



Molecular Screening of Hepatitis C virus in Anti HCV Negative Blood Donors

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Sequence

- Introduction
- **Materials and Methods**
- Results
- Discussion
- **Conclusion**
- **Acknowledgements**

Real Challenge

- The discovery of presence of transmittable virus
 - This challenge appears never ending
 - Initial Survivor succumbs to death due to disease transmission
 - Pakistan HBV, and HCV screening is mandatory
 - HBV is now preventable through vaccination
 - In case of HCV effective vaccine is not available.
 - An anti HCV negative blood may be a potential source
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- In future, blood transfusion services will face HCV as main threat if sensitive methods to screen blood and methods to eliminate the immunological window period are not adopted in time.

Introduction

- The initial test was enzyme immunoassay (EIA) for anti HCV (IgG).
- 3 generations of EIAs has been developed
- Same is true for recombinant immunoblot assay (RIBA).
- Detectable levels of anti-HCV takes many weeks.

- HCV core antigen reduced window period up to 21.5-30 days.

- HCV RNA detection showed promising results.
- Methods able to pick 5-10 IU/ml has been devised
- Detect HCV RNA as early as 1-3 weeks post exposure.

- HCV RNA testing by a sensitive and reliable method is expected to possibly interdict and virtually prevent most if not all transfusion associated HCV.

Blood safety

- Detection of HCV RNA by sensitive method is costly
- It requires sophisticated equipment
- Special environment, and technically experienced staff
- Routine use and availability is difficult
- The cost of blood screened through molecular methods would be beyond the reach of common people in Pakistan, and can prove a limiting factor
- Is it justified to screen blood through molecular methods in our setup?

How safe the safe blood is?

Aim & Objectives

- To screen the anti HCV negative blood donors for HCV RNA.
- To find out the frequency of HCV RNA in anti HCV non reactive blood donor population.
- To define the association of raised hepatic enzymes as markers of HCV infection in our blood donor population.

Materials & Methods

- All blood donors volunteering for blood donation
- Donors interviewed as per specified proforma
 - Age between 18-58 years
 - Weight minimum 50 kg
 - Normal pulse, arterial blood pressure and body temperature
 - No history of cardiac, pulmonary, liver and renal disease.
 - No history of jaundice in past
 - No history of drug addiction
 - Reactive to Anti HCV, HBsAg, HIV
 - Reactive to syphilis serology or, malaria

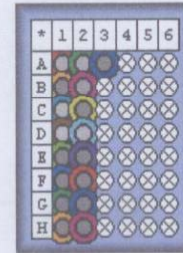
Materials & Methods

- 400 donors with ALT within the reference range
- 400 donors with raised ALT
- Real Time Polymerase Chain Reaction (RT-PCR)
- 5 sample mini pools

Materials & Methods

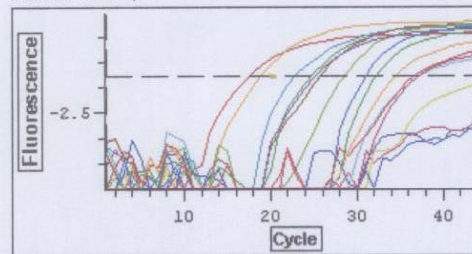
HCV RNA RT-PCR

- Bio-Rad Real time PCR instrument
- MiniOpticon™ System BIO-RAD Thermal Cycler
- 48 X 0.2 ml reaction module
- Amplified product measured as reaction progresses "real time".
- Linear measurement between 6,000 IU/ml to 600 million IU/ml
- Can detect as low as 172 IU/ml HCV RNA.

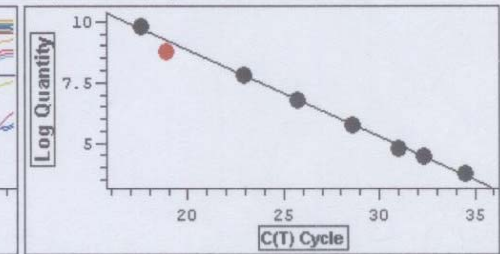


User: Shared
 Data File: C:\x1 Annex A.tad
 Active Dye: (FAM)
 C(T) Threshold: 0.016379
 Threshold has been set manually.

