Research Article

N-acetyl cystein, ascorbic acid and intravenous hydration in reducing risk of contrast induced nephropathy: AJMER CIN study (Acetylcystein ascorbic acid to rejuvenate Medulla at high Risk for CIN): interim analysis.

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ABSTRACT

Background: Combination of two highly antioxidant drug, N-acetylcysteine and ascorbic acid along with hydration may help renal medulla to sustain oxidative stress (due to contrast agent) better when compared to intravenous hydration only.

Methods: It represents an interim analysis of *ongoing* ethically approved randomized study, with a plan to enroll 500 patients undergoing coronary catheterization who are at risk of developing contrast induced nephropathy. Patients received either combination of N-acetylcysteine, ascorbic acid and intravenous hydration (group A) or only hydration (group B).

Results: At first look analysis Z value (0.47) for interim analysis fell within the Haybittle-Peto boundary of +2.58 and -2.58 on either side favoring continuation of study.

Conclusion: Interim analysis favors continuation of ongoing study so as to provide statement after analysis of adequate sample size.

Introduction

Acute deterioration in renal function caused by radiographic contrast agents is generally mild and transient but can result in long lasting renal dysfunction and the need for renal replacement therapy. CIN is the third leading cause of new-onset renal failure in hospitalized patients.[1] Moreover, contrast-induced nephropathy (CIN) has been associated with increased in-hospital and long-term morbidity, mortality, and extended hospitalization.[2–7] The incidence of CIN in the general patient population undergoing coronary angiography is low and has been estimated to be 2%.[9] However, patients with preexisting renal impairment and diabetes mellitus are at substantially greater risk of developing CIN, in the range of 20% to 80%.[2,5,7,8]Other factors may also contribute to risk, including volume depletion, the volume and osmolality of the contrast agent used, and congestive heart failure.[10]Proposed mechanism of contrast induced nephropathy (CIN) is oxidant stress injury to renal medulla. Various antioxidant therapies have been proposed previously to reduce risk of contrast induced nephropathy. N-acetylcysteine (NAC) in seminal study markedly reduce the risk of CIN, however subsequent studies did not give promising results, poor absorption of NAC was blamed for this[8]. Same is true for ascorbic acid (AA). Individually various trial had not showed much beneficial effect of either NAC or AA when used alone. We proposed that combination of two highly antioxidant drug along with hydration may help renal medulla to sustain the oxidative stress (due to contrast agent) better when compared to intravenous hydration only, thereby reduced physiological transient injury too, which may be deleterious in high risk cohort.

Methods

It is the interim analysis of ongoing randomized single centre study. Institutional Ethical Committee (IEC) approval was taken before the start of study.CIN was defined as an absolute rise in 0.5mg% of s.creatinine *or* 25% increase from the baseline value. It is a randomized, single centre study; with proposed sample size of 500 patients undergoing coronary intervention (diagnostinc or therapeutic) and fulfilling the high risk criteria for contrast induced nephropathy. Patients received either combination of N-acetylcysteine, ascorbic acid and intravenous hydration (group A) or only hydration (group B).Patient at high risk for CIN were defined as patients with serum creatinine

concentration of >1.2 mg/dL. Patients with acute renal failure, end-stage renal disease requiring dialysis, intravascular administration of contrast medium within the previous 6 days, anticipated re-administration of contrast medium within the following 6 days, use of vitamin C supplements on a daily basis during the week before the procedure, inability to administer the study medication at least 2 hours before the procedure were excluded from the study. Written, informed consent in understandable language as per ICMR (Indian Council of Medical Research) guidelines was taken from all patients before inclusion in the study. N-acetylcysteine was given in the dosage schedule: 600 mg twice daily on the day before procedure and 600 mg twice daily on day of exposure to contrast. Ascorbic acid was given in dosage schedule of 3 gm at least 2 hours before the procedure and 2 gm in the night and the morning after the procedure. Isotonic saline was given in dose of 1 ml/kg/h (0.9% sodium chloride) for 12 h before and after the procedure. Dose of saline was reduced to 0.5 ml/kg/h for patients with EF<35%. High-contrast load was defined as administered contrast agent volume >150 ml. Primary End Point was an absolute increase of at least 0.5 mg/dl relative increase or 25% over baseline serum creatinine in 72 hr after contrast agent (Iomeprol 400; non-ionic, water soluble contrast with osmolality of 726±34 mosmol/kg water) administration over baseline. Additional end points were: adverse clinical events, acute pulmonary edema, need for dialysis, hemofiltration and in-hospital death.

