Profile of Transforming Growth Factor Beta 1 Gene (TGF-b1) Nucleotide 29 T869C Polymorphism in Malaysian Rheumatoid Arthritis patients

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Abstract

Introduction:Various studies on Rheumatoid arthritis (RA) patients suggested that polymorphism in the Transforming Growth Factor-Beta 1 (TGF- b1) gene may be associated with increased risk of RA severity. Results: Fifty-nine patients (52.68%%) were found to have non-severe disease and 53 patients (47.32%) were found to have severe disease. The frequency of CT genotype was the highest among the 3 genotypes (78 out of 112). For the Malays, 6 patients (12.5%) were CC genotype, 33 patients (68.7%) were CT genotype and 9 patients (18.8%) were TT genotype. For the Chinese, 7 patients (21.2%) were CC genotype, 22 patients (66.7%) were CT genotype and 4 patients (12.1%) were TT genotype. For the Indians, 5 patients (17.9%) were CC genotype, 20 patients (71.4%) were CT genotype and 3 patients (10.7%) were TT genotype. For the other ethnic groups, all 3 patients were CT genotype.
Conclusion: CT genotype has the highest frequency (69.6%)

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INTRODUCTION

Rheumatoid arthritis (RA) is an inflammatory rheumatic disease of unknown aetiology which manifests by chronic inflammatory reaction in the synovia. As the disease progresses it results in progressive joint destruction, eventual severity and can lead to morbidity and even mortality. According to the Malaysian Society of Rheumatology, RA affects about 5 in 1000 people in Malaysia.

Attempts have been made to identify predictors of outcome but as yet no prognostic markers are available which can predict disease course in individual patients. Being able to predict an early outcome in the disease would enable more aggressive managements e.g. biologics in order to combat the complications of RA. This would then help in employment, financial and social plans for the patients.

Polymorphism of the transforming growth factor beta 1 (TGF- 1) gene had long been an issue of debate.Various studies on RA patients suggested that polymorphism in the signal sequence at position +869 (T869C) of the transforming growth factor beta 1 (TGF- 1) gene may be associated with an increased risk of RA(2), determine the progression of joint destruction in RA(3) and increased risk of RA severity(4).

TGF- has been considered an important modulator of the immune response in RA. It is a dimeric protein of 25 kDa molecular weight, and is abundant in the human bone matrix(5-6). After its release by bone cells as an inactive complex, TGF- is stored in the extracellular matrix where it may be activated by proteases. It exists as 3 isoforms which are TGF- 1, TGF- 2 and TGF- 3, with TGF- 1(7-8). TGF- 1 has been reported to have an important role in many diseases, affecting the regulation of tissue repair, fibrosing processes, and angiogenesis(9). Seven TGF- 1 gene polymorphisms have been described, of which 5 had been confirmed in several studies(10-11) (Figure 1). Two signal sequence polymorphisms at positions +869 and +915 are linked to disease outcomes (10-15). The +869 polymorphism at codon 10 is a T C substitution, resulting in a leucine proline substitution. The +915 polymorphism at codon 25 is a G C substitution, resulting in arginine proline substitution. With regards to TGF- 1 gene polymorphism at position +869, it is further divided into 3 genotypes which

are genotype CC, CT and TT.

TGF- 1 is also a pluripotent cytokine that is known to participate in both pro- and anti- inflammatory processes(16). Many cells (including T lymphocytes, monocytes, endothelial cells, fibroblasts, and others) secrete this cytokine, but the main source in normal circumstances is platelets, from which it is released in granules(17,18).

TGF- 1 also has immunosuppressive properties, including down regulation of IL- 1 receptor(19) and simultaneous induction of IL- 1 receptor antagonist expression(20), suppression of pro- inflammatory cytokine production by various cell types(21,22), macrophage deactivation(23,24), and suppression of T lymphocyte proliferation in response to mitogenic stimulation(25) . TGF- 1 can inhibit the production and response to cytokines associated with both Th1 and Th2 cells(26),and also suppresses the expression of class II MHC antigens induced by interferon- gamma(27).

Therefore, this sub-study was aimed to explore the profile of TGF- 1 polymorphism in the the Malaysian RA population and looking at some parameters previously studied such as HAQ scoring and ESR as well as the usage of Disease Modifying Anti- Rheumatic Drugs (DMARDs).

OBJECTIVES

To explore the profile of TGF- 1 polymorphism in the the Malaysian RA population and its relation to disease severity.