Quantitation Graph



C(T) Control Graph $y = -0.3571x + 15.99; r^2 = 0.999$



Note

Set	Dye	Type	Content	Description	Efficiency	C (t)	IU/ml	Avg C (t)	Max C (t)	Min C (t)	C(t) SD	Avg IU/ml	Max IU/ml	Min IU/ml	IU/ml SD
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Well	Dye	Content	Description	Efficiency	C(t)	IU/ml
A1	FAM	Standard	HCV CONTROL1	1.44	17.55	6e+009
A2	FAM	Sample	1	1.60	24.73	1.439e+007
A3	FAM	Blank	NTC	N/A	N/A	N/A
B1	FAM	Standard	HCV CONTROL2	1.77	18.91	6e+008
B2	FAM	Sample	2	N/A	N/A	N/A
C1	FAM	Standard	HCV CONTROL3	1.55	22.91	6e+007
C2	FAM	Sample	2	N/A	N/A	N/A
D1	FAM	Standard	HCV CONTROL4	1.67	25.73	6e+006
D2	FAM	Sample	4	1.24	37.32	456.5
E1	FAM	Standard	HCV CONTROL5	1.72	28.58	6e+005
E2	FAM	Sample	1	1.49	25.15	1.016e+007
F1	FAM	Standard	HCV CONTROL6	1.73	31.01	6e+004
F2	FAM	Sample	3	1.36	36.69	771.1
G1	FAM	Standard	HCV CONTROL7	1.71	32.29	3e+004
G2	FAM	Sample	3	N/A	N/A	N/A
H1	FAM	Standard	HCV CONTROL8	1.48	34.51	6000
H2	FAM	Sample	4	1.29	37.27	478.1

Materials & Methods

Purification of HCV RNA

- In extraction tube 450 ml lysis solution was added
- 150 ml serum of sample to be analyzed was added
- To the 'lysed' sample 600 ml binding solution was added
- In a spin filter receiver tube lysed mix was filtered
- The spin filter was placed into a 1.5 ml elution tube
- 60 ml RNase-free water was added.
- Mix was incubated at room temperature for 2 min
- Centrifuged at 6,000 x g for 1 minute.
- The elution tube containing purified RNA was placed on ice while waiting for amplification process

Materials & Methods

RNA quantification

- All pipetting steps were performed in rack placed on ice
- 40 μ l PCR grade water was added to lyophilized HCV/IC reagent mix
- Control strip was loaded on reaction base for 0.2 ml tubes.
- Sample RNA strips were placed on reaction base
- Master mix was made using calculated quantity of PCR grade water, dye solution, buffer, Mg sulfate, primers/probe and RT-PCR Enzyme mix.
- 5 ml aliquots of PCR grade water was added to non template control tubes
- 5 ml purified RNA sample was added to sample tubes.
- Tubes were now sealed, centrifuged at 200 x g for 1 minute

Materials & Methods

Annealing and Amplification

- Strips were placed in Real Time PCR thermocycler
- On screen wells were selected
 - Assigned respective number for identification
 - Standards
 - Controls
 - Samples
- Ramping rate was set
- Thermal cycles were setup
- The process of annealing and amplification was completed in 2-3 hours

Data management and analysis

- Statistical Package for Social Sciences (SPSS) version 11.0, and 13
- Descriptive statistics of socio-demographic variables
- Means and standard deviations (SD) for quantitative variables
- Proportions for categorical variables
- Logistic regression analysis to measure association between outcome and each independent variable.
- A Logistic regression model was employed with stepwise backward elimination of non-significant variables, with HCV status as the dependent variable.
- P values < 0.05 were considered as statistically significant.



