto MA, Eggers P, et al. Prevalence of Symptoms of Bladder Pain Syndrome/Interstitial Cystitis Among Adult Females in the United States. Journal of Urology. 2011 Aug;186(2):540–4.
3. Kim HJ. Update on the Pathology and Diagnosis of Interstitial Cystitis/Bladder Pain Syndrome: A Review. Int Neurourol J. 2006 Mar 31;20(1):13–7.
4. Hanno P, Dmochowski R. Status of international consensus on interstitial cystitis/bladder pain syndrome/painful bladder syndrome: 2008 snapshot. Neurourol Urodyn. 2009;28(4):274–86.
5. Messing EM, Stamey TA. Interstitial cystitis early diagnosis, pathology, and treatment. Urology. 1978 Oct;12(4):381–92.
6. Hunner GL. A rare type of bladder ulcer: further notes, with a report of eighteen cases. Jama. 1918 Jan 26;70(4):203.
7. Hanno PM, Burks DA, Clemens JQ, Dmochowski RR, Erickson D, FitzGerald MP, et al. AUA Guideline for the Diagnosis and Treatment of Interstitial Cystitis/Bladder Pain Syndrome. Journal of Urology. 2011 Jun;185(6):2162–70.
8. Grover S, Srivastava A, Lee R, Tewari AK, Te AE. Role of inflammation in bladder function and interstitial cystitis. Therapeutic Advances in Urology. 2011 Feb;3(1):19–33.

9. Wilkins EGL, Payne SR, Pead PJ, Moss ST, Maskell RM. Interstitial Cystitis and the Urethral Syndrome: a Possible Answer. British Journal of Urology. 1989 Jul;64(1):39–44.
10. Akiyama Y. Biomarkers in Interstitial Cystitis/Bladder Pain Syndrome with and without Hunner Lesion: A Review and Future Perspectives. Diagnostics. 2021 Nov 30;11(12):2238.
11. Gamper M, Viereck V, Eberhard J, Binder J, Moll C, Welter J, et al. Local immune response in bladder pain syndrome/interstitial cystitis ESSIC type 3C. Int Urogynecol J. 2013 Dec;24(12):2049–57.
12. Divatia M, Ro J. IgG4-related Disease of the Genitourinary Tract. J Interdiscipl Histopathol. 2014;2(1):3.
13. Montaño-Roca BE, Vanacore D, Gallegos-Sánchez G, Rosales-Velázquez CE, Ruvalcaba-Oceguera GE, Aragón-Castro MA, et al. Urologic manifestations of IgG4-related disease Manifestaciones urológicas de la enfermedad relacionada a IgG4. 80.
14. Stone JH, Zen Y, Deshpande V. IgG4-Related Disease. N Engl J Med. 2012 Feb 9;366(6):539–51.
15. Langman J, Sadler TW, editors. Langman's medical embryology. 9. ed. Philadelphia: Lippincott Williams & Wilkins; 2004. 534 p.
16. Dudek RW. Embryology. Sixth edition. Philadelphia: Wolters Kluwer Health; 2014.
17. Moore KL, Dalley AF, Agur AMR. Clinically oriented anatomy. 7th ed. Philadelphia: Wolters Kluwer/Lippincott Williams & Wilkins Health; 2014. 1134 p.
18. Chaurasia BD. Human Anatomy: Volume 2: Lower Limb, Abdomen and Pelvis. 8th ed. Vol. 2. CBS Publishers & Distributors Pvt Ltd; 2019.

