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Liver Enzymes in Metabolic Syndrome

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is a frequent accompaniment of metabolic syndrome (MS). It is associated with increased levels of serum enzymes alanine aminotransferase (ALT) and gamma-glutamyl transferase (ãGT). We studied abnormalities of liver enzymes in 1437 apparently healthy subjects of whom 259 had MS. ALT, ãGT and alkaline phosphatase (SAP) were significantly increased in MS. Aspartate aminotransferase (AST), on the other hand, was lower in MS after adjustment for other variables. Of the various components of MS, ALT was most strongly related to body-mass index, ãGT to serum triglycerides and SAP to systolic blood pressure. All liver enzymes decreased with age except for SAP. Extra-hepatic sites of origin of AST and SAP appear likely explanation for their negative or weaker association with MS.

Key words : Non-alcoholic fatty liver disease, alkaline phosphatase, cholesterol, triglycerides, body-mass index

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Introduction

Metabolic syndrome (MS) is a constellation of cardiovascular risk factors including obesity, hyperglycemia, insulin resistance, dyslipidemia and hypertension (1,2). Subjects with MS are reported to have elevated levels of serum ALT, AST, ãGT and alkaline phosphatase (3,4). This is believed to be due to fatty infiltration of the liver or non-alcoholic fatty liver disease (NAFLD).

Alcohol consumption is well recognized to have adverse effect on liver enzymes. It also increases HDL-cholesterol and triglycerides, which are defining components of MS. Vitamin D status influences bone turnover and serum alkaline phosphatase. Prevalence of infection with hepatitis viruses is high in India. Several drugs including those used in dyslipidemia have adverse effect on liver enzymes. Individuals undergoing routine health check up are often found to have abnormal values of liver enzymes. Although the topics of MS (5) and NAFLD (6) have been reviewed extensively, the influence of components of MS and alcohol ingestion on liver enzymes is complex and has not been reported from India. We report here our findings in a large number of subjects undergoing routine health check at our institution.

Subjects and Methods

Over a period of 18 months starting January 2004, we screened 1,712 individuals who presented themselves for general health check-up at the Sitaram Bhartia Institute of Science and Research, New Delhi. Most of them were apparently healthy adults and free from symptoms except for common life-style disorders. Patients with history of chronic liver disease, congestive heart failure, chronic renal disease, metabolic bone disease, alcohol and substance abuse, and current use of lipid lowering drugs were excluded from analysis. Participants in the study underwent clinical evaluation including history, blood pressure measurement and recording of body mass index. History of alcohol intake was carefully recorded. It was categorized as 0 if the current weekly intake was less than 26 g ethanol in a week or 1 if it ranged from 26-255 g per week. Individuals with higher intake were excluded. Laboratory investigations included estimation of plasma glucose in the fasting state (FBS) and 2 hours after 75 g glucose ingestion (PP), lipid profile including total cholesterol, triglycerides (TG), HDL, LDL and VLDL cholesterol, liver enzymes (AST, ALT, ãGT and total alkaline phosphatase) and serum uric acid in

addition to certain other investigations included in the preventive health package.

Subjects were categorized as having MS or no-MS on the basis of the WHO criteria (7). It was considered to be present if any three of the following 5 criteria were fulfilled: BMI>30 Kg/M^2, systolic blood pressure >140 mm Hg, fasting plasma glucose =126 mg/dl, triglycerides >150 mg/dl, and HDL-cholesterol <40 mg/dl in males and <50 mg/dl in females. We used BMI as a surrogate for central obesity as the waist circumference was not recorded in all participants. Complete information for categorizing MS status and results of all four liver enzymes was available for 1,437 individuals.

Statistical Analysis :

Means of continuous variables in two groups were compared using Student's t-test, with modification if the variances in the two groups were different. $\div^2$ statistic was used for comparison of categorical variables. Data which were significantly skewed were log transformed. Univariate and multivariable regression techniques were used. The former identified significant variables to be used in multivariable regression. Logistic regression or linear multivariable regression models were used as appropriate. Backward elimination strategy was used for arriving at the final model. Certain variables (age, gender and alcohol intake) were forced in the model even if non-significant. Stata® version 9.0 was used for statistical analysis.

Results :

The prevalence of various life style disorders was high amongst these individuals. 259 subjects had MS (18.0%), 643 (44.8%) had hypertension grade I or higher, 267 (18.6%) had BMI = 30 kg/m^2, 478 (33.3%) had TG = 150 mg/dl, 590 (41.1%) had low HDL-cholesterol (< 40 in men or <50 in women), and 362 (25.2%) had fasting plasma glucose >110 mg/dl.

Physical characteristics of the subjects and the results of selected investigations are shown in Table 1. Continuous variables have been summarized as mean ± SD. Subjects with MS were older. There was no gender difference in its occurrence. Individuals with MS were heavier, had a higher BMI, systolic and diastolic blood pressures as well as higher pulse pressure. Increase in plasma glucose (δ plasma glucose) following ingestion of 75 G glucose was higher in subjects with MS. They also had higher fasting

Table-1 : Basic characteristics of subjects with and without MS

Parameters	Normal (1178) (mean ± SD)	Metabolic syndrome (259) (mean ± SD)	Two tailed P value
Age (years)	47.2±12.6	49.7±10.8	0.0012
Female Sex	38.3%	37.1%	0.724
Height (cm)	165.4±8.84	165.3±9.40	0.9046
Weight (kg)	71.37±12.3	82.0±13.6	0.0000
BMI (kg/m^2)	26.04±3.72	30.00±4.45	0.0000
Systolic BP (mm Hg)	128.7±17.9	141.8±20.0	0.0000
Diastolic BP (mm Hg)	80.46±10.29	88.45±11.89	0.0000
Fasting plasma glucose (mg/dl)	102.4±231	128.23±40.85	0.0000
Post-prandial plasma glucose	102.6±46.2	146.2±77.8	0.0000
Alcohol Use	43.9%	38.2%	0.0010
Triglycerides	130±87.1	211.5±145.8	0.0000
HDL-C	48.0±11.4	39.2±8.2	0.0000
LDL-C	112.1±30.78	118.2±31.7	0.0056
Total Cholesterol	185.9±38.7	195.5±38.4	0.0003
AST	25.5±13.2	27.0±13.4	0.1061
ALT	31.1±23.2	35.5±21.8	0.0037
γGT	28.9±32.4	36.0±38.9	0.0062
Alkaline Phosphatase	75.7±30.6	81.8±29.9	0.0029
Uric Acid	5.7±1.56	6.17±1.42	0.0000
δ plasma glucose	0.15±32.28	18.1±45.73	0.0000
Pulse pressure (mm Hg)	48.3±14.3	53.4±15.0	0.0000

and post-prandial blood sugars, higher total and LDL-cholesterol, higher triglycerides and lower HDL-cholesterol. No significant difference existed in AST between subjects with or without MS, but the former had higher ALT, ãGT and SAP. Uric acid levels were also higher in MS. Fewer subjects with MS consumed alcohol.

On multiple logistic regression analysis (Table 2), MS was significantly and positively related to ãGT and ALT

Table-2 : Logistic regression of liver enzymes on MS

Subjects	Both sexes (n=1437)		Females (n=547)		Males (n=890)	
MS	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Age	1.02 (1.01-1.03)	0.001	1.03 (1.01-1.05)	0.005	1.01 (1.00-1.03)	0.087
Gender	.82 (0.59-1.13)	0.223				
Alcohol	.73 (0.53-0.99)	0.045	.88 (0.46-1.68)	0.695	0.71 (0.50-1.00)	0.055
AST*	.45 (0.28-0.75)	0.002	.36 (0.16-0.80)	0.013	0.51 (0.27-0.97)	0.039
ALT*	2.07 (1.38-3.10)	0.000	2.72 (1.33-5.56)	0.006	1.73 (1.05-2.86)	0.031
SAP*	1.47 (0.96-2.24)	0.074	1.59 (0.78-3.26)	0.202	1.26 (0.73-2.16)	0.402
ãGT*	1.59 (1.24-2.05)	0.000	1.99 (1.27-3.12)	0.003	1.42 (1.04-1.94)	0.025

• Log Transformed Data

and negatively related to AST after adjusting for age, gender and alcohol intake. Its association with SAP fell short of statistical significance. These relationships were noted in both female and male subjects as sub-groups as well.

We looked for association between the five defining components of MS (fasting plasma glucose, systolic blood pressure, body-mass index, HDL-C and plasma TG) and the four liver enzymes (AST, ALT, ãGT and SAP), after adjusting for age, gender and alcohol intake in combined as well as sub-group analysis of men and women.

Linear regression analysis was performed after log transformation of fasting blood sugar, systolic blood pressure, plasma triglycerides, HDL-C as the dependent variables and log transforms of all four liver enzymes as the predictor variables. Age and BMI were used without transformation.

Fasting blood sugar (Table 3) was related significantly to all four liver enzymes in combined analysis for both male and female subjects; its association with AST was inverse. Similar associations were observed in women, but in men only ãGT was significant. It was noted that on univariate analysis, AST was positively correlated with fasting plasma glucose in women.

On combined analysis, BMI was related to all liver enzymes except ãGT; again the relation with AST was inverse (Table 4). In women, all four liver enzymes were significantly related to BMI, but in men, ãGT and SAP were not significant.

Table-3 : Linear regression of liver enzymes on fasting plasma glucose (FPG)

Subjects	Both sexes (n=1437)		Females (n=547)		Males (n=890)	
FBS*	ß (95% CI)	P	ß (95% CI)	P	ß (95% CI)	P
Age	.004 (.003-.005)	0.000	.004 (.003-.005)	0.000	.004 (.003-.005)	0.000
Gender	.035 (.011-.059)	0.004				
Alcohol	-.007 (-.030-.015)	0.519	-.021 (-.056-.015)	0.251	-.001 (-.029-.028)	0.951
AST*	-.055 (-.091-.018)	0.003	-.062 (-.109-.015)	0.009	-.048 (-.101-.004)	0.069
ALT*	.051(.022-.079)	0.001	.064 (.024-.103)	0.002	.040 (.000-.080)	0.051
SAP*	.041 (.009-.072)	0.012	.054 (.012-.097)	0.012	.026 (-.019-.071)	0.261
ãGT*	.036 (.016-.055)	0.000	.042 (.015-.069)	0.002	.033 (.007-.060)	0.014

• Log Transformed Data

Table-4 : Linear regression of liver enzymes on body mass index

Subjects	Both sexes (n=1437)		Females (n=547)		Males (n=890)	
MS	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Age	.046 (.029-.063)	0.000	.033 (.001-.064)	0.041	.047 (.026-.068)	0.000
Gender	-.927 (-1.415-.440)	0.000				
Alcohol	-.348 (-.801-.105)	0.132	-1.04 (-1.99-.08)	0.033	-.07 (-.57-.426)	0.778
AST*	-1.708(-2.44-.973)	0.000	-2.28 (-3.54-1.02)	0.000	-1.25(-2.14-.35)	0.006
ALT*	2.484(1.90-3.06)	0.000	2.09 (1.03-3.15)	0.000	2.53 (1.84-3.22)	0.000
SAP*	1.26 (.624-1.90)	0.000	2.32 (1.18-3.45)	0.000	.58 (-.20-1.36)	0.146
ãGT*	.362 (-.027-.751)	0.069	.650 (-.067-1.37)	0.075	.197(-.26-.65)	0.393

• Log Transformed Data

For HDL, all liver enzymes except ãGT exhibited significant association on combined analysis (Table 5). The relationship was positive with AST and negative with ALT and SAP. Strong positive associations were also observed with female sex and consumption of alcohol. Similar observations were made in men, in whom ãGT was also significantly and positively correlated. In women, however, significant (inverse) relationship was observed only with SAP out of the four liver enzymes.

Table-5 : Linear regression of liver enzymes on HDL cholesterol

Subjects	Both sexes (n=1437)		Females (n=547)		Males (n=890)	
MS	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Agex10	.016 (.006-.025)	0.001	.018 (.001-.035)	0.039	.015(.003-.027)	0.017
Gender	-.170 (-.197-.142)	0.000				
Alcohol	.045 (.020-.071)	0.001	.052 (.001-.104)	0.047	.043 (.014-.072)	0.004
AST*	.078 (.037-.120)	0.000	.031 (-.034 -.099)	0.374	.110(.058-.162)	0.000
ALT*	-.049 (-.08-.02)	0.003	-.02 (-.08 - .04)	0.488	-.068(-.11-.03)	0.001
SAP*	-.078 (-.11-.04)	0.000	-.07 (-.13-.004)	0.036	-.08 (-.13-.04)	0.000
ãGT*	.018 (-.004-.04)	0.108	-.003 (-.04-.04)	0.861	.029 (.002-.055)	0.034

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Triglycerides were strongly related to ãGT and ALT, and negatively to AST while SAP was not a significant correlate (Table 6). In women only the first two showed significant correlations, whereas in men the associations were similar to the findings on combined analysis.

Systolic blood pressure was positively correlated with levels of serum alkaline phosphatase after adjustment for age, gender and alcohol intake (Table 7). This association was particularly striking in women, whereas in men it fell short of significance.

Table-6 : Linear regression of liver enzymes on serum triglyceride levels

Subjects	Both sexes (n=1437)		Females (n=547)		Males (n=890)	
MS	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Age	.002 (.000-.004)	0.050	.008 (.004-.011)	0.000	-.001 (-.004-.001)	0.033
Gender	.078 (.020-.138)	0.009				
Alcohol	-.004 (-.060-.051)	0.892	.012 (-.09-.114)	0.817	-.003 (-.07-.06)	0.924
AST*	-.144(.233—.054)	0.002	-.101 (-.24-.04)	0.146	-.158 (-.28—.04)	0.009
ALT*	.174(.104-.245)	0.000	.123 (.009-.237)	0.035	.17 (-.08—.26)	0.000
SAP*	.051 (-.027-.128)	0.202	.097 (-.026-.22)	0.122	-.155 (-.118-.09)	0.766
ãGT*	.206 (.159-.253)	0.000	.177 (.10-.25)	0.000	.0218 (.159-.278)	0.000

• Log Transformed Data

Table-7 : Linear regression of systolic blood pressure on liver enzymes

Subjects	Both sexes (n=1437)		Females (n=547)		Males (n=890)	
MS	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Age	.004 (.003-.005)	0.000	.005(.004-.006)	0.000	.003(.002-.004)	0.000
Gender	.018 (.002-.034)	0.025				
Alcohol	-.007 (-.022-.007)	0.329	-.007(-.04-.023)	0.640	-.005(-.02-.01)	0.565
AST*	-.017(-.007-.041)	0.157	-.008 (-.03-.05)	0.713	.028 (-.002-.06)	0.069
ALT*	.009(-.009-.03)	0.338	.011 (-.022-.05)	0.508	-.001 (-.024-.02)	0.907
SAP*	.050 (-.029-.071)	0.000	.071 (.035-.107)	0.000	.026 (-.001-.05)	0.055
ãGT*	.014 (.001-.027)	0.033	.014(-.009-.037)	0.240	.013 (-.002-.03)	0.081

• Log Transformed Data

Liver Enzymes :

AST : On univariate analysis (results not tabulated), AST was higher in males and consumers of alcohol; it decreased with age, and increased with BMI, TG and systolic blood pressure. It was not related to fasting plasma glucose, HDL-cholesterol, or the presence of MS. On multivariate analysis it was significantly related to male sex, age (negative association), systolic blood pressure, BMI, HDL-cholesterol and TG (Table 8).

