<u>*Original Article*</u>

Correlation of Serum Immunoglobulin A and Immunoglobulin A/Complement 3 Ratio with IgA Nephropathy: One Year Prospective Study

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Abstract

Background
IgA nephropathy is the most prevalent primary glomerulonephritis worldwide, characterized by the mesangial deposition of IgA immune complexes. Its clinical course is highly variable, ranging from indolent to rapidly progressive forms, and it represents a significant challenge in nephrology. The diagnostic potential of the IgA/C3 ratio, can provide valuable insights into the underlying immunological processes.

Materials and Methods
This is a one year prospective study done in the department of renal pathology at Pratham pathology private limited during the period of 1st November 2021-30th October 2022. Data of all patients were evaluated for the histopathological diagnosis. Report of all cases were reviewed for prebiopsy report of serum IgA and C3 level. All cases of which serum IgA and C3 done were included for the study. Those cases, which didn't perform serum IgA and C3, were excluded from the study.

Results
A total of 182 cases of kidney biopsies were received for evaluation during this period. Out of it 22 cases (12.08 %) were those of IgA Nephropathy. Of which, 13 patients had done a blood level of IgA and C3. There was no statistical difference between serum C3 level, IgA and/or IgA/C3 between IgA nephropathy and non IgA nephropathy

Conclusion
There is no significance variation between serum IgA, C3 and /or IgA/C3 level between IGA nephropathy and other glomerular diseases.

Keywords: *Complement, IgA, Kidney*

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Introduction

IgA nephropathy (IgAN) is the most prevalent primary glomerulonephritis worldwide, characterized by mesangial deposition of IgA immune complexes [1,2].Its clinical course is variable, ranging from indolent to rapidly progressive forms[3]. Understanding its pathophysiology, diagnostic markers, and therapeutic strategies is crucial for management.The pathophysiology of IgAN revolves around abnormal glycosylation and size of IgA1 molecules [4]. Complement C3 exacerbates inflammation and tissue damage[2-4].Recent research has highlighted diagnostic potential of the IgA/C3 ratio[5].

The Oxford Classification of IgAN provides a standardized framework for evaluating renal biopsies and predicting long-term outcomes [5,6].Therapeutic strategies for IgAN encompass a spectrum of approaches, including immunosuppressive agents, angiotensin-converting enzyme inhibitors, and angiotensin II receptor blockers [7].

Several publications shows that serum levels of IgA are significantly increased in patients with IgA nephropathy [8, 9] and it has been recommended that elevated serum IgA levels are valuable in the diagnosis of IgA nephropathy [10]. Joint committee of special study group has used serum IgA of > 350 mg/dl in adults as one of the diagnostic criteria for IgA nephropathy [11]. In current study, we're looking to identify if, IgA or IgA/C3 ratio can be a good predictorfor a diagnosis of IgA nephropathy.

Material and Methods

This is a one year prospective study done in the department of renal pathology at Pratham pathology private limited during the period of 1st November 2021-30th October 2022. Prevalence of IgA nephropathy was 9.8 % among kidney biopsy performed population in Nepal [12], based on this data, the sample size was calculated as 136 using formula n=Z2 p x q/e2 (where, Z=1.96 at 95% CI, p=.098, q=1-p, e=5%). However, a total of 182 samples in the study period were taken into consideration for the study. An IgA nephropathy diagnosis of 22 (12.08%) was selected at first for the study. Data of all patients were evaluated for the histopathological diagnosis. Report of all cases were reviewed for prebiopsy report of serum IgA and C3 level All cases of which serum IgA andC3 done were included for the study.Those cases, which didn't perform serum IgA and C3, were excluded from the study. Histopathological diagnosis of IgAN was kept in one group and non IGAN diagnosis was kept in other group to see the significant difference. Serum IgA and C3 were measured using Mispa i3 nephelopmetry instrument. All renal biopsies were evaluated using light microscopy and immunofluorescence microscopy examination. Light microscopic features, immunofluorescence microscopic features, biochemical, immunological and clinical findings were evaluated while making a diagnosis. Mesangial expansion and hyper cellularity, endocapillary hyper cellularity, crescents, interstitial fibrosis and partial sclerosis of glomeruli were mainly considered while making a preliminary diagnosis of IgA nephropathy in light microscope. Immunofluorescence positivity of 2+ or more was used as a bench mark tool to diagnose IgA nephropathy. Recorded value of serum IgA and C3 was tabulated in an excel sheet. Data were entered in Excel v 11 and was checked after every 10 day entry and exported to SPSS (Statistical Package for Social Sciences) 2020 for statistical analysis.