19. Wheater PR, O'Dowd G, Woodford P, Young B. Wheater's functional histology: a text and colour atlas ; [study smart with student consult]. 6. ed., internat. ed., repr. Philadelphia, Pa: Elsevier Churchill Livingstone; 2014. 452 p. (4190 Study smart with student consult).
20. Eroschenko VP, Fiore MSH di. DiFiore's atlas of histology with functional correlations. 12th ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2013.
21. Rauniyar SK, Agrawal A, Shah A. A Study Of Cystoscopic Biopsies In Kathmandu Valley. J Nepal Med Assoc. 2003 Oct 1;40(140):181–5.
22. Mills SE, Sternberg SS, Longacre TA. Mills and Sternberg's diagnostic surgical pathology. 7th ed. Philadelphia: Wolters Kluwer; 2022.
23. Echols RM, Tosiello RL, Haverstock DC, Tice AD. Demographic, clinical, and treatment parameters influencing the outcome of acute cystitis. Clin Infect Dis. 1999 Jul;29(1):113–9.
24. Pfeifer JD, Dehner LP, Humphrey PA, Ovid Technologies, Inc, editors. The Washington manual of surgical pathology. 3rd ed. Philadelphia: Wolters Kluwer; 2020. 1 p.
25. Zhou M, Epstein JI, Netto GJ. Uropathology: high-yield pathology ; [get full access and more at ExpertConsult.com]. Philadelphia, Pa: Elsevier, Saunders; 2012. 539 p. (Recommemded shelving classifications).
26. Sparks S, Kaplan A, DeCambre M, Kaplan G, Holmes N. Eosinophilic cystitis in the pediatric population: A case series and review of the literature. Journal of Pediatric Urology. 2013 Dec;9(6):738–44.
27. Hasan A, Abdel-Al I, Nafie K, Rashad MF, Abozied H, Elhussiny MEA, et al. Clinicopathological characteristics and outcomes of eosinophilic cystitis: A retrospective study. Annals of Medicine & Surgery [Internet]. 2021 Aug [cited

2025 Mar 14];68. Available from: https://journals.lww.com/10.1016/j.amsu.2021.102626

28. Park H. Eosinophilic cystitis with recurrent urinary retention: case report. RRU. 2017 Mar;Volume 9:51–3.
29. Hamza EA, Reda T, Salim O, Hachem ES, Yassine N. Follicular Cystitis, a Rare Diagnosis in an Emergency Setting: A case report and literature review. International Journal of Surgery Case Reports. 2025 Jan;126:110776.
30. Harrison S, Lamchahab H, Djamal J, Jabbour Y, Alae T, Karmouni T, et al. Cystitis Glandularis: A Rare Benign Condition Presenting as Bladder Tumor. OJU. 2018;08(12):336–43.
31. Meijlink JM. Interstitial cystitis and the painful bladder: A brief history of nomenclature, definitions and criteria. Int J of Urology. 2014 Apr;21(S1):4–12.
32. Homma Y, Akiyama Y, Tomoe H, Furuta A, Ueda T, Maeda D, et al. Clinical guidelines for interstitial cystitis/bladder pain syndrome. Int J of Urology. 2020 Jul;27(7):578–89.
33. Akiyama Y, Luo Y, Hanno PM, Maeda D, Homma Y. Interstitial cystitis/bladder pain syndrome: The evolving landscape, animal models and future perspectives. Int J of Urology. 2020 Jun;27(6):491–503.
34. Van De Merwe JP, Nordling J, Bouchelouche P, Bouchelouche K, Cervigni M, Daha LK, et al. Diagnostic Criteria, Classification, and Nomenclature for Painful Bladder Syndrome/Interstitial Cystitis: An ESSIC Proposal. European Urology. 2008 Jan;53(1):60–7.
35. Hanno PM. Interstitial cystitis-epidemiology, diagnostic criteria, clinical markers. Rev Urol. 2002;4 Suppl 1(Suppl 1):S3-8.

36. Goldblum JR, Lamps LW, McKenney JK, Myers JL, Ackerman LV, Rosai J, editors. Rosai and Ackerman's surgical pathology. Eleventh edition. Philadelphia, PA: Elsevier; 2018. 2 p.
37. Maeda D, Akiyama Y, Morikawa T, Kunita A, Ota Y, Katoh H, et al. Hunner-Type (Classic) Interstitial Cystitis: A Distinct Inflammatory Disorder Characterized by Pancystitis, with Frequent Expansion of Clonal B-Cells and Epithelial Denudation. Hurst R, editor. PLoS ONE. 2015 Nov 20;10(11):e0143316.
38. Peters KM, Killinger KA, Mounayer MH, Boura JA. Are Ulcerative and Nonulcerative Interstitial Cystitis/Painful Bladder Syndrome 2 Distinct Diseases? A Study of Coexisting Conditions. Urology. 2011 Aug;78(2):301–8.
39. McLennan MT. Interstitial Cystitis. Obstetrics and Gynecology Clinics of North America. 2014 Sep;41(3):385–95.
40. Meijlink J. Interstitial cystitis/bladder pain syndrome: interstitial cystitis, bladder pain syndrome, hypersensitive bladder, hunner lesion disease chronic pelvic pain, associated disorders : an overview of diagnosis & treatment. Revised edition. Nederland: International Painful Bladder Foundation; 2020.
41. Sant GR, Kempuraj D, Marchand JE, Theoharides TC. The Mast Cell in Interstitial Cystitis: Role in Pathophysiology and Pathogenesis. Urology. 2007 Apr;69(4):S34–40.
42. Hanno PM. Diagnosis and Treatment of Interstitial Cystitis/Bladder Pain Syndrome: AUA Guideline Amendment.
43. Sutcliffe S, Bradley CS, Clemens JQ, James AS, Konkle KS, Kreder KJ, et al. Urological chronic pelvic pain syndrome flares and their impact: qualitative analysis in the MAPP network. Int Urogynecol J. 2015 Jul;26(7):1047–60.