Statistical analysis

Sample size

The sample size calculated by assuming a 15% incidence of the study end point in the isotonic saline hydration group; 500 patients will be required (250 per treatment group) to detect a 50% relative reduction in the incidence of the end point in combination group with 90% power at the conventional, 2-sided significance level of 5%.Three look interim analysis using Haybittle-Peto boundary was proposed at 40% of sample size recruitment, at 70% of sample size recruitment and completion of analysis. Current analysis represents the result of pre-specified first interim analysis of study after enrollment of 40% sample size i.e first 200 patients. The interim analysis was planned to find if there is clear *early* evidence of efficacy, clear *early* evidence of harm or *early* evidence of harm based on additional safety data. Data was locked after enrollment of 40% of sample size and interim analysis was run using Haybittle-Peto boundary (it was decided to discontinue study if *Z* value will fall outside the Haybittle-Peto stopping boundary of 2.58 on either side with p value of 0.010). Pre-specified subgroup analysis was run for age≤70 versus >70 year, gender, Diabetes mellitus (yes/no), left ventricle dysfunction (EF≤35% or >35%),volume of contrast(<150ml/>150ml), eGFR (<30,30-60 and >60 ml/min).

Results

Study Participants

Between December 2013 and may 2014, a total of 200 patients were enrolled: 100 patients were allocated to N-acetylcysteine, ascorbic acid and intravenous hydration and 100 patients to intravenous hydration only. Patients with incomplete data record in any form were excluded from study. The baseline characteristics were well balanced between the groups (Table 1). All patients underwent percutaneous coronary procedure.

Study End Points

The primary end point of contrast induced nephropathy

Table 1: Baseline characteristics in two groups

Characteristics	Group A (N=100)[$]	Group B (N=100) [$]	P value
Age(yr)			
Median	61	59	NS
IQR	46-65	49-67	
Male(%)	59	56	NS
Weight(Kg)			
Median	71	67	NS
IQR	50-71	53-71	
eGFR at base line(±2SD) ml/min	48(36-60)	50(32-68)	NS
Anemia(% of patients)[#]	36	39	
High Volume Contrast(% of patient)[##]	22	19	
IABP	-	-	
Diabetes Mellitus (%)	36%	39%	NS
Prior IHD (%)	30%	37%	NS
Killip Class ≥II(on Admission)	18%	19%	NS
HT (%)	33	36	NS
EF (%)			
Median	46	48	NS
IQR	32-56	36-50	

[$] Group A received N-acetylcysteine, ascorbic acid and intravenous hydration; group B received only hydration. [#]Anemia defined as Hb <10 gm%. [##]High volume contrast defined as >150 ml/min. EF: Ejection fraction, HT: hypertension, IABP: Intraaortic balloon pump, IHD: ischemic heart disease, IQR: interquartile range(25th and 75th percentile), SBP: systolic blood pressure.

was 9% in group A versus 11% in group B (Relative Risk[RR]:0.81;confidence interval[CI]:0.35-1.88; *P value*=0.63, Z value=0.470). Adverse events, all cause death and cardiovascular death also showed no difference in two groups. Z value being well within the stopping boundary (+2.58 & -2.58; Figure 1), current interim analysis favors continuation of study till predefined statistically adequate sample size is achieved or second look interim analysis does not allow it. Analysis for other end points (cardiovascular death, need for dialysis) also showed no difference in two groups (Table 2).

Subgroup Analysis

The neutral effect of N-acetylcysteine, ascorbic acid in addition to hydration on the risk of contrast induced acute kidney injury was consistent in those with or without diabetes mellitus (P value =NS), and across other subgroups such as patients aged 70 or 70 years (P value =NS), male or female patients (P value =NS), or exposure to high (>150 mL) or low (<150 mL) volumes of contrast media (P value =NS), as shown in Figure 2.