METHODOLOGY

This sub-study (1) was based on a cross- sectional study involving RA patients attending the Rheumatology Clinic at Hospital Universiti Kebangsaan Malaysia (HUKM). The study was completed in November 2007. The HAQ- DI used in this study had been validated for local use. It was also written in English and Bahasa Malaysia.

By taking an anticipated population proportion of 60%, a sample size of 92 patients would be required to detect a statistically significant difference with a power of 95% (= 0.05).

The blood samples for gene analysis were then sent to the Insitute for Medical Research (IMR) for TGF- 1 genotyping.

Venous blood (5 millilitres) was collected from each subject into EDTA tubes. Genomic DNA was isolated with a DNA blood extraction kit (Qiagen, Hilden, Germany).

The codon 10 (T869C) polymorphism was determined by amplification refractory mutation system (ARMS) polymerase chain reaction (PCR) method. The common anti-sense primer 5' GTT GTG GGT TTC CAC CAT TAG 3' was used with the T allele specific primer 5' CTC CGG GCT GCG GCT GCT GCT 3' and the C allele specific primer 5' CTC CGG GCT GCG GCT GCT GCC 3' seperately for the amplification of the codon 10 (nucleotide 29 in exon 1) containing segment.

The PCR reaction mixture contained 0.5 l of each primer (20 pmol/l), 3mM magnesium chloride, 1.25U/l dNTP mixture and Taq polymerase, in a reaction volume of 30 l. The thermal cycling parameters consisted of 35 cycles of denaturing at 95C for 40 seconds, annealing at 60C for 30 seconds and extension at 72C for 30 seconds. The PCR products were analysed by 1% agarose gel electrophoresis and visualized by ethidium bromide staining. The expected size of the specific amplification product was 346 bp.

The HAQ- DI scoring system assessed the functional severity (0- none/ without much difficulty, 1- mild/ with some difficulty, 2- moderate/ with much difficulty, 3- severe/ unable to do).

The inflammatory assessment looked for presence of a raised ESR (ESR > 40 mm/hour) or otherwise (ESR < 40 mm/hour).

The usage of DMARDs noted how many DMARDs that the patient was using (whether before or after being seen in the study).

INCLUSION CRITERIA

RA patients attending the Rheumatology Clinic at HUKM.

EXCLUSION CRITERIA

Infections, other connective tissue diseases and other inflammatory conditions that were untreated or uncontrolled in spite of treatment at the time of the patient's appointment or visit to the Rheumatology Clinic.

DEFINITION OF SEVERE SEVERITY

Severe severity was defined as the presence of any two of the three following criteria and/or usage of 2 or more DMARDs:

1. HAQ- DI score of 2 or more(28)
2. Raised ESR (40 mm/hour and above)

3. Usage of 2 or more DMARDs (whether before or after being seen in the study)
4. Presence of joint erosions
5. Presence of extra-articular disease

RESULTS

This sub-study was based on the study which was started in December 2006. A total of 112 patients were recruited.

AGE, GENDER, RACE, AGE AT DIAGNOSIS AND DURATION OF DISEASE

The mean age of the study population was 57.22 + 11.71 years, ranging from 27 to 106 years. The population consisted of 11 (9.8%) males and 101 (90.2%) females in the study, 48 (42.9%) Malay patients, 33 (29.5%) Chinese patients, 28 (25%) Indian patients and 3 (2.7%) Sikh patients. This reflected the ethnic composition in the general Malaysian population. Age at diagnosis ranged from 18 to 96 years, with a median of 47 (39.25- 54) years. Duration of disease ranged from 1 to 38 years, with a median of 8 (3- 16.75) years.

HAQ- DI, ESR, DMARDS AND GENOTYPE

The HAQ- DI of the study population ranged from 0 to 3, with a median of 1.38 (0.88- 1.97) and ESR of the study population ranged from 5 to 120 mm/hour, with a median of 56.5 (36.25- 81) mm/hour. The number of DMARDs of the study population ranged from 0 to 3, with a median of 1 (1- 2) DMARD. 10 patients (8.9%) were not on any DMARDs, 67 patients (59.8%) used only 1 DMARD, 34 patients (30.4%) used 2 DMARDs and 1 patient (0.9%) used 3 DMARDs. None of the patients were on biological response modifiers such as etanercept and infliximab.

There were 18 patients (16.1%) with CC genotype, 78 patients (69.6%) with CT genotype and 16 patients (14.3%) with TT genotype.

