Light Chain Antigenic Determinants (κ and γ) Influence on the Treatment Outcome of Chronic Hepatitis C Infection in Egypt

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Abstract: Background: HCV is an endemic problem in Egypt with genotype 4 affecting about 90% of the infected patients. The treatment with interferon and ribavirin is costly and has various side effects, so there is an urgent need for predictors of interferon response. Of the proved to predict the response is IL-28B genotype, but there are need for other predictors. **Aim of the study:** To detect GM 3/17, GM 23 +/– and KM 1/3 ; which are γ and κ immunoglobulin light chain antigenic determinants; in the patients with hepatitis C virus and to assess their influence on the outcome of chronic hepatitis C treatment. **Results:** There was a significant association between the KM 1/3 allotypes and the treatment outcome. KM homozygousity is associated with six fold increase in response to treatment. **Conclusion:** KM 1/3 genotypes can be used a marker for prediction of chronic HCV treatment outcome.

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1. Introduction:

Hepatitis C virus (HCV) is a common infection and a major cause of chronic hepatitis. Worldwide >170 million people are affected. Of persons acutely infected with HCV, about 20% spontaneously clear the virus. Both viral and host genetic factors play important roles in the clearance of HCV ***(Singh et al., 2007).*** GM and KM allotypes are associated with the susceptibility to and outcome of infection by several infectious agents. GM allotypes are strongly associated with IgG subclass concentrations, making them relevant to viral immunity, as the antibody responses to most viral epitopes appear to be IgG subclass (IgG1 and IgG3) restricted ***(Pandey et al., 2004).***

KM3 could be a marker for enhanced HCV-specific CD8+T-cell activity through significant linkage disequilibrium with a ***cis*-**acting enhancer element in the CD8 gene complex. Increased HCV-specific CD8+ T cell responses are associated with SVR to peginterferon-ribavirin therapy for chronic HCV infection ***(Tatsumi et al., 2010).***

Recent genome wide association studies (GWAS) have revealed that single nucleotide polymorphisms (SNPs) in the promoter region of IL28B (the gene encoding IFN λ3) are strongly associated with response to SOC (Standard of Care) therapy (although the negative predictive value of the current IL28B region SNPs alone is still only ~20-30%) ***(Gelman and Glenn, 2011).*** The SNP rs12979860 is strongly associated with SVR in patients infected with HCV-4, but not with liver disease severity. Analysis of IL28B genotype might be used to guide treatment for these patients ***(Asselah et al., 2011).***

2. Subjects and Methods:

Forty one patients with chronic HCV were enrolled in this study after oral consent; they included two groups. Group I included 20 patients SVR received treatment with combination interferon α2a or α2b and ribavirin for one year. Group II included 21 patients; 17 were non responder for the same treatment after 12 weeks, other 4 relapsed after 6 months of end of treatment.

PCR for determination of the immunoglobulin GM23/23, KM 1/3 and GM 3/17 allotypic polymorphism. This was done in the Immunology Lab in the Clinical pathology department in Ain Shams University.

For the determination of IgG1 allelic markers GM3 and GM17 (arginine-to-lysine substitution, a G→A transition in the CH1 region of the γ1 gene). PCR purification and DNA sequencing for determination of GM 3/17 allotype polymorphism was done using Applied Biosystems technology. This was done using primers 5'-CCCCTGGCACCCTCCTCCAA-3' and 5'-

GCCCTGGACTGGGGCTGCAT-3'. For determination of GM23 (valine-to-methionine substitution, a G→A transition in the CH2 region of the γ2 gene). Nested-PCR–restriction fragment length polymorphism (PCR-RFLP) for Gm 23 (valine-to-methionine substitution, a G→A transition in the CH2 region of the γ2 gene). This was done as described by ***Brusco et al.(1995)***. The primers used were 5'-AAATGTTGTGTCGAGTGCCC-3' and 5'-GGCTTGCCGGCCGTGGCAC-3'. A 197-bp segment was further amplified from this 915-bp fragment, by use of primers 5'-GCACCACCTGTGGCAGGACC-3'and 5'-TTGAACTGCTCCTCCCGTGG-3'. Digestion of the amplified product by the restriction enzyme HSP 92 II resulted in the following products corresponding to the following 3 genotypes: GM23+, 90 bp, 63 bp, and 44 bp; GM23−, 134 bp and 63 bp; and GM23+,23−, 134 bp, 90 bp, 63 bp, and 44 bp. Restriction digestion (PCR-RFLP technique) for KM1 and KM3 was done using the method described by ***Moxley and Gibbs (1992).*** The primers used 5'-ACTGTGGCTGCACCATCTGTCT-3' and 5'-TCAGGCTGGAACTGAGGAGCAG-3'. Digestion of the amplified product (360 bp) by the restriction enzyme *Acc*I resulted in the following products corresponding to the following 3 genotypes: KM1, 360 bp; KM3, 247 bp and 113 bp; and KM1/3, 360 bp, 247 bp, and 113 bp

Statistical analysis:

Analysis of data was done by IBM-compatible computer using SPSS (statistical program for social science version 20) and R statistical package (Version 2.13.2), Spearman correlation and logistic regression tests were done for prediction of binary dependent variable.