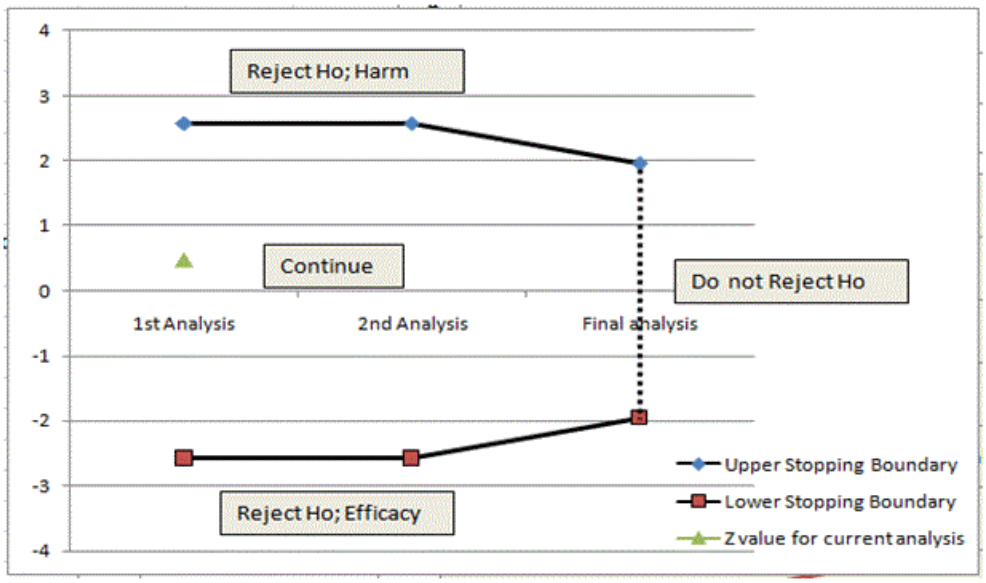

Figure 1. Intermin analysis using three look Haybittle-Peto stopping boundary, At first look analysis after locking sample at 40% of total sample, z value (0.47) fitted well within the stopping boundary; favoring continuation of study.

Table2. End Points.

Outcomes	Group A*	Group B*	Relative risk (95% CI)	P value
Primary end point(CIN)	9(9%)	11(11%)	0.81(0.35-1.88)	0.63
All cause death	3(3%)	2(2%)	1.50(0.26-8.78)	0.65
Cardiovascular death	1(1%)	2(2%)	0.50(0.05-5.54)	0.57

*Group A received N-acetylcysteine, Ascorbic acid along with intravenous hydration. *Group B received intravenous hydration only.CI indicate confidence interval.

