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INVESTIGATIONS ON THE EFFECT OF FLUORESCEIN ANGIOGRAPHY ON RENAL FUNCTION IN DIABETIC RETINOPATHY PATIENTS

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ABSTRACT: Fluorescein angiography is a commonly used procedure for diagnosing and staging diabetic retinopathy. Diabetic retinopathy is one of the major complications of type 2 Diabetes Mellitus (DM). Patients with diabetic retinopathy are likely to have Chronic Kidney Disease (CKD) as degree of retinopathy correlates with the degree of nephropathy in diabetes. Contrast Induced Nephropathy (CIN) is now defined as an increase of 25% or more in serum creatinine, or an absolute increase of 0.5 mg/dl or more from baseline value, at 48-72 h after exposure to Contrast Media (CM). Although several studies reported the effect of iodinated contrast media on kidney functions especially in diabetic CKD patients, very few conflicting data had been published regarding fluorescein (non-iodinated dye) induced renal injury. Contradictory studies have been reported regarding the effect of fluorescein angiography on renal functions using serum creatinine, estimated GFR (eGFR), so the current research study has been taken to investigate the actual effect of fluorescein on kidney function based on the serum creatinine and early sensitive acute kidney injury biomarkers *i.e.* Serum Cystatin-C level in pre and post angiography samples of diabetic patients. A total of 70 diabetic patients (38 male and 32 female) met the inclusion criteria and were studied; the mean age of participants were 53.1 ± 9.2 years; range 30-72 years (male, 58.3 ± 9.5 and female, and 45.7 ± 9.0). Mean of Serum Creatinine before fluorescein angiography was 0.89±0.20 mg/dl (male, 0.85 ± 0.20 and female, 0.89 ± 0.23), and after angiography was 0.92 ±0.23 mg/dl (male, 0.92 ± 0.62 and female, 0.93 ± 0.22). Seven patients (20.5%) had an increase in Serum Creatinine from baseline within 72 hours of fluorescein administration (5 male and 2 female). The present study observed no significant effect of fluorescein angiography on serum creatinine and Serum Cystatin-C level.

INTRODUCTION: Five hundred and ninety-two million people worldwide are projected to have diabetes by 2035, according to the International Diabetes Federation [1]. Adolf von Baeyer initially synthesized fluorescein [2] dye in 1871. Novotny and Alvis introduced fluorescein angiography in clinical ophthalmology [3] in 1960. Since that moment, fluorescein angiography is one of the most common diagnostic procedures employed in ocular pathology [4].

Sodium fluorescein ($C_{20}H_{10}O_5Na_2$) is an organic dye with a molecular weight of 376 daltons and 80% bound to plasma albumin. The remaining 20% is seen during angiography. The dye absorbs light in the blue range of the visible spectrum, with absorption peak at 490nm and it emits light at

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530nm [5]. Fluorescein angiography (FA) is the current gold standard for visualizing retinal vasculature. It allows the staging of Diabetic Retinopathy, identification of non-perfusion areas, macular edema, ischemia, and microaneurysms [6]. Fluorescein sodium dye is metabolized by the kidneys and eliminated through the urine within 48–72 h of administration [7]. The fast sequence retinal fluorescein angiography is considered to be a relatively safe diagnostic test, however, several adverse reactions have been reported. These range from mild (nausea, vomiting, sneezing, pruritus, vasovagal phenomena, inadvertent arterial injection), moderate (urticaria, rash, syncope, pyrexia, nerve palsy, local tissue necrosis, thrombophlebitis at the injection site, gastrointestinal distress) to severe laryngeal edema, bronchospasm, angioneurotic edema, cardiac arrest, myocardial infarction, basilar artery ischemia and seizures [3-5].

According to the European Society of Urogenital Radiology (2011), CI-AKI is defined as an increase of 25% or more in serum creatinine or an absolute increase of 0.5 mg/dL or more from baseline value at 48–72 h following the exposure to intravascular contrast media (CM) [9, 10]. This definition was criticized by lack of sensitivity and non-inclusion of urine output changes, as used in RIFLE, AKIN, and KDIGO [8, 9, 10] definitions. However, it is still the most acceptable and has the advantage of being widely used as an endpoint in many clinical trials, thus allowing comparison between different trials' results. Similarly, a 25% increase in serum cystatin C or uNGAL was considered a marker for renal injury. Few studies tested a 25% increase in serum cystatin C or uNGAL for earlier CI- AKI 10-11 diagnosis.