44. Tyagi P, Killinger K, Tyagi V, Nirmal J, Chancellor M, Peters KM. Urinary Chemokines as Noninvasive Predictors of Ulcerative Interstitial Cystitis. Journal of Urology. 2012 Jun;187(6):2243–8.
45. Deshpande V, Zen Y, Chan JK, Yi EE, Sato Y, Yoshino T, et al. Consensus statement on the pathology of IgG4-related disease. Modern Pathology. 2012 Sep;25(9):1181–92.
46. Wolfson AR, Hamilos DL. Recent advances in understanding and managing IgG4-related disease. F1000Res. 2017 Feb 23;6:185.
47. Umehara H, Okazaki K, Kawa S, Takahashi H, Goto H, Matsui S, et al. The 2020 revised comprehensive diagnostic (RCD) criteria for IgG4-RD. Modern Rheumatology. 2021 May 4;31(3):529–33.
48. Carlson BM. Human embryology and developmental biology. Elsevier Health Sciences; 2018 Nov 30.

# ANNEXURES

## ANNEXURE I

## PROFORMA

PATIENT HISTORY

Name: Age:

IP No.: Sex:

Date:

BRIEF CLINICAL HISTORY:

EXAMINATION FINDINGS:

CLINICAL DIAGNOSIS:

INVESTIGATIONS DONE:

1. Serum IgG4 Level:
2. Cystoscopic Findings:

HISTOPATHOLOGICAL DIAGNOSIS:

1. Hematoxylin & Eosin staining:

| **Grade** | Severity | Histopathological description |
|---|---|---|
| **1+** | **Mild** | |
| **2+** | **Moderate** | |
| **3+** | **Severe** | |

2. IHC staining:

| **Score** | **No. of plasma cells/HPF** |
|---|---|
| Score 0 | |
| Score 1 | |
| Score 2 | |
| Score 3 | |

## ANNEXURE II

## INFORMED CONSENT FORM

## "THE EXPRESSION OF IgG4 IN INTERSTITIAL CYSTITIS IN A TERTIARY CARE CENTRE - A HOSPITAL BASED PROSPECTIVE STUDY"

**Name of Student/Principal Investigator: _____**

**Name of Guide/Co Investigators: _____**

**Introduction**: You are being asked to enroll in this study as you are eligible for participation in this study. If you undergo cystoscopy for interstitial cystitis you will be included for the study.

The purpose of the study is to evaluate the expression of IgG4 immunostaining in urinary bladder biopsies of Interstitial cystitis. This study will help in determining a better diagnostic tool for Interstitial cystitis.

**Explanation of procedure:** During this study, you will be asked questions regarding history and background and you are supposed to answer to the best of your knowledge. The principal investigator of the study is Dr. Akshara Somaraj Nair under the guidance of Dr. Ranjit P Kangle(guide) and Dr. R B Nerli(co guide).

If you agree to enroll yourself in this study, you will be interviewed regarding your present, past and family history and your clinical manifestations.

**Withdrawal from participation in the study:** Participation in this study in voluntary. You will be free to decide whether to participate in this study or continue participation once enrolled. In case you decide to withdraw your participation, you are free to do so. However, please convey the decision to the principal investigator.

**Possible benefits from participating in the study:** You will not get any benefits by participating in this study. The data gathered will help the population at large.

**Privacy and confidentiality:** The information collected from you will he coded, to prevent any person from identifying you. Your identity will never be revealed. The data collected from you will be kept confidential and only processed or aggregated data will be used for publication. No information about you or information provided by you during research will be disclosed to other without your written permission except:

1. In emergency to protect your rights and welfare.
2. If required by law.

**Financial incentives:** You will not receive any payment for participating in this study.

Cost of investigations done during the course of study will be paid by the principal investigator.