ALT : On univariate analysis, it was negatively correlated with age, and HDL-cholesterol, and positively with

Table-8 : Regression of AST on Age, Gender, BMI, Alcohol Intake, HDL, TG, FBS and Systolic blood pressure (Adjusted R^2 = 0.0755)

AST	â (95% CI)	t	P
Age	-.003 (-.005 - -.001)	-3.37	0.001
Gender	.170 (.123 - .218)	7.07	0.000
BMI	.009 (.004 - .014)	3.49	0.000
Alcohol	-.016 (-.060 - .028)	-0.72	0.472
HDL	.003 (.002 - .005)	3.49	0.000
TG	.0004 (.0002-.0006)	4.14	0.000
FBS	.000 (-.001- .001)	0.02	0.983
Bpsystol	.003 (.001 - .004)	4.36	0.000

male sex, alcohol consumption, BMI, systolic blood pressure, fasting blood sugar and TG. On multivariate analysis, ALT was related to all above except alcohol but its association with HDL-cholesterol became positive (Table 9).

ãGT : On univariate analysis ãGT was positively related to male sex, alcohol consumption, BMI, fasting plasma glucose, systolic blood pressure and triglycerides. On multivariate analysis all above continued to be significantly associated with ãGT; additionally HDL-cholesterol also showed a positive correlation (Table 10).

Alkaline phosphatase : Serum alkaline phosphatase was higher in

Table-9 : Regression of ALT on Age, Gender, BMI, Alcohol intake, HDL, Tg, FBS and Systolic blood pressure (Adjusted R^2 = 0.2396)

AST	â (95% CI)	t	P
Age	-.010 (-.012 - -.008)	-8.44	0.000
Gender	.411 (.349 - .472)	13.10	0.000
BMI	029 (.023-.036)	8.82	0.000
Alcohol	-.011 (-.068- .046)	-0.38	0.701
HDL	.003 (.000 - .005)	2.02	0.043
TG	.001 (.001 - .001)	5.89	0.000
FBS	.001 (.000 - .002)	2.18	0.029
Bpsystol	.003 (.001 - .004)	3.33	0.001

Table-10 : Regression of ãGT on Age, Gender, BMI, Alcohol intake, HDL, Tg, FBS and Systolic blood pressure (Adjusted R^2 = 0.2363)

ãGT	â (95% CI)	t	P
Age	-.003 (-.006- -.000)	-2.29	0.022
Gender	.362 (.290 - .434)	9.89	0.000
BMI	.022 (.015 - .030)	5.78	0.000
Alcohol	.147 (.080 - .213)	4.34	0.000
HDL	.006 (.004 - .009)	4.29	0.000
TG	.002 (.001 - .002)	11.30	0.000
FBS	.0018 (.001 - .003)	3.20	0.001
Bpsystol	.003 (.001 - .005)	3.64	0.000

males, but lower in consumers of alcohol. It correlated positively with BMI, fasting plasma glucose, systolic blood pressure, triglycerides and negatively with HDL-cholesterol (Table 11). All these associations were noted on univariate as well as multivariate analysis.

The strength of association between the liver enzymes and the selected predictor variables (age, gender, alcohol intake, BMI, TG, HDL, FBS and systolic blood pressure) was weak for AST and SAP (adjusted R^2 0.08 and 0.07, respectively); whereas it was much stronger for ALT and ãGT (adjusted R^2 0.24 for both).

Effect of alcohol on liver enzymes: Alcohol consumption was associated with higher ãGT, ALT and AST, and lower SAP in males and females combined. In sex-wise analysis, these differences were significant for ãGT and SAP in males only. In multivariate model after adjusting for age and gender, alcohol intake was associated with higher ãGT, and lower SAP without significant effect on ALT or AST. The ALT/AST ratio was significantly higher in those who consumed alcohol.

Discussion :

Subjects included in the present analysis belong to a relatively higher socio-economic stratum of the population of Delhi. It is a non-random sample of subjects who volunteered for preventive health check-up. The prevalence of various cardiovascular risk factors in this sample is not representative of the population of Delhi. The primary objective of this analysis was to look for abnormalities of liver enzymes in subjects with

Table-11 : Regression of SAP on Age, Gender, BMI, Alcohol intake, HDL, Tg, FBS and Systolic blood pressure (Adjusted R^2 = 0.0684)

SAP	â (95% CI)	t	P
Age	-.001 (-.002 - .001)	-0.76	0.446
Gender	.054 (.014 - .095)	2.62	0.009
BMI	.009 (.005 - .014)	4.26	0.000
Alcohol	-.061 (-.099 - -.024)	-3.22	0.001
HDL	-.002 (-.004 -.000)	-2.36	0.019
TG	. 000 (-.000 - .000)	1.19	0.236
FBS	.001(.000 - .001)	2.11	0.035
Bpsystol	.003 (.002 - .004)	5.19	0.000

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Laparoscopic kidney retrieval in donor with an extended criterion : Assessing the safety and outcome

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Abstract

The term marginal kidney donor / extended donor criterion is not clearly defined. In this article we assess the safety and outcome of laparoscopic donor nephrectomy (LDN) in donors with an extended criterion. A retrospective analysis of our database was done to assess the outcome of extended donor. Analysis was done between normal donors (group 1) and donors with extended criterion (group 2). The parameters analyzed were pre and post operative serum creatinine *in donors and recipients*, serum creatinine at day 1, 7, 30 days and 1 year *in recipients*, operative time, warm ischemia time, analgesia requirement in donors and impact of extended criteria *on recipient outcome*. Group I and group II had comparable nadir creatinine at 1 year in *recipients*. Donors with BMI more than 30 kg/m^2 required more number of ports and hospital stay. Recipients' nadir creatinine with single vs. multiple vessel donors was comparable at 1 year. Donors with urolithiasis had good recipient outcome. The donor creatinine was comparable in extended and non extended indication donors. In our study, LDN was found to be safe, feasible and efficacious in donors with extended indications such as old age, BMI more

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than 30, multiple vessels and anatomical anomalies. *Recipient outcome for donors with normal vs. extended criterion was comparable at one year follow up. All donors with extended criterion had normal post operative creatinine levels with maintained nadir stable creatinine at two year follow up. Long term follow up would be of interest.*

Key words: Laproscopic donor nephrectomy, extended indications

Introduction

In India, 80,0000 patients are added to the end stage renal disease (ESRD) pool of which 2.4% undergo transplantation every year(1). This is partly due to the lack of proper cadaver donation program and the cost involved in transplantation. Annually 7% of waiting list patients die of ESRD (2). An extended donor criterion is one such effort to increase the availability of organs. The term marginal kidney donor or expanded donor criteria or extended indications is not clearly defined. The term marginal kidney donor was first used by Matas and complex living donor was used by Resse (3, 4).

In our study we defined graft function according to the following definition: Early graft function was defined as greater than 25% decline of two separate serum creatinine samples taken within first 24 hours. Delayed graft function was defined as requirement of hemodialysis within 7-post transplantation (5). Extended criteria have been defined in various studies as donors with old age, hypertension, diabetes and donors with urolithiasis(2). Laparoscopic donor Nephrectomy(LDN) is emerging as a gold standard in kidney retrieval for transplantation since its first description by Ratner(6) .Although numerous studies have proved the efficacy and outcome of LDN in non extended indications(6), there is a paucity of literature describing results in the "extended indications". In this article we assess the safety and outcome of extended indication in LDN.

Material and Methods

Since 2001, we have performed 428 LDN at our centre. We did a retrospective analysis of our database to assess the outcome of extended donors. An extended donor criterion was defined as either of the following: age more than 60 year with a GFR of 80 ml/ min or more; patient with a body mass index (BMI) of more than 30 Kg/

m^2; a donor with hypertension (acceptable if age was more than 50 years, GFR more 80 ml/min and urine albumin excretion less than 30mg/24 hours); a diabetic patient (acceptable if the sugars were under control, with no target organ damage); a patient with multiple vessels, patients with anatomical anomaly such as double ureter and donors with *urolithiasis.*

All prospective donors with diabetes and hypertension were assessed for target organ damage. They were evaluated with dobutamine stress echocardiography, fundoscopy and microalbuminuria and not accepted if any of them were abnormal.

For analysis, the patients were divided in two groups Group 1 (n = 294) – normal criterion donor and group 2 (n=154) - extended criterion donor. Significant number of donors had overlapping co-morbidities (Table 1).

The parameters analyzed were preoperative and post operative serum creatinine in *donors and recipients*, serum creatinine at day 1, 7, 30 days and 1 year in *recipients*, operative time, warm ischemia time, analgesia

Table 1: Extended indications in donors

Extended Indications (n=154)	Donor > 60 years (n=31)	BMI> 30 kg/m^2 (n=42)	Hyper-tension (n=10)	Diabetes (n=4)	Multiple vessels (n=66)	Double ureter (n=1)
Donor	24	2	2	1	2	0
> 60 years (n=31 BMI> 30 kg/m^2 (n=42)	2	35	2	1	2	0
Hypertension (n=10)	2	2	0	0	6	0
Diabetes (n=4)	1	1	0	0	2	0
Multiple vessels (n=66)	2	2	6	2	54	0
Double ureter (n=1)	0	0	0	0	0	1

requirement, impact of donor BMI on outcome, impact of single versus multiple vessels in recipients.

All donors underwent LDN by a defined technique (7). Pneumoperitoneum was created with veress needle, dissection commenced by reflecting the colon after incising the white line of Todlt, the ureterogonadal packet was lifted off the psoas and dissection proceeded to the hilum. It is our policy to tackle the adrenal vein first and dissect the adrenal gland from the upper pole, this is followed by the dissection of the lumbar veins, and the hilar dissection is done with the combination of harmonic scalpel and monopolar hook. Once the hilum is dissected, it is bathed with papavarine (Fig. 1).The ureter, renal artery and the renal vein are secured with hemlock clips and cut. The kidney is retrieved through a preplaced pfannensteil incision.

Double vessel Triple vessel Single vessel

Fig. 1: Dissection of Hilum

Results

The nadir creatinine at 1 year was comparable in the recipients of both the groups I and II (1.04 ± 0.13 Vs 1.12 ± 0.2), (Fig. 2). Donor Age did not affect the *recipient* outcome (Fig. 3). Donors with BMI more than 30 kg/m^2 required more number of ports (3.2±0.5 Vs 4.0±0.7, $p<0.05$) and hospital stay (3.0±2.0 Vs 4.0±0.7 days) (Table 2).On comparison of recipient outcome for donors having single vessel vs. multiple vessels, the nadir creatinine at 1 year was comparable (1.0± 0.4 vs. 1.0± 0.2, $p<0.05$) (Fig. 4). 6 donors with urolithiasis had uneventful recipient outcome (Fig. 5). In 5 patients the stones were in the contralateral kidney, they were treated with ESWL. Satisfactory stone clearance was ensured before LDN. In one patient the stone was in the kidney to be harvested,

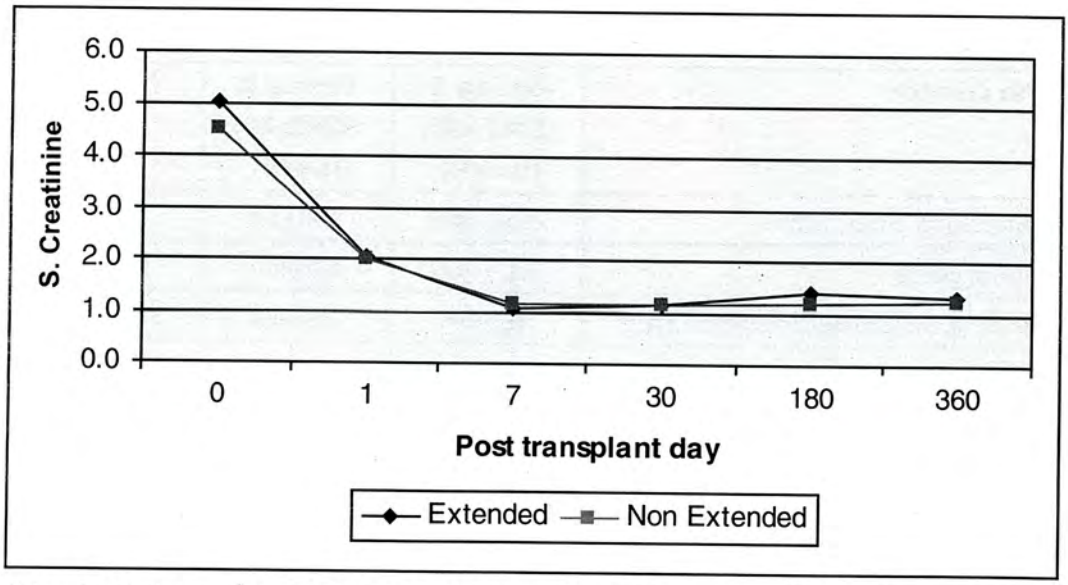


Fig. 2: Outcome in recipients: Comparing the outcome in non extended (group 1) and extended (group 2) indications