Results

A total of 182 cases of kidney biopsies were received for evaluation during this period. Out of it 22 cases (12.08 %) were those of IgA Nephropathy (picture 1 and 2). Of which, 13 patients had done a blood level of IgA and C3. On Non- IgA nephropathy 26 patients has done blood level on IgA and C3, which were taken as control group. Age of the patient with IgAN ranged from ranged from 3 years to 72 years with a mean age of 35.3 years. Male to female ratio was 1.2 (male 12, female 10). 19 (86.36%) patients presented with hematuria with or without proteinuria, while 3 (13.64%)patient presented only with proteinuria.

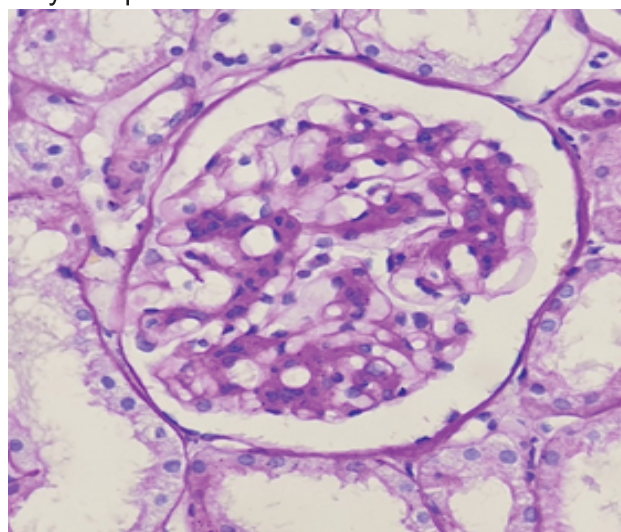**Figure 1: PAS section showing increase in mesangial matrix and cellularity associated with IgA Nephropathy (40X).**
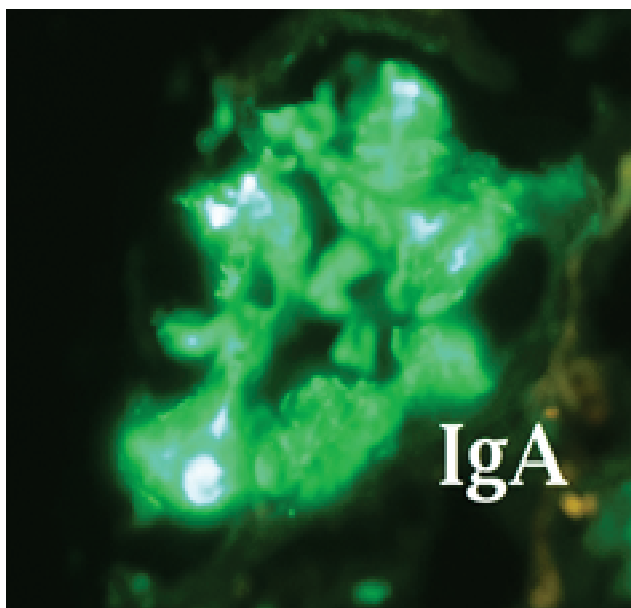
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Figure 2: Immunofluorescence staining showing strong mesangial deposits of IgA (20X).

Detection of Serum IgA and C3

The levels of serum IgA and C3 were measured by the automated Mispa I3 In order to distinguish IgA nephropathy and non-IgA nephropathy; the serum IgA/C3 ratio was calculated. Reference range of serum IgA 70-410 mg/dl was and C3 was 90-180 mg/dl.

Table 1: Levels of serum IgA (mg/dl) in patients with IgA nephropathy and non-IgA nephropathy.

	IgA Nephropathy	Non IgA nephropathy
Number	13	26
Mean	310	263
Lower limit	98	111
Upperlimit	610	520

There was no statistical difference between serum IgA level between IgA nephropathy and non IgA nephropathy

Table 2: Levels of serum C3 (mg/dl) in patients with IgA nephropathy and non-IgA nephropathy

	IgA Nephropathy	Non IgA nephropathy
Number	13	26
Mean	121	127
Lower limit	74	82
Upper limit	164	204

There was no statistical difference between serum C3 level between IgA nephropathy and non IgA nephropathy

Table 3: Levels of serum IgA/C3 in patients with IgA nephropathy and non-IgA nephropathy.