Results & Discussion

Results

- Voluntary donors 3160
- Regretted on interview 160 (5.06%)
- Reactive to anti HCV 55 (1.83%)
- Reactive to HBsAg 75 (2.5%)
- Detected raised ALT 400/2870 (13.93%)

- All donors were male
- Repeat donors 4.5%
- First time donors 95.4%
- Age 18-54 years mean 27 SD \pm 6.2
- Weight 50 -105 kg mean 68.36 SD \pm 8.7
- Hemoglobin 13.6-17 g/dl mean 14.68 SD \pm 0.54
- Serum bilirubin 7-16 mmol/L mean 11.46 SD \pm 1.72
- ALT 16-96 U/L mean 42.86 SD \pm 14.84

Results

- Normal ALT group
- Serum ALT 15-41 U/L SD \pm 6.4, mean 31.5 U/L
- Serum AST 8 -52 U/L SD \pm 6.5, mean 26.19 U/L
- Serum LDH 211-637 U/L SD \pm 66 , mean 359.5 U/L

- Raised ALT group
- Serum ALT 42-96U/L SD \pm 11.7, mean 54.3 U/L
- Serum AST 15-63 U/L SD \pm 7.11, mean 36 U/L
- Serum LDH 171-760 U/L SD \pm 63.85, mean 360 U/L

- Serum ALT was raised in 36% of HBsAg reactive blood donors
- Serum ALT was raised in 75% anti-HCV reactive blood donors

Results

- HCV RNA was detected in 2 blood donors out of 400 with normal ALT (0.5%)
- In 1st HCV RNA positive donor
- All three liver enzymes were within normal range
- HCV RNA 3.7 million IU/ml were detected in his blood.
- The donor was called after 90 days
- Repeat liver enzymes were normal
- A repeat anti HCV by 3rd generation ELISA non reactive.
- HCV RNA 1.3 million IU/ml of viral RNA were detectable
- In 2nd HCV RNA positive donor
- All three liver enzymes were normal
- HCV RNA 1.1 million IU/ml were detected

Results

- HCV RNA was detected in 1 blood donor out of 400 with Raised ALT (0.25%)
- ALT 68 U/L
- AST 40 U/l
- LDH 334 U/l
- HCV RNA 0.3 million IU/ml
- No risk factor

Discussion

Cost effects

Study	Year	Samples	Minipool	Prevalence	Place
Our study	2008	800	5	0.37%	Pakistan
Seme	2007	6432	24	0.28%	Ljubljana
Bamaga	2007	3288	Nil	1%	Saudi Arabia
Palomäki P	2005	232,600	96	0.04%	Finland
Forčić D	2005	2647	Nil	0.45%	Croatia
Koppelman	2005	2912	8	0.38%	Netherlands
Chiquete E	2005	100	Nil	1%	Mexico

Discussion

Cost effects

Parameter	Cost Rupees	Cost US Dollars
Screening one blood bag for HCV RNA	3,600/00	47/00
Screening mini pool of 5 samples	750/00	9.5/00
Screening mini pools of 10 samples	400/00	5.2/00
Screening mini pools of 50 samples*	75 /00	1.0
Treatment of one missed case	150,000/	1950/00

No of samples in minipool may be decided considering seroprevalence of virus

*Limit calculated / recommended by WHO

Discussion

Cost effects

- The core issue is HCV RNA screening requires:
 - Specially designed laboratory environment
 - Costly equipment
 - Devoted trained staff.
 - Test can not run as stat
 - Minimum size of 8 mini pools / batch to keep it cost effective.
 - Beyond the reach of most blood banking facilities
 - Not possible at town health facilities
- If possibility of HCV RNA screening is excluded at this stage
 - Likely to transfuse unsafe blood
 - Finally to embark on HCV RNA screening.
- Ways and means to centralize the system may be required in future after study of risk over benefit ratio

Shortcomings & Limitations

- The study was based on screening of 800 samples (Statistically estimated sample size 650 samples)
 - Not representative of all donor population of the country.
 - Study belongs to low seroprevalence region of country.
 - Donors with raised ALT were not followed for occult HCV
 - Not evaluated for non viral causes of raised transaminases.
 - HBV as a cause of raised ALT was not brought into consideration.
 - The limitations of study should not undermine the importance of HCV RNA screening in Pakistan. Rather should open more research avenues.
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- A risk of HCV transmission in 1 in 266 transfusions is alarming
 - The risk increases 3 times if blood bags are used to prepare components