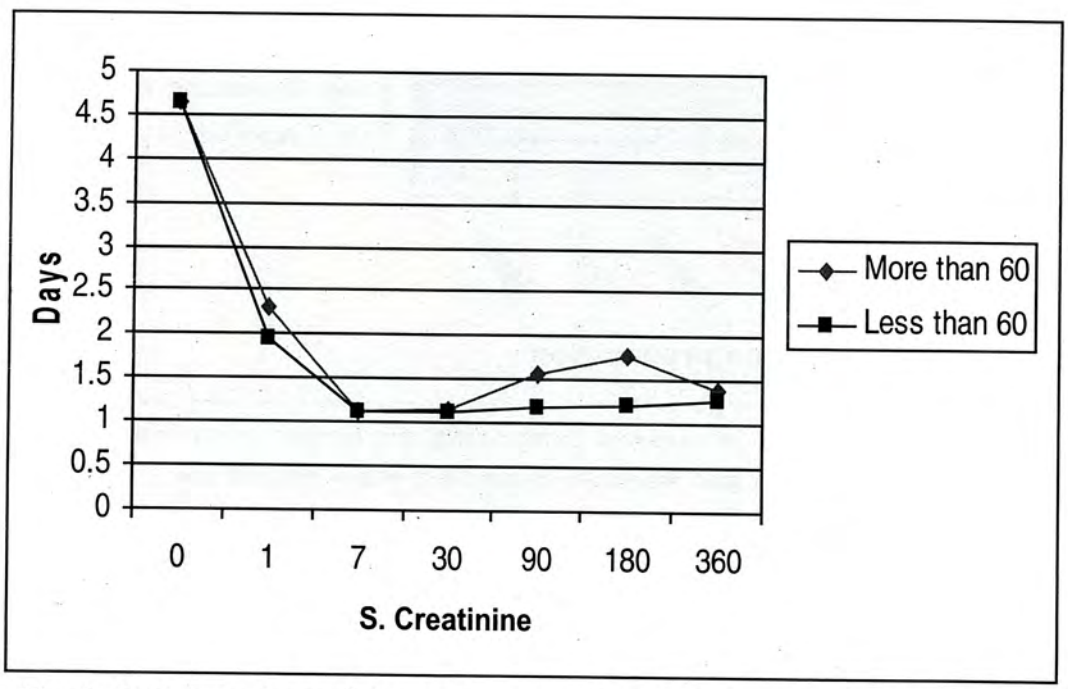


Fig. 3: Outcome in recipient: Comparing outcome with relation to age

Table 2 : Donor outcome: comparing the outcome with relation to BMI

Parameter	Group 1 (BMI <30) (n=386)	Group 2 (BMI≥30) (n=42)	P-value
Operative time(min)	156± 46.5	160±54	NS
No. of ports	3.2 ± 0.5	4.0±0.7	**P<0.05**
Dose of analgesia(Tramadol)(mg)	99± 64	110±64	NS
Starting oral fluids(**hours**)	19.7±3	20±3	NS
Drop in PCV (%)	4.8±2.3	6.2±3.2	NS
Duration of hospital stay(days)	3.0±.2	4.5±0.6	**P<0.05**
Warm ischaemia (min)	5.8 ± 1.9	6.0±2.0	NS
Total ischaemia (min)	55±8.7	58 ±16	NS

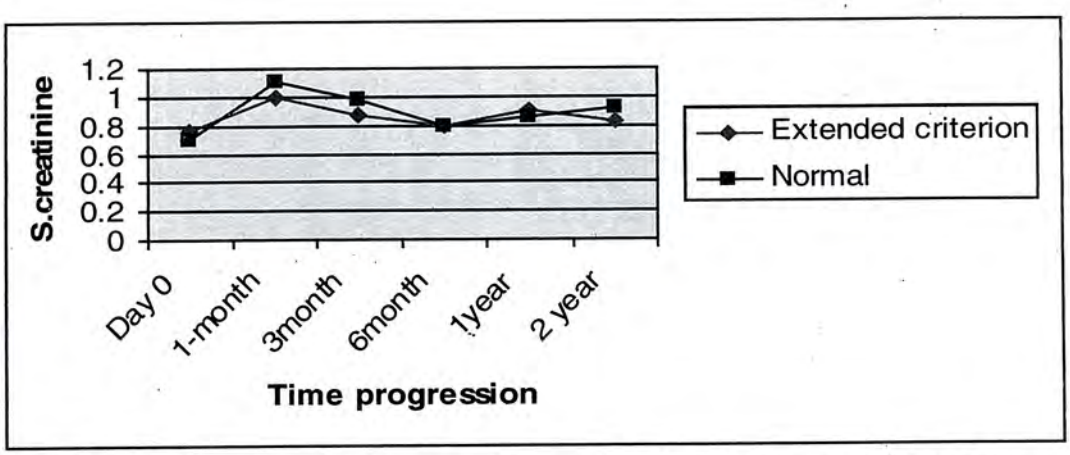
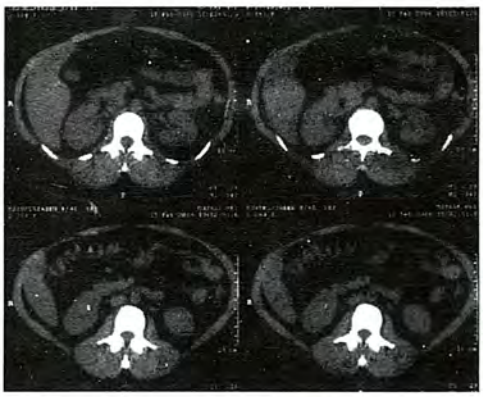
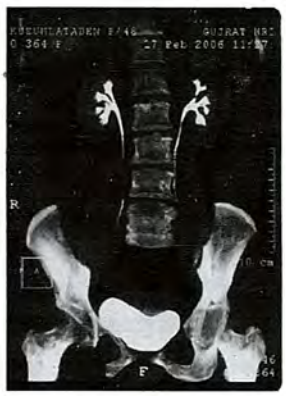


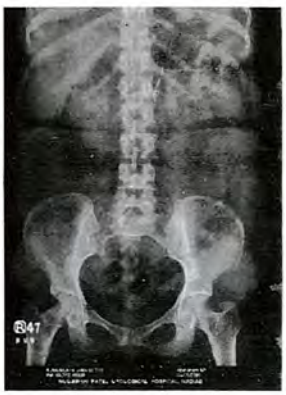
Fig. 4 : Outcome in donors: comparing the donor creatinine in extended and normal group at 2 years follow up



CT-evidence of 8mm right middle calyx stone with left gravel (2mm) Right ESWL done-1500 shocks



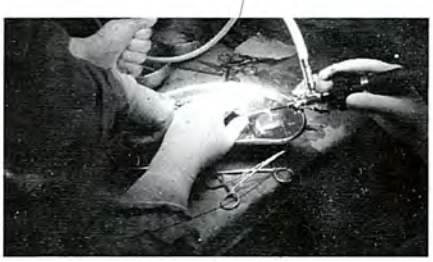
Urography



Post Left donor Nephrectomy - Complete stone clearance on Right side

Fig. 5 : Recipient outcome in donors with urolithiasis

the stone was retrieved on table with pediatric cystoscope and dormia basket from the graft kidney with *ex vivo* ureteroscopy (Fig. 6). All these recipients, who received kidneys from donors with urolithiasis, had uneventful post operative course with normal graft function. Fig. 7 shows donor with double ureter on the left side. The ureter was cut with common sheath to preserve the vascularity and routine uretero-neocystostomy was done with common sheath reimplantation. Post operative course was uneventful.



Bench Surgery

Stone Removal

Fig. 6 : *Ex vivo* ureteroscopy

Discussion

There is an ever increasing need to accommodate deserving transplant recipients coupled with the relative paucity of active cadaver transplant programme. *The terms extended donor criterion and marginal donors are confusing, they may simply mean usage of suboptimal quality cadaver renal grafts, non heart beating donors and living donors with some acceptable medical risks (2). We have identified old age, hypertension, diabetes mellitus, obesity, and anatomical anomalies such as multiple vessels and double ureters as extended indications for LDN as we feel these donors require considerable surgical / medical expertise and acumen to attain good results.*

LDN has the advantage of better cosmetic outcome, minimal morbidity and comparable recipient outcome. It can be performed with minimal morbidity and comparable outcome as open donor nephrectomy (7). Over the past 400 cases, we have observed a strict operative protocol for donor nephrectomy. We have also applied a few risk reduction strategies as follows: a transperitoneal approach, proper port placement, adequate division of splenorenal ligament and renocolic ligament, identification of the ureterogonadal packet, tackling the upper pole and adrenal first, use of harmonic scalpel at the hilum and manual removal of the graft(7) for optimizing the results. Although the presence of double vessels or early branching possesses a technical challenge, it is feasible and safe (7). In such patients in our series, the recipient creatinine levels were higher at day 1; however at 1 month and 1 year the difference was not statistically significant. Moreover the graft outcome was similar in both the groups (8) (Table 3).

Increased body mass index is associated with increased risk of proteinuria and focal sclerosing glomerulonephritis (FSGS). In addition, these donors are more likely to suffer from diabetes, hypertension and metabolic disorders (9). Obese patients with a BMI of less than 35 are acceptable (10). With the advent of LDN, there are concerns with donor nephrectomy owing to the technical challenge. In our experience in patients with BMI more than 30 or associated truncal obesity, the port needed to be placed more laterally. Similarly there was a need for an additional port for retraction. The only significant factor on comparing patients with higher BMI was need for longer hospitalization with rest of the outcome being comparable.

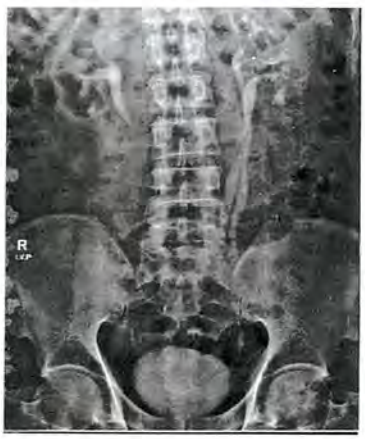
Table 3 : Comparing the recipient creatinine in donors with double artery, early branching and single artery.

	Double artery	Early branching	Single artery
Age(years)	34 ± 13.7	33.9 ± 12.5	37 ± 11.7
Total ischemia time(TIT)	74 ± 18.5	63 ± 13.4	55.1 ± 11.7
Creatinine day 0 (mg/dl)	4.7 ± 1.6	4.8 ± 1.4	4.6 ± 0.8
Creatinine day 1 (mg/dl)	2.4± 1.4	2.1± 0.8	1.9 ± 0.7
Creatinine day 5 (mg/dl)	1.1 ± 0.7	1.0 ± 0.5	1.1 ± 0.7
Creatinine month 1 (mg/dl)	1.1 ± 0.4	1.1 ± 0.2	1.2 ± 0.5
Creatinine month 6 (mg/dl)	1.3 ± 0.5	1.2 ± 0.3	1.3 ± 0.5
Creatinine year 1 (mg/dl)	1.3 ± 0.5	1.2 ± 0.5	1.3 ± 0.4

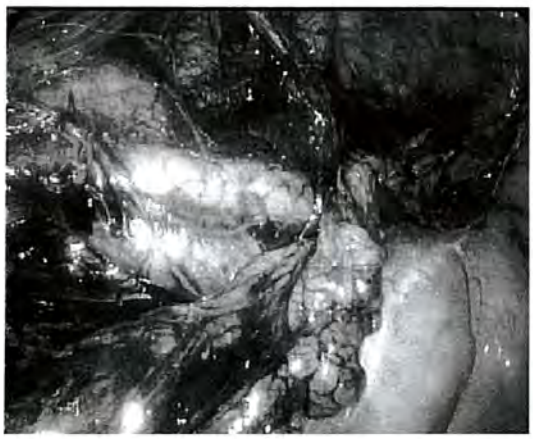
International forum on living donor 2004 at Amsterdam did not comment on acceptance of any related donor of a diabetic nephropathy individual; however it was recommended that prospective donors with blood sugar levels of more than 126 mg% on two occasions should not donate (10). Similarly on ambulatory blood pressure monitoring the BP>140/90 is not acceptable as donors (2). Our analysis shows that patients with age more than 60, hypertensive and diabetics have a good outcome at 1 year.

LDN was feasible in donors with double ureter and double vessel. In our case, the kidney was retrieved laproscopically and a common sheath reimplantation done (Figure 4).

Review of literature shows *ex vivo* semi rigid ureteroscopy as a technically feasible endoscopic option for rendering a stone bearing renal graft kidney stone clearance without compromising ureteral integrity and allograft function. Rashid and colleagues report 10 cases of stones in the kidney. 9 were successfully tackled with *ex vivo* URS. Trivedi *et al* have also reported *ex vivo* URS to be feasible and safe in 3 grafts (11, 12). In our experience, we performed ureteroscopy in one case with a pediatric cystoscope and stone was retrieved with dormia after harvesting the graft on bench. There was no ureteric injury and good functioning allograft at 1 month. Literature suggests that shock wave lithotripsy (ESWL) to the graft does not



35 years old mother donating to son



Double ureter with common sheath

Fig. 7 : Donor with double ureter on the left side

have any adverse outcome. In our series we did not give ESWL to any allograft post transplantation. However, ESWL was performed prior to transplantation in 5 patients to stones in the contralateral kidney; complete stone clearance was ensured prior to transplantation.

Conclusion

In our study, LDN was found to be safe, feasible and efficacious in donors with extended indications such as old age, BMI more than 30, multiple vessels and anatomical anomalies.

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Fetal Liver Cytokines : Potential Therapeutic Value

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Abstract

Due to the paucity of lymphoid cells, weak expression of HLA antigens, higher proliferative potential as well as higher long term repopulating ability of fetal liver cells compared to adult bone marrow (BM) and the minimal potential to induce graft versus host disease (GvHD), fetal liver cells (FLC) have been utilized to treat severe combined immune deficiency, aplastic anemia and other disorders. Of the 41 patients of aplastic anemia infused with FLC by us, 40% demonstrated slow and incomplete autologous hematopoietic recovery. Stable engraftment was not demonstrable as the preconditioning regimen was not utilized. To dissect the role of fetal liver infusion (FLI) in autologous hematopoietic recovery, the colony supporting potential of fetal liver conditioned medium (FLCM) was evaluated in BM cells of normal adult and aplastic anemia patients. In both cases, each sample of FLCM supported the growth of colony supporting cells in semisolid culture medium. The FLCM was analyzed for the presence of colony stimulating cytokines namely stem cell factor (SCF), Granulocyte macrophage colony stimulating factor (GM-

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CSF), interleukin 3 (IL-3), erythropoietin (EPO), IL-6 and FLt-3. GM- CSF and IL-3 were absent in most FLCM samples. EPO in small quantity was present in 30% of samples. SCF was present in more than 50% of samples. Flt-3 and IL-6 were present in majority in more than 80% of the samples. Bioneutralization assay confirmed 42% suppression of CFU-GEMM colonies with the addition of anti SCF antibody to FLCM. Suppression was 42% with anti IL-6 and 20% with anti Flt-3 antibody. Using RT-PCR method gene expression for SCF, GMCSF, Epo, IL-6 and Flt-3 was demonstrable. Suitable combination of cytokines may be a useful management strategy for the treatment of aplastic anemia.