**Authorization for publication of aggregated data:** Results obtained after processing of the aggregated data will be published for scientific purposes and or presented to scientific groups. However, your identity will never be revealed.

**Questions:** If you have any question or complaints with regard to your right as study participant you may contact Dr. Harsha Hegde, Chairperson, Ethical committee of JNMC, 0831-2473777 Extension 4052.

**Legal rights:** By signing this consent form, we ure not waving any of your legal rights

## ANNEXURE III

## CONSENT STATEMENT

I am making a voluntary decision to participate in the study **"THE EXPRESSION OF IgG4 IN INTERSTITIAL CYSTITIS IN A TERTIARY CARE CENTRE- A HOSPITAL BASED PROSPECTIVE STUDY"**. My signature below indicates that I have decided to participate and I have read the information provided above or the information provided above has been read to me in the language that I understand best. I was given the opportunity to ask questions and that they have been answered to my satisfaction.

Name of the participant:

Signature or left thumb impression of the participant:

Name of the witness:

Signature or left thumb impression of the witness:

Name of the investigator:

Signature of the investigator:

## ANNEXURE IV

## HEMATOXYLIN AND EOSIN STAIN

**REAGENTS**

1. Erhlich's Haematoxylin solution

2. Eosin Y solution 1%

3. 1% acid alcohol solution

**HEMATOXYLIN AND EOSIN STAIN – PROCEDURE**

1. Deparaffinise the tissue sections in xylene (Xylene 1 for 5 mins + Xylene 2 for 5 mins
2. Subject the tissue section to water through reducing grades of alcohol (90% alcohol for 5 mins + 70% alcohol for 5 mins)
3. Keep it in hematoxylin for 8 to 10 minutes
4. Rinse it in tap water for 2 mins
5. Differentiate with 1% acid alcohol for 10 sec
6. For bluing - place in tap water for about 10 minutes
7. Counter stain by eosin 1-2 minutes
8. Rinse in water
9. Dehydration increasing grades of alcohol (70% alcohol for 30 sec + 90% alcohol for 30 sec)
10. Clearing is done by Xylene (Xylene 1 for 5 mins + Xylene 2 for 5 mins
11. Mount it with Dibutylphalate Polystyrene Xylene (DPX).

## ANNEXURE V

### IMMUNOHISTOCHEMICAL STAINING PROCEDURE FOR IgG4:

1. Cut the tissue sections on microtome with the thickness of 3u and collect them on coated slides
2. Bake the sections at 37 degrees Celsius for overnight.
3. Before the test bake it at 60 degrees Celsius for 1 hour.

Deparaffinize step:

4. Xylene I for 10 minutes
5. Xylene II for 10 minutes
6. Absolute alcohol I for 10 minutes
7. Absolute alcohol II for 10 minutes
8. Rinse in water - 5 minutes
9. Rinse in distilled water- 1 minute
10. Antigen retrieval (Tris Buffer+ EDTA)
11. Prepare the required amount of buffer and cook the slides in a pressure cooker for 3 whistles
12. Allow it to cool to room temperature for 1 hour
13. Wash it with wash buffer 3 times with a gap of 2 minutes each.
14. Apply 3% Hydrogen peroxide- 8 to 10 minutes
15. Wash with wash buffer 3 times with a gap of 2 minutes each
16. Primary antibody IgG4 incubated for 45 to 60 minutes in a closed chamber at room temperature.
17. Wash with wash buffer 3 times with a gap of 2 minutes each.
18. Apply the primary antibody amplifier master for 15 minutes in a closed chamber.
19. Wash with wash buffer 3 times with a gap of 2 minutes each.

20. Apply polymer HRP for 25-30 minutes in a closed chamber.
21. Wash with wash buffer 3 times with a gap of 2 minutes each.
22. Apply DAB chromogen for 10 minutes.
23. Wash slowly under running tap water from the backside.
24. Counterstain with Haematoxylin for 2 minutes.
25. Blueing in warm water for 1 minute.
26. Clear in xylene and mount with DPX.