Conclusions

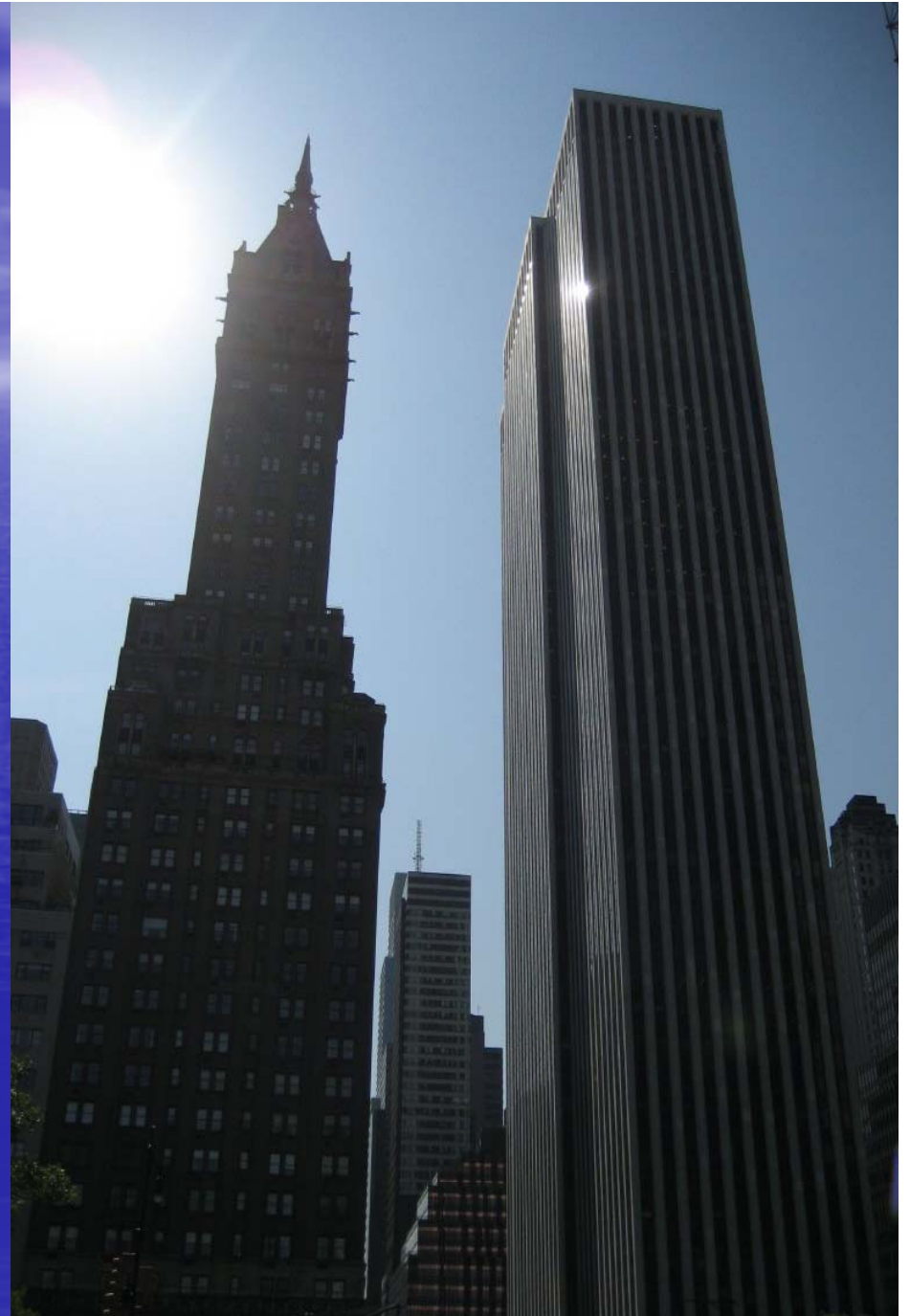
- 1. Prevalence of HCV RNA in blood donors is 0.5% out of 400 normal and 0.25% in raised ALT anti HCV non reactive blood donors.
- 2. Study represents of a cross section of donor population with a relatively small sample size, but raises the question for further studies.
- 3. There is a poor correlation between raised ALT and prior seropositive HCV RNA in donors.

Search for state of
the art facilities to
screen blood

&

Existing simple
devoted affordable
measures

shall go side by side



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