Keywords: lymphoid cells, fetal liver cells, cytokines, aplastic anemia, hematopoiesis

Introduction

Hematopoiesis begins extra embryonically in the blood islands of yolk sac. Intra embryonic hematopoiesis is first observed in the aorto- gonad- mesonephros region and moves to the liver, the primary site of hematopoiesis during much of fetal life (1, 2).

Pluripotent hematopoietic progenitors (CFU- GEMM), myeloid progenitors (CFU- GM) and erythroid progenitors (BFU- E) are detected at high levels in the fetal liver from 12 to 23 weeks of gestation. Stem cells are first observed in the bone marrow (BM) at 15 to 16 weeks of gestation. Spleen cultures show growth of stem cells at 18 to 19 weeks. Peripheral blood also shows high levels of all 3 progenitors during 2nd trimester (3).

Studies carried out in our laboratory (4) have demonstrated higher proliferative potential of fetal liver hematopoietic stem cells compared to adult bone marrow (BM). This was evidenced by the formation of more number of colonies, higher plating efficiency and larger colony size of CFU – GEMM, BFU – E and CFU – GM colonies. Studies by Rebel *et al* (5) demonstrated that mice fetal liver (FL) competitive repopulation units (CRU) exhibit a greater proliferative activity *in vivo* than similar cells from adult BM accompanied by a greater production of daughter CRU.

Besides, it has been shown that 14 day mice fetal liver contains several times more long term repopulating cells relative to short term repopulating cells compared to adult marrow. Concentration of primitive stem cells was 7 fold higher in fetal liver than adult marrow, an increase that accounts for 4 to 9 fold higher concentration of long term repopulating ability in fetal cells (6). This may be due to the existence of asymmetric division in primitive hematopoietic cells in which proliferative potential and cell cycle properties are unevenly distributed among daughter cells (7). The continuous generation of functional heterogeneity among the clonal progeny of hematopoietic stem cells (HSC) suggests intrinsic control of stem cell fate and provides a model for the long term maintenance of hematopoiesis *in vitro* and *in vivo*.

Human fetal livers between 16 and 24 weeks of gestation contain a median 1.9x 10^9 viable cells and a mean of 1.3 x 10^8 CD 34 +\ ++cells (range 1.1x 10^7 to 4.7x 10^8). Regardless of gestational age , no significant differences are apparent in the number of total progenitors or in the number of candidate stem cells (CD 34++ CD 38 – and CD34 ++ CD 4 +) , suggesting that the expansion in the liver of the early compartments of hematopoietic progenitors reaches a plateau after the sixteenth week of gestation (8).

Studies of FL cells from abortuses of 9 – 24 weeks of gestation by Gilles *et al* (9) revealed that percentage of CD 123 positive cells increased significantly with gestational age ($p < 0.01$). The percentage of triple positive cells (CD34 +, Cd117 + and CD 123 +) increased with age but did not reach significance ($p = 0.05$). Human leukocyte A, B and C antigens were expressed on all nucleated cells from 9 to 24 weeks of gestation. Human leukocyte DR antigen however was expressed only on 50% of these cells. The percentage of cells that expressed both hematopoietic stem cell markers and DR antigen did not vary with gestational age. Other studies including the one done by our group (10) also suggests that determination of full house HLA antigens on fetal liver hematopoietic cells was possible only after 18 weeks of gestation.

Fetal red blood cells, white blood cells and platelet counts all increase with gestation, reflecting hematological development. Circulating and hepatic T lymphocytes increased in number shortly before the 13th week of gestation, which reflect thymic maturation. Fetal liver contains fewer

T lymphocytes than fetal blood (2.5% vs 18.6%; p= 0. 003) but more CD 34 + hematopoietic stem and progenitor cells (17.5% vs 4.3%; p= 0.004). Fetal liver contains more of the primitive CD 34 + and CD 38 negative hematopoietic stem and progenitor cells than fetal blood (32% vs. 17%; p = 0.04). Based on these studies Pahal *et al* (11) suggest the use of fetal livers before the 13th week of gestation for transplantation purposes. Golfier *et al* (8) however, are of the view that at this stage of fetal development it may not be possible to get sufficient quantity of FL cells safe for transplantation, and this could explain the failure of *in utero* transplants in several instances. These authors suggest the use of mid trimester abortuses in clinical practice. Positive selection of CD 34 + \ ++ cells yield progenitors essentially free of mature T cells. 1.3 x 10^8 CD 34 +\++ cells present in an average FL of 16 to 24 weeks gestation compares well with a typical BM graft that contains 1 to 4 x 10^8 CD 34 +\++ cells and surpasses a typical umbilical cord blood donation containing 3 to 8 x 10^6 CD 34 +\++ cells (7).This number of cells could allow for a transplantation of a recipient weighing 50 to 100 kgs.

Paucity of lymphoid cells , week expression of HLA antigens and higher proliferative potential as well as higher long term repopulating ability of fetal liver cells compared to adult BM, initiated the use of FL cells for the management of immune deficiencies, leukemias, aplastic anemia and other disease entities such as Fabry's disease (12) with encouraging results.

During the late 70's, when we did not have the facilities to perform bone marrow transplants, we used fetal liver cells to treat patients of aplastic anemia and acute myeloid leukemia (AML).

Fetal Liver Infusion in Acute Myeloid Leukemia (AML)

Fetal liver cells (FLC) were infused in 12 patients of AML after completing standard chemotherapy protocol. The plan was to infuse FLC within 48 hours of last dose of chemotherapy. However, since availability of appropriate abortuses was unpredictable, 9 patients received FL infusion (FLI) beyond 48 hours of last dose of chemotherapy; 3 received it after 6 days. In view of this, it was difficult to interpret the data and to get any conclusive results. The role of FLI in the management of AML remains uncertain (13).

Fetal Liver Infusion in Aplastic Anemia

Forty one (38 males, 3 females) patients with aplastic anemia received an intravenous (IV) infusion of 0.004 to

11.1 x10^8 (median 8 x 10^8) hematopoietic cells prepared from the fetal livers of 8 to 32 weeks gestation. There was a slow and incomplete autologous hematopoitic improvement in 40% and expected survival of 52% at one year and 37% at 5 years. Six patients with good response were alive after 9 to 44 months (median 20 months); one died 106 months after FLI due to renal lithiasis. Four with moderate response were alive after 9 to 31 months. Of the patients with minimum response, one was lost to follow up and the others died in 3.4 to 10 months (median 6 months). Median survival (MS) of responding patients was 15.7 months. Seven patients relapsed; 6 regained a normal BM cellularity after a second or a third FLI. These data suggest a role of FLI in inducing BM recovery among patients suffering from aplastic anemia (14, 15).

Response to FLI was slow, often requiring 10 to 30 days or more. The BM cellularity increased to 48% in about 18 weeks with repopulation of both erythroid and myeloid cells. The erythroid response was predominant and the earliest to occur (1.5 to 6.1 weeks); it lasted for 7.5 to 100 weeks. Marked dyserythropoiesis was observed. Myeloid response occurred simultaneously in 45% of the respondents, it was delayed by 1.5 to 4 weeks in others. Significant dysmyelopoiesis with shift to the left, unrelated to infection, was seen 1.5 to 9.5 weeks after FLI lasting for 9 to 26 weeks. Megakaryocytic response either did not occur or was delayed, less marked and often transient (16).

Fetal Liver Growth Factors

It was noteworthy that no preconditioning had been employed; stable engraftment by fetal liver cells was not demonstrable; 3 patients however did show temporary engraftment (17). FLI thus appeared to have led to autologous hematopoietic recovery in those who responded. However whether this recovery was spontaneous or the effect of the infused fetal liver cells was not clear. To dissect the role of FLI in autologous hematopoietic recovery, the colony supporting potential of fetal liver conditioned medium (FLCM) was evaluated in BM cells of normal adult and aplastic anemia patients. In both cases, each sample of FLCM supported the growth of colony supporting cells in semi solid culture medium. The FLCM was assayed for the presence of four principle colony stimulating cytokines namely stem cell factor (SCF), granulocyte macrophage colony stimulating factor (GM-CSF),

interleukin- 3 (IL-3) and erythropoietin (EPO). GM- CSF, IL- 3 and EPO were present in insignificant amounts or were altogether absent, 50 to 635 pg/ml of SCF was found in 8 of the 13 FLCM samples tested. Preliminary results of bioneutralization assay indicated the possible role of SCF secreted by the FL cells, in colony supporting activity of aplastic anemia and normal BM cells. Overall, this *in vitro* study implicated the paracrine role of infused fetal liver cells in regenerating autologous hematopoiesis in aplastic anemia patients (18).

Of the 13 FLCM samples, 5 did not show the presence of SCF and yet had stimulated CFU MIX colonies in normal as well as aplastic marrow samples. This observation raised the possibility that FLCM might contain additional colony factors which were not studied during earlier investigation. Possibility also existed that SCF might exhibit a synergistic effect with other growth factors to stimulate colony formation of aplastic anemia marrow cells. Amano *et al* (19) have reported that the combination of EPO and SCF but not IL- 3stimulated colony formation upto 45% greater than normal in 11 of the 19 cases of aplastic anemia. Flt- 3 ligand and thrombopoietin (TPO) have also been implicated in working synergistically with SCF (20). Another study showed that Flt – 3 signal as well as c- kit signal synergizes with gp- 130 signal to stimulate human myelopoiesis, erythropoiesis, and megakaryopoiesis (21).

Irrespective of the presence or absence of SCF, all FLCM samples supported colony formation in aplastic anemia marrow samples. This suggests that in all likelihood, the stimulatory effect was not due to SCF alone but in combination with other cytokines. Also clinical recovery of 40% is inconsistent with *in vitro* recovery of 100%. It is likely that while certain factors act synergistically, others might be acting antagonistically. The concentration of growth factors might also make a difference. For instance, transforming growth factor ß1 (TGF ß1) at concentration of 1 to 50 pg/ml stimulated colony formation but at higher concentration it had an inhibitory effect (22).

Additional factors IL-6 and Flt-3 were evaluated by us in 24 FLCM samples in addition to SCF, GM- CSF and EPO. GM – CSF as absent in most FLCM samples, EPO in small quantities was present in 30% of samples. SCF was present in more than 50% of FLCM samples. Flt- 3 and IL – 6 were present in majority in more than

80% of samples. Bioneutralization assay confirmed 40% suppression with the addition of anti SCF antibody to FLCM. Suppression was 42% with anti IL – 6 antibody and 20% with anti Flt-3 antibody.

All the 5 cytokines studied by us were checked for the gene expression by RT-PCR method. The gestation age of the fetuses was between 8-18 wks. Out of 12 fetuses analyzed till now, SCF (100%), GM-CSF (68%), IL-6 (92%) and Flt-3 (83%) were demonstrable in most of the samples while Epo (25%) was present in only 5 samples(8-9weeks only). The study indicates that there could be a correlation between the cytokine gene expression and gestation age of the fetuses.

Ongoing Studies

Further studies are required to work out the optimum concentration and combination of various cytokines which will aid hematopoiesis in AA patients and also patients who are undergoing high dose chemotherapy. FL may be of benefit for the management of various clinical disorders, however, the exact mechanism involved in the regulation of hematopoiesis, cytokine interaction and signal transduction is still elusive. Up and down regulation of various cytokines by gene expression studies is needed.

The main hindrance in carrying out further studies is relatively small number of cells available from a single fetal liver. Therefore the future clinical potential of these cells would be strongly enhanced if a reliable method allowing expansion of stem cells is established. To do various *in vitro* studies and to get homogeneous supply of fetal cells there is also need to prepare cell line. It may help in various studies like gene expression of various cytokines, intracellular cytokine studies, vaccine development, cell growth and regulation, carcinogenesis and the immune response. A cell line provides a useful model for studying cell biology of specific tissues, tumorogenecity, genetic abnormalities or to help screen for effective methods of gene therapy. They can also be used to test a drug's ability to damage genetic material or to test the effects of specific viral infections

Ongoing work in our laboratory comprises of isolation of fetal liver hematopoietic stem cells, expansion of these cells under *in vitro* culture conditions and establishing transformed and non-transformed cell lines. Till now we have succeeded in getting 50- fold expansion of these cells using different cytokine combinations.

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Tobacco Smoking and Chronic Obstructive Lung Disease – The Indian Evidence

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Abstract

There is a large body of data on adverse effects of tobacco on non-neoplastic lung diseases. Adequate evidence on these effects is now available from studies in India. Tobacco smoking is the most important cause of chronic obstructive pulmonary disease. It is also responsible for increased morbidity and progressive deterioration of lung function in these patients. Passive exposure of nonsmoker individuals, especially women to smoking from others is a significant risk factor for the development of COPD.

Exposure to smoking is also known to adversely influence asthma. Childhood parental exposure to maternal smoking is important in the development of asthma. Passive smoking exposure is responsible for an increase in morbidity indices and acute exacerbation of asthma in both children and adults.

Introduction

Tobacco smoking is established as the most important risk factor in the development as well as in worsening the morbidity and mortality from various lung diseases. Not only the respiratory malignancies such as lung cancer, but several other non-neoplastic lung diseases are also caused and/or adversely influenced by tobacco.

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Chronic obstructive pulmonary disease (COPD) is the most important tobacco related lung disease. The others include bronchial asthma, respiratory infections and some forms of interstitial lung diseases (ILD). There is a fair amount of work now available from India on this association (1-6).

Chronic Obstructive Pulmonary Disease

COPD is a leading cause of morbidity and mortality worldwide (7, 8). It is also an important cause of economic burden in both the developed and the developing countries, including India (9, 10). In a study on assessment of costs of tobacco related COPD in India, on an average, a COPD patient spent about 15 percent of his income on smoking products and up to 30 percent on disease management (10). Tobacco smoking was also the most frequent cause of chronic cor pulmonale which occurred as a long term complication of COPD both amongst men and women patient (1). COPD globally accounted for 4.4% of total deaths in 1990, expected to rise to 6.9% of total deaths by 2020[7].

Chronic obstructive pulmonary disease which was hitherto underdiagnosed in the Indian subcontinent, is now recognized in 4-10% of adult male population of India and several other Asian countries. The Regional COPD Working Group for 12 Asia Pacific Countries and Regions used a COPD prevalence model and estimated an overall prevalence rate of 6.3% with a range from 3.5% (Hongkong and Singapore) to 6.7% (Vietnam). The smoking associations with COPD were high from most countries – 2.65 in India, 2.57 in China and 2.12 in Japan.