In one study, defining CI-AKI as a 25% increase in serum cystatin C increased the prevalence of diagnosed cases than when a 25% increase in serum creatinine was used (44% vs. 11%, respectively), with 78% sensitivity and 81% specificity [11-12]. Another study proved that a 25% increase in uNGAL 8 h after contrast administration was able to identify cases with CI-AKI with a 94.1% sensitivity and 97.7% specificity at the time when creatinine level did not show statistically significant changes ($P = 0.11$) until 48 h after contrast material administration. We adopted a 25% increase in serum cystatin C as marker of kidney injury following FA.

MATERIAL AND METHODS: This study was conducted at Rajiv Gandhi Biotechnology Center, Laxminarayan Institute of Technology Campus, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, in collaboration with the Central Diagnostic Laboratory, Star Super-speciality Hospital Nagpur. With due ethical clearance and providing informed consent to all the patients, a cross-sectional study was done on 70 patients with the following inclusion and exclusion criteria.

Inclusion Criteria:

- ❖ All patients without a pre-existing renal disease.
- ❖ Diabetic patients are well-controlled with oral medications.
- ❖ Hypertensive patients are well-controlled with oral medications.

Exclusion Criteria:

- ➢ Un co-operative patients.
- ➢ Severely debilitated patients.
- ➢ Patients with pre-existing renal disease.
- ➢ Patients allergic to contrast media.
- ➢ Pregnant patients.

Blood Sample Collection: Blood samples were collected from all the participants for the analysis of serum creatinine under aseptic precautions using standard phlebotomy techniques 4 – 6 hours before Fluorescein angiography, after which the patients were conveyed about non-consumption of any new medicine other than regular medicine related to anti-diabetic and patients were asked to revisit anytime between 24 – 72 hours after the Fluorescein angiographic examination and again 3 ml blood samples were collected as per the procedure mentioned above.

Fluorescein Angiography: Fundus fluorescein angiography was performed by injecting 5ml of 10% sodium fluorescein in the antecubital vein, and sequential fundus photographs were taken after a delay of nine seconds. The observations were recorded [13].

Measurement of Serum Creatinine: Creatinine concentrations in serum samples were measured with a modified Jaffé method in which picric acid with pH 1.2 was used as reagent 1 and sodium hydroxide with pH 13 as reagent 2. This method is based on kinetic test without deproteinization according to the Jaffé method. Creatinine forms colored complex in an alkaline picrate solution. The difference in absorption at fixed time during conversion is proportional to the concentration of creatinine in the sample.

Procedure: All samples were brought to room temperature before use, and further reagent 1 and reagent 2 were mixed in equal parts just before use. Samples were analyzed by autoanalyzer Cobus C111 using the following parameters.

Method	Fix time
Slope of reaction	Increasing
Wavelength	505 nm (500-520 nm)
Flow cell temp.	37 degree C
Delay time	30 Sec
Delta time	60 Sec
Sample	Serum
Sample volume	50 ul
Working reagent	1000 ul
Slandered Conc.	2 mg/dl
Normal range	0.6-1.1 mg / dl (women) 0.8-1.5 mg/dl (men)
Linearity	25 mg / dl

Measurement of Serum Cystatin-C: Serum marker Cystatin-C was measured using particle-enhanced immunoturbidimetry method by kit from Accurax procedure followed as per the instruction manual.

Statistical Methods: Descriptive statistical analysis has been carried out in the present study using medcalc software. Results on continuous measurements are presented on Mean ± SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance. Student t-test (two tailed, dependent) has been used to find the significance of study parameters on the continuous scale within each group. Effect size has been computed to find the effect of contrast agents.