	IgA Nephropathy	Non IgA nephropathy
Number	13	26
Mean	2.96	2.40
Lower limit	2.45	2.26
Upper limit	3.12	2.83

There was no statistical difference between IgA/C3 ratio between IgA nephropathy and non IgA nephropathy

Discussion

This one-year prospective study, conducted at Pratham Pathology Private Limited in the department of renal pathology, delved into the histopathological landscape of kidney biopsies. The primary focus was on cases where serum IgA and C3 assessments were integral. The study, spanning from November 1, 2021, to October 30, 2022, meticulously examined 182 kidney biopsy cases, revealing a noteworthy 22 cases (12.08%) diagnosed with IgA Nephropathy.

Demographic Insights: Within this cohort, IgA Nephropathy patients exhibited a diverse age range, spanning from 3 to 72 years, with a mean age of 35.3 years. Notably, the clinical presentation manifested predominantly as hematuria, observed in 86.36% of cases, either alone or in conjunction with proteinuria. A smaller yet significant subset (13.64%) presented solely with proteinuria.

Serum IgA and C3 Dynamics: Serum IgA and C3 levels were recorded using the automated Mispa I3 platform. In the subset of IgA Nephropathy patients, serum IgA levels averaged 310 mg/dl, while non-IgA nephropathy cases showed a mean of 263 mg/dl. Similarly, serum C3 levels displayed no statistically significant variance between IgA Nephropathy (121 mg/dl) and non-IgA nephropathy (127 mg/dl) cases.

The calculated IgA/C3 ratio, designed to distinguish between the two groups, yielded mean values of 2.96 in IgA Nephropathy and 2.40 in non-IgA nephropathy, with no significant statistical difference observed.

In the study done by Tomino Y, *et al*[13]. the mean values of serum IgA in patients with IgA nephropathy (336 ± 129 mg/dl, mean ± SD were significantly higher than those in patients with non-IgA nephropathy (270 ± 112 mg/dl) which is

quite near values as compared to our study. Similarly in the same study, the 95% range of serum C3 in patients with IgA nephropathy was from 75 to 175 mg/dl. The mean value of serum C3 in patients with IgA nephropathy was 114 mg/dl, while that in patients with non-IgA nephropathy was 131 mg/dl.[12] so; both the markers had no significant differences between the patients with IgA nephropathy and non-IgA nephropathy.

It appeared that the serum IgA/C3 ratio is a more useful marker to distinguish IgA nephropathy from non-IgA nephropathy together with serum IgA levels but the serum IgA/C3 ratio and discriminant function for serum IgA and C3 were used for distinguishing IgA nephropathy from non-IgA nephropathy more efficiently because statistical evaluation by factor analysis showed that the levels of serum IgA and C3 are related to IgA nephropathy [13]. The authors reported that serum IgA levels in patients with IgA nephropathy were significantly higher than those in patients with other glomerular diseases [13,14]. Their results showed that the serum IgA/C3 ratio was also a useful marker for distinguishing IgA nephropathy from non-IgA nephropathy [15],but in our study the results were not statistically significant.

In the study by Gong, Wy., Liu, M., Luo, D. *et al* [16]. the serum IgA/C3 ratio of the IgA nephropathy was significantly higher than that of the non-IgA nephropathy in which they calculated the 95% cut-off point for the IgA/C3 ratio in the non-IgA nephropathy population, which was 3.5304, and the diagnostic accordance rate of an IgA nephropathy diagnosis among all patients (1095) with an IgA/C3 ratio > 3.5304 was as high as 92.02%.So, the cut-off assumes that patients with an IgA/C3 ratio > 3.5304 are predicted to be IgA nephropathy, and patients with an IgA/C3 ratio < 1.0546 are non-IgA nephropathy.

Acknowledging study limitations, including sample size constraints and biomarker-focused analysis, propel us to envision future investigations. A broader exploration of immunological and genetic factors, considering the intricate nature of renal disorders, beckons collaborative efforts and continuous research initiatives.

Conclusion

In conclusion, this one-year prospective study enriches our understanding of the histopathological nuances in kidney biopsies, particularly when serum IgA and C3 assessments are at the forefront. While our findings align with existing literature, the dynamic nature of diagnostic approaches underscores the need for comprehensive assessments.

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