Chronic obstructive pulmonary disease which includes chronic bronchitis (CB) and emphysema is a common clinical condition but frequently misdiagnosed. Both asthma and COPD which may present similarly with several overlapping features bear a common name in Hindi and several other Indian languages. It is not always possible to separate the two conditions from each other especially in epidemiological studies in adults. But a good study-questionnaire, especially designed and validated for their diagnosis is generally adequate to arrive at epidemiological conclusions.

India

There are a large number of clinical and epidemiological reports from India on the prevalence of COPD (11). A review of different population reports for prevalence rates of COPD and the smoking association shows a wide range (Table 1) (11-26). The disease was

Table 1
Studies on the prevalence of chronic obstructive pulmonary disease (COPD) and its association

Authors	Population studied	Total Number	Prevalence of COPD (%)		Ratio of Sm:NSm
			M	F	Males
Viswanathan *et al*, 1964 (12)	Patna	15,905	2.1	1.3	7.4
Wig *et al*,1964 (13)	Delhi: Urban	2366	3.1	1.9	8.8
	Delhi: Rural	1401	8.4	2.4	4.7
Jindal 1993 (15)	Chandigarh	1473	5.0	2.7	9.6
Sikand *et al*, 1966 (16)	Delhi	14,460	3.3	2.6	5.9
Bhattacharya *et al*, 1975 (17)	Lucknow: Rural	1140	6.7	4.5	1.7
Joshi *et al*, 1975 (18)	Punjab (industrial workers	473	12.5*		5.3
Viswanathan and Singh 1977 (19)	Delhi: Urban	993	8.0	4.3	4.1
	Delhi: Rural		4.7	3.5	9.6
Thiruvengadam *et al*, 1977 (20)	Madras (Chennai)	817	1.9	1.2	10.2
Radha *et al*, 1977 (21)	Delhi	2098	4.2	2.1	1.8
Malik *et al*, 1977 (22)	Chandigarh	1154	8.3	5.3	4.8
Nigam *et al*, 1982 (23)	Jhansi: Rural	1424	8.1	4.5	1.4
Malik 1986 (24)	Chandigarh: Urban	1450	10.8	1.6	11.0
	Chandigarh: Rural	671	20.5	4.9	4.0
Malik & Kashyap 1986 (25)	Himachal Pradesh: Rural	446	21.7	19.0	5.5
Behera and Malik 1987 (26)	Chandigarh: School teachers	681	3.3	2.1	1.6
Ray *et al*, 1995 (14)	Tamil Nadu	9946	4.1	2.6	

Sm: Smokers: NSm: Nonsmokers
*Data for both combined

first highlighted in the 1960s from analyses of hospital records of patients attending outpatient chest clinics (12). Chronic bronchitis comprised 2.5% of the total admissions of several hospitals in north India (11). COPD accounted for 31% of the clinic attendance in 1952-1954 in Punjab (13). A prevalence rate of 4.1% in males was reported in a large prospective study from Tamil Nadu (14). Epidemiological studies in the 1990s reported prevalence data from cross-sectional community surveys. The overall median prevalence of COPD was found to be 5% in male and 2.8% in female subjects in the different population studies from India.

Tobacco Association

Tobacco smoking is the most important cause of the development of COPD. This relationship was first established in the 1960s through several population-based epidemiological studies (27, 28). It is universally accepted now that tobacco smoking accounts for over 80%-90% of cases of COPD (29). Tobacco smoking is included as an important criterion for the diagnosis of COPD (30). Evidence favoring a causal relationship between tobacco and COPD is based on valid and consistent epidemiological, clinical and laboratory data from multiple studies (31).

Active Smoking

Most of the epidemiological reports on the prevalence of COPD clearly talk about smoking as the most important risk factor. The association between active smoking and COPD has been variably reported in the Indian studies (12-26). There was a large variation in the methodology employed in these surveys. Moreover, the populations surveyed also varied from hospital employees and patients' attendants to clusters of industrial workers or the general population. Smokers, however, were classified as separate groups in most of these studies. The prevalence ratio of smokers to nonsmokers among those with COPD was invariably more than 1 in all the surveys reported.

To overcome the problem of variation, a large multicentric epidemiological study was undertaken at 4 different centers at Chandigarh, Delhi, Bangalore and Kanpur (2). The overall population prevalence of chronic bronchitis as a prototype of COPD was 4.1% of 35295 subjects with a male to female ratio of 1.56:1. In smokers, the prevalence was 7.7% with smoker: nonsmoker ratio of 2.65. Almost all forms of smoking products such as the cigarettes and the 'bidis' used in different countries were found to be significantly associated with COPD.

Odds ratio for COPD for cigarettes, bidis and 'hookah' were 1.952, 2.654 and 2.847 respectively (2).

The relationship of COPD with tobacco smoking seems independent of the type of product smoked, i.e. cigarettes, *bidis* or *chutta*, on the basis of some data available on *bidi* smoking and COPD. In an earlier study specifically examining *bidi* smoking, COPD was observed in 34.5% of *bidi* and 45.4% of cigarette smokers versus 3% of nonsmokers, the difference in the prevalence of COPD among cigarette and *bidi* smokers was not significant (32).

Passive Smoking and COPD

COPD was diagnosed in 2.9 percent of nonsmoker individuals. An important factor in these patients is the exposure to smoking from others e.g. spouses, colleagues, sibs and parents. In particular, nonsmoker women exposed to smoking from husbands are at significant risk. The odds ratio (1.535) for risk from passive smoking i.e. the environmental tobacco smoke (ETS) exposure in nonsmoker patients was significant during both the childhood and the adulthood (2). The other risk factor for COPD in nonsmoker women is the exposure to indoor air pollution from domestic combustion of solid (i.e. biomass) fuels emanating a lot of smoke containing particulate and gaseous air pollutants.

Bronchial asthma

Asthma is a common respiratory disease in both children and adults. There have been widely different prevalence rates reported from different parts of India varying from about 1.8 to 3.5% in adults and from 2.8 to 16.6% in children (Table 2) (5, 33, 34). The most authentic data on asthma prevalence in adults has now become available from the Indian Council of Medical Research (ICMR) study which was conducted simultaneously at four different centers in the country with the help of the above mentioned questionnaire and methodology on 73605 individuals aged 15 years or over (43). The overall presence of ever asthma was diagnosed in 2.4% individuals while one or more respiratory symptoms were present in 4.3 - 10.5% of subjects (43).

Tobacco smoking has got important effects on asthma. Exposure to smoking is not only responsible for aggravation of asthma but is also important as a risk factor in the development of asthma. A few studies on the adverse role of environmental tobacco smoke (ETS) exposure in the development as well as aggravation of symptoms of asthma are available from India (4, 6, 40, 45). The

Table 2

Prevalence studies on asthma from India*

	Author	Study Population			Age (yrs)	Definition/ Methodology	Prevalence (%)
		Region	Group	No.			
1.	Viswanathan (1966) (33)	North (P)	Urban	15805	All ages	Symptoms on interview	1.8
	Children						
2.	Shah (2000) (34)	Multicentric	Schools	37171 31697	13-14 6-7	Self reported, (ISAAC)	3.7 4.5
3.	Awasthi (2004) (35)	North (L)	Schools	3000	13-14 6-7	do	3.3 2.3
4.	Mistry (2004) (36)	North (C)	Schools	575	13-14	Q. Wheezing	12.5
5.	Chakravarthy (2002) (37)	South (TN)	Field	855	< 12	Q. Diagnosed Asthma	5
6.	Chhabra (1998) (38)	North (D)	Schools	2609	4-17	Q. Current	11.6
7.	Paramesh (2002) (39)	South (B)	Schools	6550	6-15		16.6
8.	Gupta (2001) (40)	North (C)	Schools	9090	9-20	IUATLD based validated Q	2.3
	Adults						
9.	Chowgule (1998) (41)	West (M)	Field	2313	20-44	ECRHS Q	3.5
10.	Jindal (2000) (42)	North (C)	Field	2016	18 - > 70	Validated Q	2.8
11.	Aggarwal (2006) (43)	Multicentric	Field	73605	> 15	Validated Q	2.4

P = Patna; ISAAC = International Study on Allergies and Asthma in Children; L = Lucknow; C = Chandigarh; Q = Questionnaire; TN = Tamil Nadu; D = Delhi; B = Bangalore; IUATLD = International Union Against Tuberculosis & Lung Disease; M = Mumbai; ECRHS = European Community Respiratory Health Survey

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odds ratio for being asthmatic for children of 9 to 20 years age exposed to ETS compared to those not exposed was 1.78 (95% C.I. 1.33 – 2.31) (4). In the ISAAC International Study on Asthma and Atopy in Children) study on 2000 school going children near Delhi, the odds ratio for ETS exposure for asthma was 3.33 (95% CI = 1.88 – 7.65) (34). The in-utero exposure to maternal smoking may also be independently responsible for early onset of asthma (46, 47).

In adults, the ETS exposure during childhood and both during childhood and adulthood was significantly associated with an increased prevalence, while exposure during only adulthood was not associated with this risk (4). Among 62109 nonsmoking individuals, prevalence of asthma was higher in the ETS exposed than the non-ETS exposed individuals (2.2% vs 1.9%, $p < 0.05$) (4). ETS exposure was also shown to be responsible for increased indices of morbidity and poorer control of asthma in adults such as in nonsmoking women exposed to smoking from their husbands (48). Acute exacerbations were also more frequent in the ETS-exposed asthmatic patients (49). Bronchial responsiveness of nonsmoking asymptomatic individuals, who were ETS exposed was higher than of the non-ETS exposed individuals (50). The increase in bronchial responsiveness was even more pronounced in nonsmoker asthmatic individuals when exposed to ETS from smokers (51).

In conclusion, there is enough data from India especially from this Centre which demonstrates an adverse relationship of both active and passive smoking with obstructive lung diseases such as COPD and asthma. This is important from point of view of an individual patient for his clinical management as well as for adoption of public health program in tobacco cessation and control activities.

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Comprehensive Management of Ischemic Stroke Based on the Concept of Ischemic Penumbra

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Abstract

Stroke is a cause of mortality and morbidity of immense magnitude, rating second only to cancer in the developing world. Humungous advances in the understanding of the pathophysiology and subtypes have resulted in paradigm shifts in approach to management. With the burgeoning stroke burden on the society it is imperative to keep pace with these advances. Treating strokes based on the concept of identifying and quantifying the critically perfused brain tissue called the "ischemic penumbra" by a rapidly available radiological marker have led to radical changes in the attitude amongst clinicians from being nihilistic to being aggressive and optimistic. The paper delineates these emerging concepts and summarizes the limited experience with the hyperacute thrombolysis of acute ischemic stroke patients in Indian setting.

Introduction

Years of nihilism later, acute stroke therapy has finally emerged, including thrombolysis with tissue plasminogen activator (rt-PA). However, rt-PA is limited by a narrow therapeutic time window period and side effects, so that only 5% of all stroke patients receive

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thrombolysis. Unimodal targeting of key events in stroke pathophysiology has not been effective in providing long-term benefits, leading to negative results in previous clinical neuro-protective stroke trials. A successful future stroke therapy needs to approach multiple pathophysiological mechanisms besides revascularization/ reperfusion including thrombolytics, related adverse side-effects, prevention of apoptosis (programmed cell death), stimulation of neuro-regeneration and neuronal plasticity.

Thrombolytic therapy is an inherently attractive treatment for acute ischemic stroke (AIS), based on the known pathologic and angiographic substrates of ischemic cerebrovascular disease. Most ischemic strokes are the direct consequence of atherothrombosis or a thromboembolism of a cerebral or pre-cerebral artery. The critical event is usually the formation of an acute thrombus. The basis for thrombolytic treatment is to achieve rapid arterial recanalization with a relatively safe agent, soon enough to improve patient outcome. Reperfusion is the most beneficial of all therapeutic strategies for acute ischemic stroke. There is Level I evidence now which shows that thrombolysis in cases of AIS is effective if administered with in 3 hours of stroke onset. This benefit has been shown to be time dependent and potentially extends beyond 3 hours, with evidence that potentially viable penumbral tissue may be present in a significant proportion of cases well-beyond 3 to 6 hours and in isolated cases, up to 48 hours.

The standard cerebral reperfusion treatment of the first decade of the reperfusion era, non-contrast CT guided = 3 hours intra-venous tissue plasminogen activator, has many limitations. This review surveys emerging strategies that have the potential to extend cerebral reperfusion therapy beyond 3 hours time window as well as the means to bridge the "stroke recovery gap" (defined as the difference observed between the clinical response to thrombolytic therapy in a given population of patients presenting with ischemic stroke and the potential clinical recovery if all of the penumbra were salvaged under ideal circumstances). Approaches to this include: 1). Intra-arterial pharmacological reperfusion approaches, combined intravenous-intra-arterial fibrinolysis and combined fibrinolytics and GPIIb-IIIa agents, 2). Emerging endovascular mechanical reperfusion strategies including intra-arterial thrombectomy (clot retrieval devices and suction

thrombectomy devices), mechanical disruption (micro-guide wire passage, laser photo acoustic emulsification, primary intracranial angioplasty), 3). Augmented fibrinolysis by endovascular ultrasound, 4). Multimodal imaging with magnetic resonance (MRI) or CT to rapidly assess the infarct core, penumbra, site of vessel occlusion and tissue hemorrhagic propensity, enabling improved selection of patients for reperfusion therapy beyond any arbitrary fixed time window, 5). Newer thrombolytic agents and 6). Adjunctive therapies such as neuroprotectants.

Intra-venous Thrombolysis in AIS

The National Institute of Neurological Disorders and Stroke (NINDS) study on the intravenous use of recombinant tissue plasminogen activator (rtPA) within 3 hours of a an acute ischemic stroke has shown the benefit of this form of treatment . In order to be effective the protocol for institution of rtPA therapy must be strictly followed. The evidence for this form of therapy is of level I. When all accumulated evidence for rtPA therapy is considered, the relative risk reduction provided by it is 44%, absolute risk reduction is 13% and the number needed to treat to save one person from death or disability is 7.