RESULTS: 70 patients were screened and scheduled to undergo Fluorescein angiography. All the patients met the requisite eligibility criteria. An Evaluation prospective clinical study with 70 patients is undertaken to study the effect of Fluoresce in angiography agent on renal functions, based on serum creatinine, which was performed before fluoresce in angiography and post-Fluoresce in angiography within 48-72 hours. In the present study, there were 38 (58.3%) male patients and 32 (41.7%) female patients ranging from 31 to more than 70 years old. The demographic data of the participants are presented in **Table 1** and **Table 2**. The effect of fluoresce in angiography on renal functions test on serum creatinine and serum cystatin-C level is presented in **Table 3.**

TABLE 1: AGE-WISE DISTRIBUTION OF PATIENTS STUDIED

Age in years	Number of patients	% population
31-40	12	17.14
41-50	18	25.71
51-60	23	32.85
61-70	15	21.42
>70	2	2.85
Total	70	100

TABLE 2: GENDER-WISE DISTRIBUTION OF PATIENTS STUDIED

Gender	Number of patients	Population%
Male	38	54.3
Female	32	45.7
Total	70	100

TABLE 3: EFFECT OF FLUORESCEIN ANGIOGRAPHY ON RENAL FUNCTIONS TEST ON SERUM CREATININE AND SERUM CYSTATIN-C LEVEL

Study variables	Pre-angiography	Post angiography	Difference	Standard error	t-statistic	P value
Serum creatinine	0.89±0.20	0.92 ±0.23	0.030	0.036	0.823	0.4131
Serum Cystatin -C	0.87±0.34	0.94 ±0.36	0.070	0.059	1.183	0.2389

Several studies have reported that the parameters of diagnostic tests were affected by the gender and age factor of the patients. In the present investigation, data related to post angiographic effect on renal function parameters according to age and gender with a p-value at 95% confidence interval is mentioned in **Tables 4** and **5,** respectively.

TABLE 4: DIFFERENCE OF PRE-ANGIOGRAPHY AND POST ANGIOGRAPHY OF RENALFUNCTIONS PARAMETERS ACCORDING TO AGE

Age in Years	Number of patients	Serum Creatinine level		p-value	Difference(D) in pre & post angiography creatinine value
		Pre angiography	Post angiography		
31-40	12	0.68 ± 0.20	0.69 ± 0. 21	0.8	0.020
41-50	18	0.71 ± 0.23	0.74 ± 0.22	0.5	0.040
51-60	23	0.70 ± 0.30	0.78 ± 0.31	0.3	0.080
61-70	15	0.89 ± 0.21	0.95 ± 0.19	0.4	0.060
>70	2	0.87 ± 0.34	0.98 ± 0.31	0.7	0.110

TABLE 5: DIFFERENCE OF PRE AND POST-FLUORESCEIN ANGIOGRAPHY OF RENAL FUNCTIONS PARAMETERS ACCORDING TO GENDER

Gender	No. of patients	Serum Creatinine level		p value	Serum Cystatin -C		p value
		Pre angiography	Post angiography		Pre angiography	Post angiography	
Male	38	0.85 ± 0.20	0.92 ±0. 31	0.2	0.83±0.31	0.93 ±0.30	0.1
Female	32	0.89± 0.23	0.93± 0.22	0.6	0.89±0.21	0.95 ±0.24	0.2

DISCUSSION: Patients with diabetes often develop ophthalmic complications, such as corneal abnormalities, glaucoma, iris neovascularization, cataracts, and neuropathies. However, the most common and potentially blinding of these complications is diabetic retinopathy [14-16]. Fluorescein angiography is a medical procedure in which a fluorescent dye is injected into the bloodstream. The dye highlights the blood vessels in the back of the eye so they can be photographed. Fundus fluorescein angiography (FFA) is an invasive common diagnostic procedure for retinopathy and other eye related complications.

In the present study a total 70 patients (n=70) were scheduled to undergo Fluorescein angiography. All the patients met requisite eligibility criteria. The Age wise distribution of patients described in **Table 1** in which 12 patients in the age group of 31-40 (17.14 %), 18 patients in the age group of 41-50 (25.71%), 23 (32.85%) patients were in the group of 50-60, 15 (21.42 %) patients in the age group of 61-70, 2 (2.85 %) patients in the age group of above 70 years. There were 38 (54.3%) male patients and 32(45.7%) female patients as mentioned in **Table 2**. All these patients were well controlled with oral medications. The minimum and maximum values of serum creatinine were 0.47 and 1.1 respectively.