3. Results:

Group I included 17 (85 %) males and 3(15 %) females, their age's ranges from 26-60 with mean of (47) years. Group II included 17(81%) males and 4(19%) females, their age's ranges from 23-66 with mean of (43) years.

Table (1) shows that there is a statistically significant difference between the two groups as regard to the KM genotypes. P_1 =0.04* .However there is no statistically significant difference between the two groups as regard to GM23 or GM3/17 in P_1. Also the table shows there is no statistically significant difference between the two groups as regard to the homozygousity and heterozygousity of different genotypes P_2, however the KM genotype homozygousity nearly reaches statistical significance with value P_2 =0.057. There is no statistically significant difference between the two groups as regard to the various KM and GM allotypic carriers and non carriers of different alleles P_3.

Table (2) shows that the homozygotes have 6.75 times (=exp (1.91)) the odds of being SVR as those who are heterozygotes. This was done by using the response as the dependent variable and the zygousity as the explanatory variable. Concluding that the zygousity is independent predictor for treatment response.

Table (3) shows assessment of predictive efficiency, cutoff 0.5, of the KM 1/3 zygousity on the treatment outcome, where the sensitivity of the test is 85.8% and the specificity of the test is 52.9% . Where is the sensitivity; is the percent correctly predicted to be SVR and the specificity is the percent correctly predicted to be with no response.

Figure (1):DNA sequencing results showing homozygous GM3 (a single G peak = a single black color peak is present at position 238).

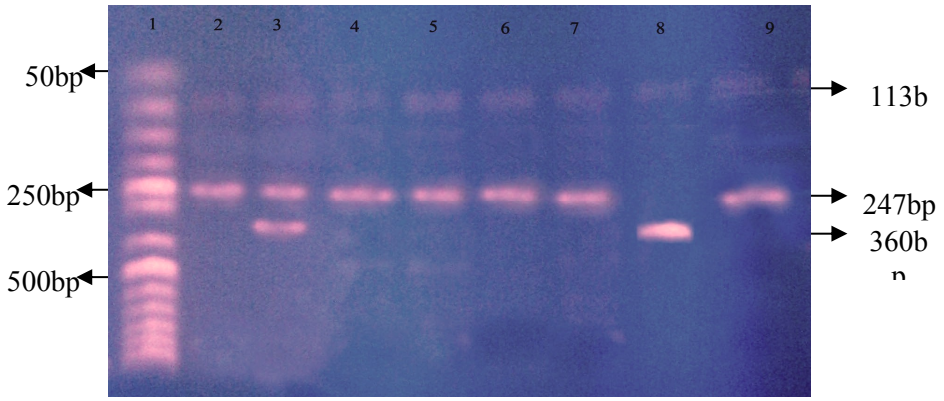
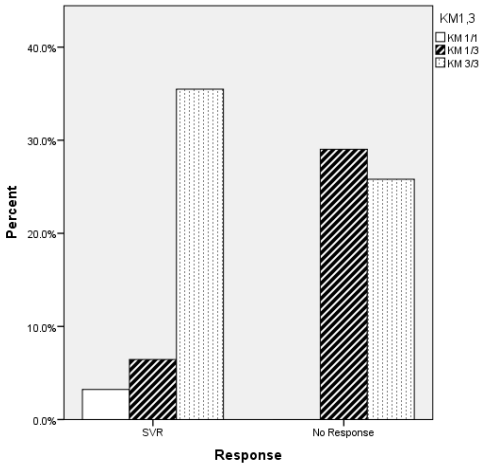


Figure (2): KM 1and KM 3 polymorphism analysis shown by agarose gel electrophoresis:
Lane (1) DNA ladder (50bp). Lane (2) KM 3→ 2 bands (247bp and 113bp).
Lane (3) KM 1,3 → 3 bands (360bp, 247bp and 113bp). Lane (8) PCR product mock (360bp).



Figure(3): (no need) Comparison between the two groups as regards the KM genotypes (KM1,KM1/3, KM3). *p <0.05* (S).*

Table (1): Comparison between the two groups as regard the allotypic carriers and homozygousity:

Genotype	No response	SVR	Global significance P_1	Significance of homozygous P_2	Significance of first carrier P_3
KM 1/1	0 (0%)	1(7%)	<0.05* S	>0.05 NS	>0.05 NS
KM 1/3	9(53%)	2(14%)			
KM 3/3	8(47%)	11(79%)			
GM23 +/+	6(32%)	3(18%)	>0.05 NS	>0.05 NS	>0.05 NS
GM23 +/–	9(47%)	10(59%)			
GM23 –/–	4(21%)	4(23%)			
GM 3/3	8(42%)	10(50%)	>0.05 NS	>0.05 NS	>0.05 NS
GM 3/17	3(16%)	5(25%)			
GM 17/17	8(42%)	5(25%)			

Because of typing failures total do not sum to 21 in case of No Response or 20 in case of SVR.