Many countries now routinely thrombolyze all patients of acute ischemic stroke who resent within the 3-hour time window and do not have any contra-indication. However, many stroke units, especially those in the developing nations, are hampered by constraints of resources and lack of awareness. Feasibility of thrombolysis is still being evaluated at these centers. The hyperacute thrombolysis for stroke was started in the year 1995-1996 in India and in March 2002 at All India Institute of Medical Sciences, a tertiary referral center in India. We assessed the outcome of patients treated with intravenous rtPA, including the incidence of intracerebral hemorrhage and other adverse events.

AIIMS is a large tertiary referral center in North India catering to almost 14 million people in Delhi. An earlier study found nearly 10-15% of patients with acute stroke to present within 3 hours to the Emergency services (unpublished). Since the catchment area is large and the population served is more than 14 million, this 10% could translate to significant numbers. Nearly 350-400 cases of acute stroke are seen every year at AIIMS.

The variables that were specifically recorded for each patient treated with rtPA: time of symptom onset, time of

arrival in the emergency department, time of CT scan examination and the time of rtPA administration. An extensive neurological examination including the baseline NIH Stroke scale (NIHSS) was performed in all patients. Other parameters noted were the demographic profile, stroke risk factors, baseline CT scan findings and the blood pressure measurements.

Potential benefits and significant risks, specifically the four fold greater risk of symptomatic intra-cerebral hemorrhage after thrombolytic treatment were discussed with all patients and /or their families and informed consent obtained. The tPA protocol was based on protocols published by the American Heart Association and the American Academy of Neurology. Inclusion and exclusion criteria are shown in Table 1.

All patients had pretreatment CT scans that were read by the attending neurologist together with the radiologist. Each of our patients received 0.9 mg/kg of intravenous rtPA upto a maximum of 90 mg, based on the estimated or actual body weight. Ten percent of this calculated dose was injected as a bolus and the remainder infused over an hour. Heparin and aspirin were withheld for the first 24 hours after rtPA administration in all our patients. Hypertension was treated using intravenous labetolol, or intravenous enalapril. Occasionally injection nitroglycerine or nitroprusside was required.

Results of follow-up CT scans or MRI scans were recorded in all patients thrombolysed. The use of aspirin, clopidogrel, heparin, acitrom and antihypertensives during the hospital stay was noted. The peak blood pressure during rtPA administration as well as during the first 24 hours after the infusion was recorded (Stroke subtype was determined by using the following classification: 1). Large artery atherosclerosis, 2). Cardio-embolic, 3). Small-vessel occlusion, 4). Stroke of other determined etiology, 5). Stroke of undetermined etiology using the TOAST criteria. We obtained telephone or clinic follow-up with the patient and caregiver in all 54 cases and assessed the Barthel Activities of Daily Living Index. For analysis, we defined a good outcome as an improved NIHSS of = 4 and a Barthel Index of = 75%. At AIIMS, due to constraints of time and resources we deviated from the recommended NINDS protocol in excluding patients with a history of liver or renal disease and in not obtaining results of platelet counts, prothrombin time and activated partial thromboplastin time in patients prior to thrombolysis.

Table 1

Criteria for use of tissue plasminogen activator (rtPA) in treating acute ischemic stroke at All India Institute of Medical Sciences

Clinical Inclusion Criteria

- Patient/care giver able to give informed consent before study procedure.
- Age = 18 years.
- Onset of symptoms of ischemic stroke within 0-3 hours.
- Measurable neurological deficit defined as impairment of language, motor function, cognition, and/or gaze, vision or neglect.
- Score for stroke severity = 4 on the National Health Stroke Scale (NIHSS).

Clinical Exclusion Criteria

- Severe symptoms (coma or severe obtundation with fixed eye deviation and complete hemiplegia or NIHSS score > 22).
- Minor stroke symptoms (NIHSS score < 4) or those that are rapidly improving.
- History of stroke in previous 6 weeks.
- Seizure at onset unless the treating physician is convinced that the neurological deficit is due to stroke and not seizure.
- Previous known intracranial hemorrhage.
- Hemorrhage in pretreatment CT scan of the brain.
- Uncontrolled baseline hypertension (> 185/110 mm Hg).
- Myocardial infarction in past 30 days.
- Recent (< 15 days) surgery, major arterial puncture, trauma or ulcerative wounds.
- Known and clinical evidence of chronic or acute hepatic or renal disease.
- Known bleeding diathesis/ coagulopathies.
- Patients on anticoagulants.
- Pregnancy, lactation, or parturition within the previous 30 days.
- Hypoglycemia, hyperglycemia (baseline serum glucose level must be between 50 mg% - 400 mg%).

Computed Tomography Exclusion Criteria

- Evidence of acute or chronic intracranial bleeding on CT.
- Likely etiology other than acute brain ischemia.
- Early signs indicating infarct of more than a third of the territory of the middle cerebral artery.

Fifty four patients received IV rtPA for AIS between March 2002 and March 2006. The stroke team was notified of 1096 patients suspected of having an acute stroke during this period. The most common reasons for disqualification from thrombolytic therapy were, exceeding the window period (38%), intracerebral hemorrhage (20%), minor or rapidly resolving symptoms (20%), and a non-stroke diagnosis (12%). Of the patients who could qualify to received thrombolysis, 26% could not be thrombolyzed since they could not afford rtPA.

The mean age of our patients was 66 years, ranging from 32 to 82. The mean blood pressure at admission was 156/88 mmHg and the mean maximum pretreatment blood pressure was 166/90 mm Hg. The mean baseline NIHSS was 14± 2 (range 8-22). Using TOAST criteria, patients were classified into: large artery atherosclerotic = 13; cardioembolic = 12; small vessel occlusion = 22; other determined etiology = 3; undetermined etiology = 4. The mean time to reach emergency was 2.4 hours (1.15 - 3.4 hours). The mean door to CT time was 24 minutes (10 - 47minutes). The mean door to rtPA injection was 26.8 minutes (25 - 67 minutes). The NIHSS scores ranged from 11 to 22 minutes (mean = 15.5 ± 2.7). Patients with history of liver or renal disease or on anticoagulants were excluded. PT, aPTT and platelet count results were not obtained prior to thrombolysis. Early signs of infarction on CT were seen in 25/54 (46.3%) patients. These were sulcal effacement in 11, insular ribbon sign in 6, and loss of gray white matter definition in 8. An infarct in the follow-up CT or MRI was present in 53 of our 54 patients (97.1%).

Mean length of hospitalization in our patients was 9 days. Hemorrhagic conversion was noted in 5(8.8%). Symptomatic intracranial hemorrhage or fatal hemorrhage did not occur in any of our patients.

Thirty five patients (65%) significantly improved on NIHSS at 48 hours (= 4 points) (mean change = 10; range = 4-17). At one month, 43 (79%) improved on Barthel Index (mean change = 45%). Of these, 19 (44% of the 43 patients improved; 35% of total number[54]), achieved = 95% scores on Barthel Index indicating near normal functional status. Of the stroke subtypes, cardioembolic and small vessel occlusions did better than others. Of the 8 patients who showed no improvement, 6 were lacunar strokes. One developed small frontal lobe hemorrhage and recurrent stroke; one died of aspiration and 8 showed no

improvement. The mean length of follow up after discharge was 9.5 ± 3 months.

We succeeded in treating 54 patients of acute ischemic stroke with IV rtPA over a period of four years. Importantly, we were unable to thrombolyse 38% of otherwise eligible patients due to delay in evaluation and treatment. The stroke subtype by final diagnosis was most often, in 35.3% patients, small vessel disease. The NINDS trial and few subsequent studies have shown that intravenous thrombolysis within the 3 hour time window is similar between different stroke subtypes. Patients arrived in the emergency department an average of 150 minutes after the symptom onset. A CT scan was performed within 22 minutes and intravenous rtPA was administered within an average of 27 minutes from the time of arrival to the emergency department respectively. A significant difference between our experience and the NINDS trial is that half of the patients randomized in the NINDS study were treated within 90 minutes of stroke onset. However, the response to treatment observed in the NINDS trial between patients treated under 90 minutes and between 90 and 180 minutes did not differ. This underlines the importance of not withholding treatment from patients even if they present beyond 90 minutes of symptom onset. We were able to treat patients within an average of 27 minutes from their time of arrival at the emergency department. This is contrary to the popular perception that it is generally not possible to treat patients within the time window of 180 minutes in busy and constrained hospitals of developing countries unless they arrive early. Recent NIH consensus guidelines recommend a "door-to-needle" time of 60 minutes or less for acute stroke patients.

An improvement of 4 points or more on the NIHSS at 48 hours was seen in 65% of our patients while a Barthel Index of 75 or more at 1 month was present in 79% patients. It is important to note here that the long term benefit as observed from the Barthel Index is significantly more impressive than the immediate benefit as reflected from the 48 hour NIHSS. The reluctance of many neurologists to treat patients is based on this premise of insufficient immediate improvement in outcome. However, it is equally important not to lose sight of the delayed benefits, especially considering the tremendous impact that stroke morbidity has on the individual, caregivers, and society as a whole.

This preliminary data from a tertiary care center from a developing country shows that hyperacute thrombolysis in acute ischemic stroke is both feasible and useful. It also emphasizes the importance of increasing awareness about stroke and its acute management. More significantly, till such time that patients start presenting more promptly for treatment, it needs to be understood that thrombolysis can still be provided in hospitals if a dedicated stroke team endeavors to reduce the "door-to-needle" time to the minimum possible.

The limitation of the present study is the lack of a control group which will impede with the assessment of efficacy of thrombolysis. However, since the study is strictly observational, it may be taken as a pilot project to evaluate essentially the feasibility and safety.

Intra - arterial thrombolysis in AIS

Intra – arterial (IA) thrombolysis provides an alternative to intravenous thrombolysis in selected patients with AIS. Recent advances in the field of neuro interventional radiology, with the development of extremely soft, complaint microcatheters and steerable microguide wires, along with high resolution fluoroscopy and digital imaging, and nonionic contrast agents, have made it feasible and safe to access the major intracranial blood vessels around the Circle of Willis from a percutaneous transfemoral approach under local anaesthesia. IA thrombolysis has been used most successfully in patients with acute middle cerebral artery (MCA) occlusion. There is evidence that the treatment window for IA thrombolysis extends to at least 6 h from stroke onset in patients with MCA occlusion. Other potential candidates for IA thrombolysis include patients with extracranial carotid artery (ICA) occlusion, intracranial carotid artery "T" occlusion or basilar artery occlusion.

Two randomized trials (1, 2) comparing intraarterial recombinant pro – urokinase (r – proUK) plus intravenous heparin vs intravenous heparin have been conducted in patients with occlusion of the MCA (M_1 & M_2) of < 6 h duration. The prolyse in Acute Cerebral Thromboembolism (PROACT) I trial treated 40 patients with MCA occlusion, with either IA r – pro UK (n = 26) or placebo (n = 14). All patients received intravenous heparin. The protocol initially specified a heparin dose of a 100 IU/ kg bolus and 1,000 U/h for 4 h. After 16 patients were randomized, the heparin dose was reduced to a 2,000IU bolus and 500 IU/ h 4 – h infusion on recommendations of the safety committee. The study drug

was started on an average, 5.5 h after symptom onset. Recanalization rates were significantally higher with r – proUK (58%) than with placebo (14%; 2 – sided p = 0.017). There was nonsignificant difference in the rate of early symptomatic hemorrhagic transformation, which occurred in 15.4 % of the r – pro UK patients and 7.1 % of the placebo – treated patients (2p = 0.64).

Ninety – day mortality rates (4% in pro UK group vs 7 % in the control group) and good clinical outcomes (30.4 % vs 21.4 %) favored treatment with r – pro UK but did not reach statistical significance. Recanalization rates and the risk of brain hemorrhage were influenced by the dose of heparin.

PROACT – II study (2) was designed to further test the efficacy and safety of IA r – pro UK in patients with MCA occlusion of < 6 h duration. More than 12,000 patients were evaluated for inclusion in the trial and 474 patients underwent a screening conventional cerebral angiogram. A total of 180 patients had angiogram – confirmed MCA occlusions and were randomized to receive 9 mg of IA r – proUK plus heparin (n = 121) or heparin alone (n = 59). The heparin dose was the same in both groups (2,000 – U bolus and a 500 U/h infusion of heparin for 4h). A clinically and statistically significant benefit favored r – proUK in the primary outcome analysis, with 40% of treated patients recovering to a mRS of ≤ 2 compared with 25 % of control patients (absolute risk reduction, 15 %; p = 0.043; relative risk reduction, 60 %). Mortality was 25 % in the r – pro UK group and 27 % in controls. Symptomatic ICH occurred in 10 % of r – proUK group and 2 % in controls (p = 0.063). The recanalization rate (Thrombolysis in Myocardial Infarction 2 or 3) was 66% for r – proUK vs 18 % for controls (p< 0.001). Patients recruited to PROACT II had moderate - to – severe strokes with a median baseline NIHSS of 17. The median time to start of intra – arterial treatment was 5.3h. Benefits of r – proUK were greatest in patients with baseline NIHSS scores of 11 to 20.

R – proUK is not available for routine clinical use. Reports of IA thrombolysis using tPA or urokinase in selected patients have generally showed favorable results, or better than anticipated clinical outcomes, despite increases in the rate of symptomatic ICH (3 -14).

Treatment had been given beyond 3 h in most cases, and patients often had severe stroke syndromes due to large – vessel occlusions. Symptomatic

ICH was more frequent with IA thrombolysis (9.5 % vs 3 %, p = 0.046).

IA thrombolysis in Vertebro basilar Territory

The natural history of basilar artery occlusion is extremely poor with mortality ranging from 83 % to 91 % (15). Because of this poor natural history, IA thrombolysis has been preferred in patients with acute basilar artery occlusion. Basilar artery occlusions are usually owing to atherothrombosis. There is a high incidence of residual stenosis after basilar artery recanalization, which often requires adjuvant therapies including angioplasty, antithrombotic and antiplatelet agents and stenting. Good outcomes are strongly associated with recanalization after thrombolytic therapy. Hacke *et al* (16) described 65 consecutive patients with vertebro – basilar occlusions treated either with local IA urokinase (UK) or IA SK plus heparin (n = 43) or conventional antiplatelet/ anticoagulation therapy (n = 22). The recanalization rate among thrombolysis cases was 44% (19 of 43). All patients without recanalization died whereas 14 of 19 with recanalization survived, 10 with favorable outcome. The mortality rate with conventional therapy was 86 % compared to 67 % with thrombolysis. The rate of brain hemorrhage with clinical deterioration was 7 % in thrombolysis patients. Schumacher *et al* (17) reported 20 patients with vertebrobasilar occlusion of < 6 h duration treated with upto 1,500, 000 IU local UK plus IV heparin. Recanalization was achieved in 66 %. There was no or minimal deficit in 45 %. The mortality rate was 45 %.