As already mentioned a base line screening of serum creatinine and Serum Cystatin C were did before and after angiography for all participants. This study found slight increases in the serum creatinine and serum Cystatin -C values of post-Fluorescein angiography compared to pre-Fluorescein angiography as per Table 3, but the change was insignificant. The age-wise differentiation **Table 4** of pre and post-Fluorescein angiography serum creatinine did not show any significant change (p-value =0.38). Similarly, the gender-wise differentiation did not yield any significant change in both screening parameters of serum creatinine and Serum Cystatin- C mentioned in **Table 5.**

Diabetes was a risk factor [7] for contrast-related renal reactions, as described by many other studies. Still, the present study did not observe any significant adverse effects after fluorescein angiography in diabetes patients. Although, Kameda and colleagues used the estimated glomerular filtration rate to show renal injury secondary to fluorescein sodium and did not find any significant effects on renal function [8]. Similar results were observed by Chung and Lee [17] using serum creatinine. Kameda *et al.* found no effect of FA on renal functions. Chung and Lee reported no significant change in serum creatinine after FA in 186 patients retrospectively. Four patients developed AKI that authors explained to be due to unrelated reasons [17].

On the other hand, Alemzadeh- Ansari *et al.* demonstrated a 25% rise in serum creatinine following FA in nine out of 44 diabetic patients, which the authors did not specify to be significant or not both serum cystatin C and serum creatinine have been established in different previous trials as novel biomarkers for early diagnosis and detection

of CI-AKI [18]. This pilot study was conducted to test the possible occurrence of fluorescein-induced renal injury rather than equivocal early detection. Furthermore, in other studies, cystatin C measured at 48 h following contrast administration detected CI- AKI (45 patients - 37.2%) better than creatinine (20 patients - 16.5%), and Mehran risk scoring correlated more strongly with a cys- tatin C increase [19].

Moreover, it was more convenient for patients to come only once after 48 h for creatinine and cystatin C measurements. Serum cystatin C significantly increased 48 h after FA (P >0.001). Moreover, when calculating eGFR using the 2012 CKD- Epi Cystatin C and CKD-Epi Creatinine-Cystatin-C equations, eGFR significantly decreased after FA (P <0.001). Rickli *et al.* found elevated cystatin C 24 h after radiocontrast media administration, whereas creatinine was elevated after 48 h. Ebru *et al.* detected CI-AKI using cystatin-C (45 patients - 37.2%) better than creatinine (20 patients 16.5%) when both were measured 48 h after Coronary diagnostic angiography [20].

Although the present study had patients with significant increases in biomarkers after FA, it did not follow up to identify any potential reversible or irreversible loss of kidney function. This omission warrants consideration in future studies. However, we cannot overlook the significant increase in biomarkers, which have been proven to be early sensitive detectors of CI-AKI. Furthermore, NGAL has been demonstrated in many studies to be useful in predicting the prognosis and outcome of AKI, even in the absence of a diagnostic rise in serum creatinine [21]. When analyzing pooled data from 2322 critically ill patients from 10 prospective observational studies of NGAL, Haase *et al.* found a significant increase in subsequent renal replacement therapy initiation and hospital mortality in the group of patients who had a rise in NGAL levels without an increase in serum creatinine level [20]. These researchers concluded that NGAL detects sub-clinical AKI and subsequent increased risk of adverse outcomes without diagnostic increases in serum creatinine. Limitations of this study include the absence of long-term follow-up of the patients, using single point measurement of biomarkers following FA instead of serial measurements, non-inclusion of more advanced CKD who might be more vulnerable to CI-AKI, and using a small sample of patients. These limitations should be addressed in future studies.

CONCLUSION: This is the first Indian population-based prospective case series looking at changes in serum creatinine and serum cystatin-C levels within a cohort of diabetic patients with known retinopathy after Fluorescein Angiography. Previous studies have used lower doses, such as 2.5 ml of 10% Fluorescein Sodium [3] or only measured a single biomarker, *i.e.,* serum creatinine. With a lack of consensus in current practice on performing FFA in patients with known retinopathy, this small study demonstrates the safety profile of fluorescein sodium at normal doses, *i.e.* 5 ml of 10% Sodium Fluorescein in patients with diabetes. The present investigations revealed no evidence that FAG induces acute renal function deterioration.

The said study was supposed to be the first study in India where patients with diabetic retinopathy showed no significant change in the Serum Creatinine and serum cystatin-C levels before and after Fluorescein angiography. few research studies have reported that the contrast medium agents used in various clinical settings for angiography are responsible for kidney injury, but from the outcome of the present investigation, Fluorescein angiography (non-iodinated contrast agent) does not show any significant effect on kidney function; hence this angiographic agent found to be safe for the diabetic patients to determine the condition of retinopathy.

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