Table (2): Logistic regression test to for prediction of KM 1/3 zygousity on the response

Variable	Regression coefficient	Significance
Zygousity of KM 1,3		
Heterozygous(0)	Reference (0)	
Homozygous (1)	1.91	<0.05* S
Constant	–1.504	>0.05 NS

Table (3): The outcome assessment of Logistic regression test to for prediction of KM 1/3 zygousity on the response

Response	Prediction percentage correct
No response	52.9%
SVR	85.8%

Table (4): Spearman rank correlation between different genotypes and response to treatment.

Variable	Variable	Rank correlation coefficient	P value	Significance
KM 1 carrier	Response	–0.3	>0.05	NS
KM1,3 zygousity	Response	0.402*	<0.05*	S
GM 3,17 zygousity	Response	–0.1	>0.05	NS
GM3 carrier	Response	0.2	>0.05	NS
GM 23 carrier	Response	0.2	>0.05	NS
GM 23 zygousity	Response	–0.1	>0.05	NS

4. Discussion:

The present study showed that the KM genotypes (global distribution) were associated with the treatment outcome. This was statistically significant were *p* Value <0.05*, on the other hand non of the KM alleles has statistically significant difference as regard the treatment outcome in this study.

The present study showed relative increase in the SVR in KM1 non carriers ; where as the KM3 homozygousity was associated with 58 % SVR, while the KM1 carriers (KM 1/3 and KM 1) showed 25 % SVR ,but the results did not reach a statistical significance. This is in comparison to ***Pandey and Kristner-Griffen (2011),*** where the percentages of SVR in AA KM1 non carriers and carriers are 33.3% and 16.7% respectively, while the percentages of SVR in CA KM1 non carriers and carriers are 51 % and 60.9% respectively, with *p* value 0.024 in AA and 0.378 in CA respectively. This may suggest a relative effect of KM3 homozygousity on the treatment outcome.

At least three mechanisms, which are not mutually exclusive, could explain these observations. The KM3 allele could be directly involved in humoral immunity to HCV. Where another study showed that IgG1 aβab levels were higher in patients with KM3/KM3 genotype compared with patients with KM3/KM1 genotype ***(Ciofu et al., 1997).*** Second, it could indirectly contribute to the cellular immunity to HCV epitopes mediated by $CD8^+$ T cells. Genes encoding CD8 glycoproteins and KM allotypes are very closely linked, both located on the same band (p12) of human chromosome 2. In addition, both CD8 α and β chains share significant homology with the κ light chain. Thus, KM3 could be a marker for enhanced HCV-specific $CD8^+$ T-cell activity through significant linkage disequilibrium with a cis-acting enhancer element in the CD8 gene complex ***(Pandey and Kristner-Griffen, 2011).*** Third, increased HCV-specific $CD8^+$ T cell responses are associated with SVR to peginterferon-ribavirin therapy for chronic HCV infection ***(Tatsumi et al., 2010).***

In the present study there was a statistically significant difference; *p* Value <0.05* , between KM genotype homozygousity and end of treatment response , where KM homozygous patients (patients were mostly homozygous to KM3 where only one patient was homozygous for KM1 genotype) showed increased end of treatment response than the KM heterozygous patients (adding the SVR and relapsers).

On the other hand the homozygousity (mostly KM3 homozygousity) approached significance in SVR versus no treatment response *p* value=0.057, however, there was significant rank correlation between the KM zygousity and treatment outcome with *p*<0.05*, where the homozygotes have 6.75 times

(=exp (1.91)) the odds of being SVR as those who are heterozygotes i.e the KM homozygousity is associated with six fold increase in treatment response. Comparable to this a previous study done where, noncarriage of KM1 allele, i.e., KM3 homozygosity, was associated with higher SVR in African Americans (odds ratio =2.50, 95% confidence interval=1.12–5.60). Thus, the KM3 allele may be a marker for higher SVR in African Americans ***(Pandey and Kristner-Griffen, 2011)***. This have a relation to a study done by ***Scherzer et al.(2011),*** where IL28B polymorphism modulate early virologic respose to peginterferon/ribavirin treatment. In contrast to HCV genotype 1 patients, no effect on SVR rates was observed in genotype 3 patients. The clinical relevance of an earlier viral decline in C/C patients needs to be determined.

This was confirmed by another study where; AA with C/C and C/T genotypes had lower week 24, 48, and 72 (SVR) rates than did CA (p = 0.03). SNP C/C predicted higher SVR rates in AA and CA with high baseline HCV RNA (600,000 IU/ml), and in CA with 1 log10 IU/ml decrease in HCV RNA from day 0 to 28 ***(Howell et al.,2011).*** This is also comparable to another study where in patients with Burkitt lymphoma, simultaneous homozygosity or heterozygosity at GM (chromosome 14) and KM (chromosome 2) loci resulted in higher antibody responses to EBV antigens than did dissimilar zygosity at the 2 loci ***(Biggar et al.,1984).***

We recommend that κ and γ chain determinants of immunoglobulin genes (KM and GM allotypes) be used as a predictor to response before treatment, but future studies are needed to assess the efficacy of this prediction. This may save time and money and decrease the burden on the patients and the health care system.

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