Recanalization rates depend upon the location of the vertebrobasilar occlusion. Distal basilar occlusions have higher recanalization rates than proximal occlusions. The timing of IA vertebrobasilar thrombolysis is often a difficult decision. The presence of coma or tetraparesis for several hours portends a poor prognosis, despite recanalization (16, 18, 19). Such symptoms do not preclude survival, and recovery has been documented after successful recanalization in such patients (18).

The time window for thrombolysis may be longer in the vertebrobasilar circulation. Many series have included upto 24 h (16), 48 h (18) and even 72 h (16) after symptom onset in patients with stuttering courses. Some studies suggested good outcomes in patients receiving delayed treatment (20). A longer time window may be because of a higher ischemic tolerance or improved collateralization in the posterior

circulation. Cross and colleagues (21) found that better collateral blood flow was correlated with improved responses to thrombolysis and with longer tolerance of ischemia. Patients with proximal basilar artery thrombosis did not seem to have the same benefit. Patients with vertebrobasilar ischemia often have chronic atherosclerotic disease which allows collaterals to develop over time.

There may be two distinct populations of patients with vertebro basilar occlusion as hypothesized by Cross *et al.* (21). Patients with a progressive stuttering course may have better collateral circulation and better outcomes despite later treatment than patients with the sudden onset of severe deficits owing to poor collaterals even though they may be brought to treatment earlier.

Table 2
Summary of the major thrombolytic trials
Randomized Trials of Hyperacute Thrombolysis in AIS

Name of study	Onset to treatment(n)	No. of patients	Therapy	Route	Dose
NINDS rtPA Stroke trial	≤ 3	624	rtPA	IV	0.9 m/kg over 1h
ECASS I	≤ 6	620	rtPA	IV	1.1 mg/kg over 1h
ECASS II	≤ 6	800	rtPA	IV	0.9 mg/kg over 1h
ATLANTIS A & B	≤ 5h ≤ 6h	613 142	rtPA	IV	0.9 mg/kg over 1h
PROACT II	≤ 6 h	180	Pro – UK + heparin	IA	9 mg over 2h
ASK	≤ 4 h	340	SK	IV	1.5 Million units over 1h
MAST I	≤ 6 h	622	SK	IV	1.5 Million units over 1h
MAST E	≤ 6 h	310	SK	IV	1.5 Million units over 1h

A major issue regarding access to IA thrombolysis to treat acute ischemic stroke is that it requires the ready availability of a neuro interventionalist and a tertiary stroke team. Such expertise is not currently available in most community hospitals. Another limitation of IA thrombolysis is the additional time required to begin treatment compared to intravenous thrombolysis. There are also concerns regarding the invasiveness of the technique and procedural risks not inherent to IV thrombolysis. However, serious procedural complications were uncommon in PROACT I & II trials.

Meta – Analysis

Two meta-analysis included data of several thrombolytic trials. Hacke *et al* (22) evaluated the data of the NINDS trial and both ECASS trials including 2044 patients (1034 – rtPA, 1010 – placebo). The effects of rtPA treatment on reducing disability and death (mRS score = 2 vs = 3), on the occurrence of symptomatic ICH and on mortality were evaluated. The analyses were stratified for both time windows: 0 to 3 and 0 to 6 hours. For the 3 hour time window, there was a 45% reduction of unfavorable outcome (OR, 0.55; 95% CI, 0.41 to 0.72); risk for ICH was significantly increased among rtPA – treated patients (OR, 2.68; 95% CI, 1.56 to 4.62); mortality associated with ICH was not increased (OR, 0.91; 95% CI, 0.63 to 1.32). For the 6 hour time window, there was a 3% reduction of disability and death (mRS score = 3) (OR, 0.63; 95% CI, 0.53 to 0.76); risk of ICH was significantly more in rtPA treated patients (OR, 3.33; 95% CI, 2.39 to 4.37), but mortality was not different between rtPA treated patients and controls (OR, 1.07; 95% CI, 0.84 to 1.36).

In the Cochrane Library, all randomized, placebo – controlled trials of any thrombolytic agent in patients with ischemic stroke were included (23) (NINDS trial, ECASS I and II, ATLANTIS A and B, PROACT I and II, ASK trial, MAST – E, MAST – I and several smaller trials). There were 5216 patients in 17 trials, 2889 of them from rtPA trials. Thrombolytic therapy significantly increased the odds of death within the first 10 days (OR, 1.85; 95% CI 1.48 to 2.32). The main cause of the increase in deaths was fatal ICH after thrombolysis (OR, 4.15; 95% CI, 2.96 to 5.84). Despite this, thrombolytic therapy, administered up to 6 hours after onset, significantly reduced the proportion of patients who were dead or dependent (mRS score = 3) (OR, 0.83; 95% CI, 0.73 to 0.94). For patients treated within 3 hours of stroke,

thrombolytic therapy appeared to be more effective (OR, 0.58; 95% CI, 0.46 to 0.74). Trials testing intravenous rtPA suggest that it may be associated with a lower mortality when given up to 6 hours after onset (OR, 1.24; 95% CI, 0.85 to 1.81). A meta-analysis with only the data of trials using intravenous rtPA for thrombolytic therapy in the 3 to 6 hour time window has not yet been published. An analysis of the appropriate data for this time window concerning dependence or death (mRS = 3) from ATLANTIS, ECASS and ECASS II, leading to an OR of 0.79 (95% CI, 0.66 to 0.96) in favor of rtPA is given in Table 4 (24).

Table 3

Meta analysis of data concerning a 3 – 6 hour time window from ATLANTIS, and ECASS

Study	rt PA	Placebo	Fisher's Exact *P*	OR	95 % CI
ATLANTIS	120/272	126/275	0.73	0.93	0.67-1.30
ECASS-I	170/226	192/279	0.24	0.80	0.56-1.15
ECASS-II	140/328	163/314	0.02	0.69	0.51-0.94
Sub Total	430/866	481/868	0.02	0.79	0.66-0.96

Since 1995, the number of patients in randomized controlled trials of thrombolysis for acute ischemic stroke has more than doubled, bringing the total of 5210 patients in 17 trials including 2955 in 8 trials of rtPA. Cumulative analysis confirms that there are net benefits despite the definite hazards. For every 1000 patients treated with rtPA upto 6 hours, ~ 55 more patients will be independent at the end of follow-up including the cost of ~ 20 extra deaths. The number – needed – to – treat (NNT) to prevent one death and disability with in 3 hour time window with rtPA is 7 or 8. Similar NNT with in 6 hour time window increases to 17 and for the 3 to 6 hour time window, it is 25. However, the statistical heterogeneity and wide CIs indicate that these estimates of rtPA effects are unreliable. The true effect of rtPA might be considerably better or considerably worse than suggested here. ECASS – III was designed to confirm the superiority of rtPA over placebo for patients with ischemic stroke when administered within a time window from 3 to 4 hours from the onset of symptoms. The study is being

performed in 80 sites in 15 European countries. IST–3 study reassesses the role of rt – PA in patients with symptoms lasting less than 6 hours.

Recommendations (25)

The current recommendations are based on the hierarchy of evidence drawn from meta and pooled analysis of randomized controlled trials, individual clinical trial data, formal phase IV studies, and reports from routine clinical practice, and incorporate the accepted importance of early treatment. Based on currently available data, and the principle of early therapy, it is appropriate to provide recommendations based on the time to treatment (0 to 3 hours, 3 to 6 hours, or 0 to 6 hours), the specific thrombolytic agent (tPA, SK, r- proUK) and the route of administration (IV, IA).

I. For eligible patients, of IV rtPA in a dose of 0.9 mg/Kg (maximum of 9 mg), with 10% of the total dose administered as an initial bolus and the remainder infused over 60 minutes, is recommended, provided that treatment is initiated within 3 hours of clearly defined symptom onset (Grade IA).

II. For unselected patients with AIS > 3 hours but < 6 hours, clinicians are recommended not to use IV rtPA (Grade 2 A).

III. For patients with AIS, clinicians are recommended against the use of SK (Grade IA).

IV. For patients with angiographically demonstrated MCA occlusion and no signs of major early infarction on the baseline CT scan, who can be treated within 6 hours of symptom onset, intra-arterial thrombolytic therapy with rtPA is recommended. (Grade 2 C).

V. For patients with acute basilar artery thrombosis and without major CT/MRI evidence of infarction, intra-arterial thrombolysis with rtPA is recommended (Grade 2 C).

Concept of Ischemic Penumbra and Expansion of Therapeutic Time-Window

After vascular occlusions, there is a heterogenous depression of cerebral blood flow (CBF) in the territory of the occluded artery. The penumbra is identified as the brain region receiving regional CBF (rCBF) between two critical values (26, 27). The first, higher, critical value is associated with neuronal paralysis: brain areas receiving rCBF less than 18 – 20 mL/ 100g/ min do not function. The second, lower, critical value is associated with cell death; brain areas receiving less than 8 – 10 in L/ 100g/ min do not

survive, and this area becomes the core of the infarction (26). Neurons in the penumbra are sometimes identified as "idling" to suggest that they are salvageable, although the mechanism of such a phenomenon is unknown. The time course of cell death in the core is rapid where as cells in the penumbra may survive up to several hours (28).

Baron obtained elegant data using positron emission tomography (PET) scanning to explore the penumbra concept. They showed that overtime after stroke an area of excessive oxygen extraction, perhaps reflecting the core, does enlarge (29). However, the temporal evolution of the penumbra can be highly variable. In some patients, a penumbra could be identified upto 48 hours (30) and in others there was no penumbra by 5 hours. Similar sorts of experiments using magnetic resonance imaging (MRI) of water diffusion (DW), a possible marker of the penumbra have also demonstrated the classic DWI – PWI mismatch, representing penumbra. The exact duration of survival is unknown, and likely different under differing circumstances. Cells in zones receiving 10 to 20 cells/ 100g/min are likely to survive for hours, but the number of hours is not known. Many factors may reduce the time such marginally perfused cells can survive. For example, it is known that hyperthermia of 1 or 2^0 C will accelerate cell death (31). Elevations of serum glucose also accelerate cell death (32). Pending recanalization and restoration of blood flow, a number of therapies could be directed at idling neurons in the penumbra. Such neuroprotectants might salvage brain by preserving neurons until blood flow is restored.

The therapeutic window for rescuing ischemic but still viable brain tissue is amenable for many patients. Neuronal death and brain infarction evolves progressively in a time – dependent fashion, but is determined by the duration and severity of the ischemic insult, the underlying cause of ischemia as well as the rate of recanalization. Therapeutic strategies designed to restore cerebral perfusion in a timely fashion, have the potential to limit, the cellular, biochemical and metabolic consequences of cerebral ischemia that ultimately lead to irreversible brain injury. It may be that the survival of the bulk of ischemic tissue is dependent upon early energy failure and ionic imbalances, whereas smaller volumes are dependent upon neurotoxic and inflammatory processes and even less on apoptosis. The concept of 'time is brain" is generally a good one, regardless of what intervention is planned.

Stroke MRI (33 - 36)

A substantial amount of information now is available from PET and MRI studies that potentially salvageable ischemic tissue exists in some patients for many hours after stroke onset. PET availability is limited and the technique is time consuming and hence likely to remain an imaging tool restricted to research centers. Diffusion-perfusion MRI and CT perfusion studies are now widely available at many clinical facilities. The PWI – DWI mismatch on MRI provides a rapidly identifiable imaging marker of potentially salvageable ischemic tissue that can be widely used to identify potentially treatable ischemic stroke patients irrespective of time from onset. Hopefully, using this approach, to identify, patient's therapy will move patient selection to a pathophysiologically based assessment and away from rigid time windows. This should expand the therapeutic time window for both thrombolysis and neuroprotection.

In the future, perfusion and diffusion absolute value maps are likely to be generated, and estimations provided as to what percentage of ischemic tissue is highly likely to be irreversibly injured, what percentage is not at a risk of infarction, and what percentage is at risk for infarction but can be salvaged with appropriate treatment. The availability of such a three–compartment assessment of ischemic tissue will move acute stroke treatment into a new era of "time – independent" decision making.

DWI may delineate infarcted brain tissue within minutes, although there is grouping evidence that in the very early stage of stroke there may be reversible DWI changes in up to 45 % of patients experiencing recanalization after treatment with rtPA (37-40). However, these lesion reversals in most instances are only minor or partial and are quite frequently not permanent. Contradictory data exist about whether a DWI/ apparent diffusion coefficient threshold for irreversible ischemia exists (40 - 42). PWI defines the area of cerebral hypoperfusion. The absolute volume difference or ratio of PWI and DWI reveals the ischemic tissue at risk of irreversible infarction (43, 44). The presence of a vessel occlusion on MRA is associated with a PWI/ DWI mismatch. Therefore, stroke MRI defines the ideal candidate for thrombolysis (45-48). In addition, early recanalization achieved by thrombolysis results in significantly smaller infarcts and a significantly better clinical outcome (49, 80). A

prospective, open–label, non randomized multicenter trial examined the MRI baseline characteristics of patients with acute ischemic stroke and studied the influence of intravenous rtPA on MR parameters and functional outcome in 139 patients (76 rtPA versus 63 controls) within 6 hours after symptom onset and on follow–up(47). Patients treated with rtPA were significantly more severely affected at baseline but still had a non significant trend toward smaller infarctions and a significantly better clinical outcome (mRS ≤ 2) regardless of the time of rtPA treatment (≤ 3 hours or 3 to 6 hours). Therefore, stroke MRI–guided rtPA therapy appears to be safe and effective beyond a 3 hour time window. Furthermore, the rather strictly defined therapeutic window may be qualified and individualized according to the findings in each patient. An interesting observation is the persistence of a PWI/ DWI mismatch even > 24 hours after stroke onset (46, 50). This implies that a late recanalization may be beneficial for a patient with tissue at risk and a persisting vessel occlusion, as shown in individual patients (51). Additionally, a protracted metabolic recovery after thrombolysis may still be associated with a good clinical result (52). Stroke MRI may be useful in identifying patients with extensive infarctions with or without a PWI/ DWI mismatch, which, if present, may be salvaged by more aggressive therapies (53-55).