The tonsil was used as a positive control

## ANNEXURE VI

## KEY TO MASTER CHART

Grade 1+: Mild Inflammation

Grade 2+: Moderate Inflammation

Grade 3+: Severe Inflammation

Score 0: No IgG4 positive plasma cells detected per hpf

Score 1: Presence of 1-5 IgG4 positive plasma cells per hpf

Score 2: Presence of 5-30 IgG4 positive plasma cells per hpf

Score 3: Presence of >30 IgG4 positive plasma cells per hpf

HP - HISTOPATHOLOGICAL

LUT - LOWER URINARY TRACT

TURBT- TRANSURETHRAL RESECTION OF BLADDER TUMOR

TB - TUBERCULOSIS

UTI - URINARY TRACT INFECTION

LGUC - LOW GRADE UROTHELIAL CARCINOMA

## ANNEXURE VII MASTER CHART

| CASE No. | AGE | SEX | OP/IP No. | SERUM IGG4 LEVEL | CLINICAL HISTORY | CLINICAL DIAGNOSIS | TYPE OF SPECIMEN | HP DIAGNOSIS | GRADING | PREDOMINANT INFLAMMATORY CELL | IGG4 EXPRESSION STATUS | IGG4 INTENSITY | IGG4 No. OF PLASMA CELL POSITIVE/HPF | IGG4 SCORE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 40 | Female | 1144320 | 156mg/dL | LUT symptoms | Interstitial cystitis | Bladder Biopsy | Eosinophilic Cystitis | 2+ | Eosinophils | Positive | Intermediate | 6-7/hpf | 2 |
| 2 | 30 | Female | 1172484 | 149mg/dL | Hematuria+Dysuria | Interstitial cystitis | Bladder Biopsy | Interstitial Cystitis | 2+ | Lymphoplasmacytes | Positive | Weak | 2-3/hpf | 1 |
| 3 | 45 | Female | 10085143 | 148mg/dL | LUT symptoms | Interstitial cystitis | Bladder Biopsy | Interstitial Cystitis | 2+ | Lymphoplasmacytes | Positive | Weak | 1-2/hpf | 1 |
| 4 | 88 | Male | 10089714 | 158mg/dL | Heamaturia+Dysuria | K/C/O Carcinoma Bladder | Cystoscopy+TURBT | Interstitial Cystitis | 2+ | Lymphoplasmacytes | Positive | Intermediate | 5-6/hpf | 2 |
| 5 | 64 | Male | 10083002 | 140mg/dL | Hematuria+Dysuria | Interstitial cystitis | Bladder Biopsy | Interstitial Cystitis | 2+ | Lymphoplasmacytes | Positive | Weak | 1-2/hpf | 1 |
| 6 | 48 | Female | 10080085 | 148mg/dL | LUT symptoms | Interstitial cystitis | Bladder Biopsy | Chronic Cystitis | 2+ | Lymphocytes | Negative | – | – | 0 |
| 7 | 72 | Female | 1096085 | 136mg/dL | LUT symptoms | K/C/O Carcinoma Cervix | Bladder Biopsy | Chronic Cystitis | 2+ | Lymphocytes | Negative | – | – | 0 |
| 8 | 22 | Male | 1185385 | 130mg/dL | LUT symptoms | Renovascular Hypertension | Bladder Biopsy | Chronic Cystitis | 2+ | Lymphocytes | Negative | – | – | 0 |
| 9 | 5 | Male | 10030223 | 95mg/dL | Recurrent UTI | Urethral Polyp | Cystostomy | Cystitis cystica Glandularis | 1+ | Lymphocytes | Negative | – | – | 0 |
| 10 | 70 | Female | 10048159 | 140mg/dL | LUT symptoms | TB Cystitis | Bladder Biopsy | Chronic Cystitis | 2+ | Lymphocytes | Negative | – | – | 0 |
| 11 | 32 | Female | 10054303 | 128mg/dL | LUT symptoms | Interstitial cystitis | Bladder Biopsy | Chronic Cystitis | 2+ | Lymphocytes | Positive | Weak | 0-1/hpf | 1 |
| 12 | 38 | Male | 10062594 | 143mg/dL | LUT symptoms | Interstitial cystitis | Bladder Biopsy | Interstitial Cystitis | 2+ | Lymphoplasmacytes | Positive | Weak | 3-4/hpf | 1 |
| 13 | 32 | Female | 10066971 | 138mg/dL | LUT symptoms | K/C/O Inverted papilloma | Bladder Biopsy | Interstitial Cystitis | 2+ | Lymphoplasmacytes | Positive | Weak | 1-2/hpf | 1 |
| 14 | 60 | Male | 10080584 | 112mg/dL | LUT symptoms | Cystitis | Bladder Biopsy | Interstitial Cystitis | 1+ | Lymphocytes | Positive | Weak | 1-2/hpf | 1 |
| 15 | 68 | Male | 7552437 | 120mg/dL | Hematuria+Dysuria | TB Cystitis | Bladder Biopsy | Interstitial Cystitis | 1+ | Lymphocytes | Positive | Weak | 0-1/hpf | 1 |