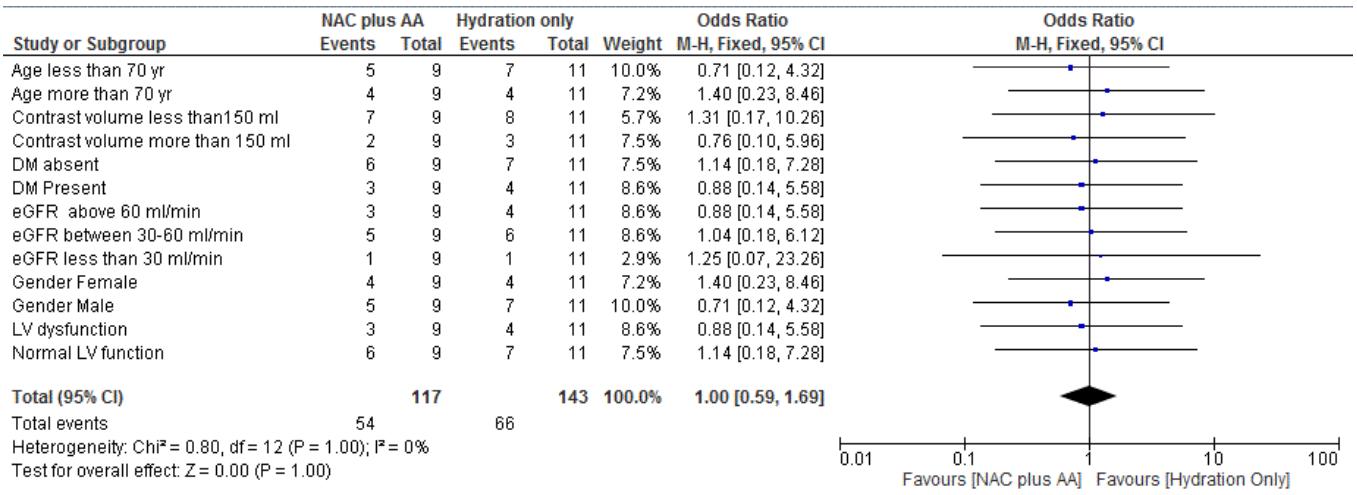

Figure 2: Forest plot for analysis of pre-specified subgroup .LV dysfunction was defined as EF<35%. AA indicates ascorbic acid, CI: confidence interval, M-H:Maental-Haenszel method. NAC: N-acetyl cystein.LV dysfunction defined as EF< 35%.

Discussion

Interim analysis of current study revealed some promising facts, but none had crossed the lower stopping boundary to prove efficacy of combination therapy at this point of analysis. Number of primary end point event (CIN) did not differ in two groups. Subgroup analysis also did not differ

in two groups at this stage. However combination therapy appears more promising in certain subset e.g. male gender, presence of LV dysfunction, high contrast burden, presence of diabetes, younger age (< 70 year) and eGFR more than 60 ml/min (Figure 2). Although absolute number slightly favors combination therapy for prevention of CIN in these subsets of high risk patients,

but it will be too premature to make any concrete statement. Proposed mechanism of CIN is vulnerability of the renal medullary circulation to stimuli that disrupt the balance between the *high metabolic needs* of its tubular segments and their *hypoxic environment*.[11] Contrast agents,

imposes high burden of osmotic load and viscosity, thus increases the hypoxia of the renal medulla and increases renal free-radical production through post-ischemic oxidative stress.[11–13] Currently, only hydration and use of iso-osmolar contrast agents have shown consistent benefit for prevention of CIN. [4,14–16]Recent studies have produced conflicting results regarding the efficacy of the antioxidant N-acetylcysteine[17–19]. NAC protect against CIN by two mechanisms: *First*, by supplementation of the body's antioxidant capacity and *second* by inducing glutathione synthesis. However after oral administration, NAC is almost completely metabolized before entering the systemic circulation. With intestinal transit and absorption, NAC is effectively deacetylated by the enzyme acylase I in the intestines and liver[20-22]**.** After deacetylation in the intestines and liver, NAC yields cysteine that supplies glutathione's active sulfhydryl group and plays a critical role as the rate-limiting factor in glutathione synthesis. This circulating cysteine may enter renal cells and serve as a precursor for glutathione production which in turn plays a central role in the body's defense against cellular oxidative damage[23-24]. The relevance for the kidney is suggested by the demonstration of glutathione depletion in ischemia reperfusion injury. [25-26] The antioxidant ascorbic acid has also been shown to attenuate renal damage caused by a variety of insults and has an extensive safety record as a dietary supplement in humans.[27-28.] Ascorbic acid is a potent, water-soluble antioxidant capable of scavenging a wide array of reactive oxygen species that can cause damage to macromolecules such as lipids, DNA, and proteins.[29] In addition, ascorbic acid can regenerate other antioxidants, acting as a coantioxidant.[29]The bioavailability of orally administered ascorbic acid doses of 2 to 3 g is 36% to 44%, and the time of its maximum excretion rate is 2.7 hours.[30-31]These doses of oral ascorbic acid have been shown to reverse endothelial vasomotor dysfunction within 2 hours after administration in patients with coronary artery disease and are therefore, biologically relevant.[32] Since both NAC and AA provide antioxidant protection to renal medulla via different mechanism, so combination of both may yield cumulative result in protecting medulla from oxidative stress of contrast media in addition to hydration, but to validation of it require completion of study as concluded by interim analysis.

Conclusion

Infusion of radiographic contrast agents, with the attendant increases in osmotic load and viscosity, increases the hypoxia of the renal medulla and increases renal free-radical production through post-ischemic oxidative stress. Two antioxidants, NAC and ascorbic acid, in addition to already proved hydration therapy may have cumulative effect on prevention of CIN. Data analysis of adequate sample size in current ongoing study may through light on this burning issue whether addition of NAC and ascorbic acid to hydration is helpful in prevention of CIN.

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