CT/ CT Angiography [CTA] – source Images [CTA – SI] (56 - 58)

A new instrument for the improvement of CT rating is ASPECTS (Alberta Stroke Program Early CT Score). ASPECTS studies divide the MCA territory into 10 regions of interest as seen on 2 standardized axial CT slices (basal ganglia and lateral ventricles). The whole MCA territory is allotted 10 points (1 for each area), and a single point is subtracted for each of the defined regions if ischemic lesions are seen. The baseline ASPECTs value correlated inversely with the severity of stroke on the NIHSS Cr = 0.056, P < 0.001) and predicted functional outcome and symptomatic ICH (P < 0.001; P = 0.012). A sharp increase in dependence and death occurs with an ASPECTs value ≤ 7. A comparison of CT/CTA/CTA–SI findings with MRI (DWI) and MRA reveals equal accuracy of CTA and MRA close to equal accuracy of infarct volumes according to CTA- SI compared with DWI, and a predictive value of poor collaterals for infarct growth. Therefore, CT/ CTA/ CTA–S1 may render information similar to that of PWI/ DWI mismatch concept.

Combined IV & IA thrombolysis (59, 60)

Combined IV and IA delivery of thrombolytic drug has several potential advantages. First, a combined approach looks to start IV thrombolytic therapy as quickly as possible in order to minimize the time from stroke onset to recanalization. The addition of IA therapy following IV rt PA has the advantage of demonstrating whether or not a clot is still present and whether more thrombolytic drug or other methods to recanalized the occluded artery are needed.

The Emergency Management of Stroke (EMS) or the bridging Study, was designed to test the feasibility and provide preliminary data regarding the relative benefits and risks of combined in rtPA (0.6 mg/kg over 30 min) and IA rtPA therapy (administered only if a clot was demonstrated by angiography at a dose of up to 20 mg at the site of the clot over 2 hours), as compared to IV placebo and IA rtPA therapy. Intravenous treatment in the EMS study had to be started within 3 hours of onset. The inclusion/exclusion criteria for the EMS pilot trial were identical to that of the NINDS rtPA randomized trial except that patients had to have an NIHSS of 5 or more. The total number of patients in the EMS study was small (total n = 35, combined IV/IA rtPA group = 17 and IV placebo and IA rtPA =18). The pilot study was not powered to examine differences in efficacy between the two treatment groups. Despite the imbalances in the baseline NIHSS scores between the two treatment groups, recanalization in combined IV/ IA rtPA treatment groups was more complete than recanalization in the IA alone group, although the difference was not significant. For these patients who had a clot at angiogram, recanalization was significantly greater in the combined IV/ IA group than in the IA alone group. The proportion of improved outcomes (prospectively defined as a seven point improvement of the NIHSS at 7 – 10 d) was not significantly greater in combined IV/ IA treatment group as compared to IA alone group (both groups = 24 %). There were no symptomatic or asymptomatic intracerebral hematomas in the 35 EMS patients. The data illustrated that a combined approach can help to minimise the time to treatment and recanalization, can be used to help titrate the amount of thrombolytic drug and is feasible in clinical practice. Interventional Management of Stroke (IMS) Study was similar in design to EMS Bridging Trial. IMS – II is currently underway and is investigating the risks and benefits of

combined IV/ IA rtPA plus low – energy ultrasound in ischemic stroke patients with an NIHSS ≥ 10, in whom treatment is started with in 3 hours of onset.

Combination of Thrombolytic Therapy with Neuroprotection (61-73)

Till date only two studies designed to evaluate the effect of combining thrombolysis and neuroprotection have been carried out, and one was terminated prematurely. In patients given intravenous rtPA within 3 hours of stroke onset according to NINDS protocol, lubeluzole had to be started 1 hour of starting rtPA. Although the number treated (80 – 90) was too small (due to premature termination) to draw definite conclusions, there was no evidence of benefit. However, the study demonstrated that such a trial design is feasible. A small study was designed to test the combination of rtPA given within 3 hours and clomethiazole given within 8 hours. The study included 97 patients given clomethiazole and 93 patients given placebo, all patients received rt – PA first according to NINDS protocol. Overall, there was no benefit seen. In the patients with the worst strokes however, there was a possible effect. These results suggest that clomethiazole may augment the beneficial effect of rtPA in patient with large strokes. Although still unproven as a means to improve on results obtained with thrombolytic therapy alone, combination therapy of thrombolysis with neuroprotective drugs has a sound theoretical and experimental basis. Although one possible benefit of such an approach may be to extend the therapeutic time window, any such combination should be started as early as possible after the onset of stroke symptoms, and it is likely that the neuroprotective agent will be most effective if it is administered before or along with lytic therapy.

Newer Thrombolytic Agents

One of the two major problems in crossing 3 – hour window is the propensity to intracerebral hemorrhage. Several second and third generation thrombolytic agents (tenecteplase, reteplase, desmoteplase, plasmin, microplasmin) are being tested. The goal is to create new thrombolytic agents with longer half–lives that permit bolus administration, with improved rates of restoration of flow and with reduced rates of ICH.

Desmoteplase: The recently completed phase–II-B (DIAS) Desmoteplase in Acute Stroke) study of a rtPA molecule derived from bat saliva

used diffusion/ perfusion MRI to enroll patients and to assess the biological activity of different doses. Patients were included in the study between 3 to 9 hours after stroke onset, if they demonstrated a perfusion lesion volume ≥ 20 % above the baseline diffusion lesion volume. MRA was also performed and all 3 MRI modalities were repeated several hours after completion of the thrombolytic therapy to evaluate reperfusion efficacy. Lower, weight–adjusted dosing demonstrated a very reasonable safety profile and quite dramatic reperfusion efficacy. Clinical outcome tended to be more favorable in treated patients as well. This pilot study is important because it confirms the safety of weight – adjusted desmoteplase in ischemic stroke patients when given upto 9 hours after, stroke onset as well as the intended biological effect of inducing reperfusion.

Tenectplase: TNK – tPA is a more fibrin specific variant of tPA. It also has a longer half – life and increased resistance to plasminogen activator inhibitor type I that allows for dosing as a single bolus. Pilot studies are underway.

Combination of Glycoprotein (GP) IIb/ IIIa Inhibitors with Thrombolysis: GP II b/ III a inhibitors block the final common pathway of platelet aggregation, and have demonstrated efficacy in preventing thrombus formation in acute coronary syndromes and percutaneous coronary interventions. In acute ischemic stroke, a dose – escalation study demonstrated that the GP II b/ III a inhibitor aboiximab is safe when given as a 0.25 mg/kg bolus and 0.125 mg/kg/ minute infusion. Abciximab in Emergent Stroke Treatment Trial (ABESTT) study suggests possible efficacy of abciximab in acute, stroke, with a longer therapeutic window and a better safety profile than rtPA within 3 hours. A phase 2 trial is underway in acute ischemic stroke of less than 6 hours duration. Some centers are using Gp IIb/ IIIa inhibitors as adjunctive therapy with intra – arterial thrombolysis in AIS.

Mechanical Clot Lysis: Mechanical approaches to ischemic stroke are attractive because they may obviate the need for thrombolytic drugs that increase the risk of reperfusion hemorrhage. The angiojet catheter has been used to fragment and suck in the clot. It uses a high-velocity stream of saline directed back into the catheter to create a localized, low-pressure zone at the distal tip. Via the Bernoulli effect, thrombus is trapped, broken up into small particles, and evacuated, literally within minutes. The system is currently

approved for use in saphenous vein graft thrombectomy but has been modified for neurovascular use. A problem has been distal embolism of particles. Other means of delivering energy to fragment the clot are being developed, including the use of ultrasound and laser devices. The current limitation of these mechanical devices is their relatively larger catheter size & less flexibility, which limit access to the tortuous vessels of the intracranial circulation. The Mechanical Embolus Removal in Cerebral Ischemia (MERCI) Trial is underway to evaluate the safety of the MERCI Retriever System. There are a few other smaller studies such as EKOS, TRUMBI and CLOT BUST that evaluate if there are additional benefits when thrombolytics are used in combination with ultrasounds exposure. Theoretically, this approach would increase the accessibility of the drug to the clot.

The search for Neuroprotective strategies in stroke (61-85)

Neuroprotective strategies can be divided into those that limit ischemic cascade or those that reduce reperfusion injury. Neurons die under ischemic conditions when delivery of oxygen and an energy source are not sufficient to meet their metabolic demands. Therefore, ischemic neurons must either receive increased blood flow or their metabolic demands need to decrease. The most important step in limiting ischemia is the quick restoration of blood flow that occurs either naturally or with the aid of thrombolytics such as rt-PA. Before complete restoration of blood flow, therapies such as hypothermia may decrease metabolic demand and extend neuron survival. Once depleted of energy, membrane-bound ion pumps fail, leading to membrane depolarization, sodium and calcium entry, and neurotransmitter release. Dendritic glutamate release activates down stream neurons, leading to calcium entry, cellular damage, apoptosis, and further release of toxic molecules. Agents that modulate ion channels and glutamate receptors target this pathway. Reperfusion injury was observed by comparing infarct volumes in animal models of transient and permanent ischemia. Reperfusion, while critical to limit ischemia, contributes to cell death by enhancing production of free radicals, inflammation and blood-brain barrier breakdown; each has been targeted by therapies. However, all the human trials on neuroprotection have been negative thus far.

On the basis of complexity of events in cerebral ischemia and the disappointing results from single-agent trials, it may not be realistic to expect that a single neuroprotective drug will have lasting benefit. Rather, effective neuroprotection may require "rational" polytherapy that combines drugs with different mechanisms of action, perhaps administerd at different poststroke intervals, to maximize efficacy and/or extend the window for reperfusion, minimize reperfusion injury or hemorrhage, or inhibit delayed cell death. Furthermore, because the failure if several neuroprotective trials has been attributed to dose-limiting toxicity, combination therapy may permit lower doses of each agent and minimize adverse effects. Combinations with nonpharmacological neuroprotective strategies such as hypothermia, insulin, and blood pressure control should also be subjected to clinical trials.

Combined thrombolysis-neuroprotective approaches have shown promise in animal studies and are beginning to be investigated in clinical trials. For example, synergistic effects have been demonstrated in animals when thrombolysis is combined with citicholine, an AMPA antagonist, an NMDA antagonist, or other agents. Administration of antileukocytic adhesion antibodies has been shown to extend the therapeutic window for thrombolysis. Recently, 2 trials have demonstrated the feasibility and safety of intra-venous t-PA treatment followed by neruoprotectant administration: the CLASS-T trial of clomethiazole and a study of lubeluzole.

In animals, synergy has been demonstrated by the combination of 2 neuroprotective agents with different actions; some examples include the NMDA antagonist MK-801 in combination with a GABA agonist, a free radical scavenger, a calcium antagonist, citicholine or basic fibroblast growth factor (bFGF). Similarly, synergy has been observed with the combination of the antioxidant tirilazad and magnesium, the combination of 2 different antioxidants and citicholine combined with bFGF.

Synergistic effects on infarct size and therapeutic window have also been found in animals through the use of antiexcitotoxic agents in combination with antiapoptotic agents, eg., the NMDA antagonist dextrorphan plus the protein synthesis inhibitor cyclohexamide, or MK-801 with the caspase inhibitor ZVAD –fmk. Caspase inhibitors given with bFGF extended the therapeutic window and lowered the required doses for neuroprotection.

Hypothermia (86-89)

It has been well documented that mild (34°C) to moderate (32°C) systemic hypothermia protects brain from some ischemic damage in various animal models. Although the exact mechanisms are unknown, a reduction of body temperature, especially brain temperature, may lead to reduced cerebral oxygen consumption, decreased intracellular lysosomal enzyme activity, suppressed free radical formation, protection of the fluidity of the cell membranes, reduced intracellular acidosis, and inhibition of cell damage mediated by excitatory neurotransmitters.

Several clinical studies suggested that mild hypothermia improved neurologic outcome in patients after cardiac arrest. A case-control multicenter trial demonstrated that cardiac arrest patients who were treated with mild externally applied hypothermia (33°C) had significantly better neurological outcome and less mortality than those who were maintained normothermic. Acute hyperthermia, on the other hand, has been associated with a higher mortality rate 1 year post-cardiac-arrest, as compared to normothermic patients.

Recently, Krieger *et al*(88) published a pilot study, investigating moderate hypothermia therapy in ischemic stroke. Nineteen patients with major acute ischemic stroke (NIHSS>15) were randomly assigned to either hypothermic (32°C) or control (maintained normothermic) groups. Patients in the hypothermic group were subjected to surface cooling with a cold blanket. The therapeutic window for hypothermic treatment was 6 h after stroke onset, and thrombolytic therapy was given whenever patients were eligible. Although complications such as bradycardia, ventricular ectopy, hypotension, melena, infections, and rebound hyperthermia were observed, the treatment was considered safe.

There are two main obstacles to surface cooling hypothermia: slow induction and intense patient shivering. To overcome these problems, endovascular cooling was introduced. In general, a catheter with a closed-loop circulation system embedded was attached to a temperature controlling system. The catheter was then inserted in the inferior vena cava to cool circulating blood and regulate body temperature. Georgiadis *et al*(89) reported 6 patients who suffered severe acute ischemic stroke and were treated with endovascular hypothermia. During the hypothermia, the minimal temperature achieved was 32.2°C. According to their study, the procedures

were safe, and the adverse effects were similar to those encountered in surface cooling. To further evaluate its safety and efficacy, several clinical trials are currently being conducted.

Combination of Hypothermia with thrombolysis:

COOL AID trial (cooling for Acute Ischemic Brain Damage) is a phase 2 trial in patients with acute ischemic stroke of less than 12 hours duration. After thrombolysis, a heat exchange catheter is introduced into the inferior vena cava, without need for paralysis or sedation that may account for much of the medical comorbidities seen with other methods of hypothermia. The body is cooled to 32° C.

Besides the advances in understanding stroke pathophysiology and development of newer means to reperfuse and apply a comprehensive multipronged approach in salvaging the jeopardized and critically perfused brain tissue, it is increasingly being recognized that provision of specialist stroke services and stroke units is associated with reduction in mortality, dependency and subsequent need for institutional care. Although it is not clear precisely which aspects of care are associated with these improvements, there is accumulating evidence that the process of care delivered by specialist stroke teams is significantly different from that of conventional care. The importance of physiological monitoring following stroke may be reflected in the fact that up to 40% of acute stroke patients suffer neurological deterioration shortly after admission to hospital. This neurological deterioration may be consequent upon natural progression of the initial stroke, but in part may also be due to changes in physiological variables such as glucose, oxygen saturation, temperature and blood pressure. Specialist stroke care, while based upon very simple principles, appears to be highly effective; comprehensive multidisciplinary assessment including the early assessment and management of swallowing disorders, early maintenance of hydration and nutrition and early mobilization underpin the principles of acute care.

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