| CASE No. | AGE | SEX | OP/IP No. | SERUM IGG4 LEVEL | CLINICAL HISTORY | CLINICAL DIAGNOSIS | TYPE OF SPECIMEN | HP DIAGNOSIS | GRADING | PREDOMINANT INFLAMMATORY CELL | IGG4 EXPRESSION STATUS | IGG4 INTENSITY | IGG4 No. OF PLASMA CELL POSITIVE/HPF | IGG4 SCORE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 16 | 61 | Female | 1166236 | 128mg/dL | Hematuria | Cystitis | Bladder Biopsy | Cystitis cystica Glandularis | 1+ | Eosinophils | Negative | – | – | 0 |
| 17 | 34 | Male | 1168436 | 97mg/dL | Hematuria | K/C/O LGUC | Bladder Biopsy | Cysitis cystica Glandularis | 2+ | Lymphocytes | Negative | – | – | 0 |
| 18 | 75 | Male | 1170023 | 96mg/dL | LUT symptoms | TB Cystitis | Bladder Biopsy | Interstitial Cystitis | 2+ | Lymphocytes | Negative | – | – | 0 |
| 19 | 67 | Female | 1004125 | 138mg/dL | Hematuria | Cystitis | Bladder Biopsy | Interstitial Cystitis | 2+ | Lymphoplasmacytes | Positive | Intermediate | 5-6/hpf | 2 |
| 20 | 33 | Female | 1181218 | 136mg/dL | LUT symptoms | Interstitial cystitis | Bladder Biopsy | Interstitial Cystitis | 1+ | Lymphocytes | Positive | Weak | 1-2/hpf | 1 |
| 21 | 76 | Male | 1007446 | 141mg/dL | Recurrent UTI | Interstitial cystitis | Bladder Biopsy | Chronic Cystitis | 2+ | Lymphocytes | Positive | Weak | 1-2/hpf | 1 |
| 22 | 48 | Male | 1205278 | 138mg/dL | Hematuria | Interstitial cystitis | Bladder Biopsy | Interstitial Cystitis | 2+ | Lymphoplasmacytes | Positive | Weak | 1-2/hpf | 1 |
| 23 | 27 | Male | 10023867 | 93mg/dL | Hematuria | Trigonitis | Bladder Biopsy | Cystitis cystica Glandularis | 1+ | Lymphocytes | Negative | – | – | 0 |
| 24 | 26 | Male | 10029481 | 124mg/dL | LUT symptoms | Dorsal chordee with Penoscrotal hypospadias | Bladder Biopsy | Chronic Cystitis | 2+ | Lymphocytes | Positive | Weak | 0-1/hpf | 1 |
| 25 | 35 | Female | 1144230 | 86mg/dL | LUT symptoms | Cystitis | Bladder Biopsy | Chronic Cystitis | 2+ | Lymphocytes | Negative | – | – | 0 |
| 26 | 45 | Female | 1157997 | 140mg/dL | LUT symptoms | Interstitial cystitis | Bladder Biopsy | Interstitial Cystitis | 2+ | Lymphoplasmacytes | Positive | Intermediate | 5-6/hpf | 2 |
| 27 | 53 | Male | 1142974 | 102mg/dL | Hematuria | K/C/O Carcinoma Bladder | TURBT | Chronic Cystitis | 2+ | Lymphocytes | Negative | – | – | 0 |
| 28 | 30 | Male | 1090699 | 113mg/dL | Hematuria | Cystitis | Bladder Biopsy | Interstitial Cystitis | 2+ | Lymphoplasmacytes | Positive | Weak | 0-1/hpf | 1 |
| 29 | 57 | Female | 1168035 | 128mg/dL | Hematuria | Cystitis | Bladder Biopsy | Chronic Cystitis | 2+ | Lymphocytes | Negative | – | – | 0 |
| 30 | 52 | Male | 10090661 | 120mg/dL | LUT symptoms | Neurogenic bladder with Cystitis | Bladder Biopsy | Interstitial Cystitis | 1+ | Lymphocytes | Negative | – | – | 0 |