COMPARISON BETWEEN THE GENOTYPES AND RACE

For the Malays, 6 patients (12.5%) were CC genotype, 33 patients (68.7%) were CT genotype and 9 patients (18.8%) were TT genotype. For the Chinese, 7 patients (21.2%) were CC genotype, 22 patients (66.7%) were CT genotype and 4 patients (12.1%) were TT genotype. For the Indians, 5 patients (17.9%) were CC genotype, 20 patients (71.4%) were CT genotype and 3 patients (10.7%) were TT genotype.

COMPARISON BETWEEN RACE AND RA SEVERITY

For severe severity, 45.8% were seen in the Malay patients, 48.5% were seen in the Chinese patients, 50% were seen in the Indian patients and 33.3% were seen in the other ethnic groups.

COMPARISON BETWEEN THE GENOTYPES AND RA SEVERITY

Comparison between the 3 genotypes showed that the CC genotype had 11 patients (61.1%) with non- severe severity and 7 patients (38.9%) had severe severity; CT genotype had 40 patients (51.3%) with non- severe severity and 38 patients (48.7%) with severe severity; TT genotype had 8 patients (50%) with non- severe severity and 8 patients (50%) with severe severity.

However, the Chi- Square equaled to 0.621 with a p value of 0.733 which was not statistically significant. Therefore, there was no association between the genotypes and RA severity.

DISCUSSION

Discussion regarding multiple ethnicity in RA patients has always been an interesting subject to research upon especially in Malaysia. Up till December 2007 there are still no published data regarding profile of RA patients according to ethnicity(1).

In this study the profiles of TGF- 1 gene polymorphism in HUKM patients revealed a high frequency of CT genotype which is comparable to those reported by Sugiura et al(2) and DL Mattey et al(4). However, our study revealed that the frequency for TT (14.29%) and CC (16.07%) genotype varied compared to those studies(2,4) (16.81% versus 19% and 14.4% for CC genotype and 14.16% versus 22% and 36.1% for TT genotype). Whether this is unique for Malaysia remained unanswered. So far, we could not find the prevalence of TGF- 1 gene polymorphism in the signal sequence at position +869 in the general population in Malaysia. We also found that CT is the major polymorphism involved in severe disease.

One trial on RA by Lars Klareskog, et al(29) used a patient population with significant disease that had an unsatisfactory response to at least one DMARD other than methotrexate. The mean of the number of DMARDs used by the patients prior to the trial was 2.3. Therefore, for the purpose of this

study usage of DMARDs of 2 or more was used to define RA severity. Usage of 2 or more DMARDs alone as a marker of severity was based on the assumption that most patients who had to be on more than 1 DMARD were having moderate severity at least.

In terms of DMARDs usage, the presence of a T allele (whether CT or TT genotype) showed a higher percentage in DMARDs usage of 2 DMARDs or more. This is comparable to a study by DL Mattey et al(4) which concluded that the T allele was associated with increased inflammatory activity and poor functional outcome as 89% of the patients recruited by DL Mattey et al were being treated with one or more DMARDs. In our study, 91.1% of the patients recruited were on DMARDs.

It was also noted that the female patients had a higher percentage of HAQ- DI score of at least 2 and above (26.7% versus 9.1%), a higher percentage of ESR levels of 40 mm/hour and above (74.3% versus 36.4%), a higher percentage of DMARDs usage of 2 and above (31.7% versus 27.3%) and a higher percentage of patients with severe disease (49.5% versus 27.3%) compared to the male patients. These findings were compatible with the fact that RA tends to affect female rather than male patients.

In order to further evaluate the reasons of our study findings, we further analyzed some of the studies conducted regarding TGF- 1 gene polymorphism in the signal sequence at position +869 (T869C) and its association with RA. A study by Sugiura et al(2) concluded that the T allele in the TGF- 1 genotypes may be associated with an increased risk of RA. This was based on several studies which showed that the T allele of the T869C polymorphism has been reported to be associated with significantly reduced production of TGF- 1 proteins(13,30). As reduced TGF- 1 may result in increased inflammation in RA, the T allele may be associated with worsening of RA. However, the association between relatively low production of TGF- 1 and T alleles of T869C polymorphism was only speculated as the association between TGF- 1 T869C polymorphisms and their protein concentrations remain unclear and the investigators had no data of their own to show TGF- 1 concentrations by genotype. The other limitation to this study was that it only recruited Japanese patients whereas our study included all 3 major ethnic groups in the Malaysian population. Even though a significantly higher proportion of patients with RA carrying the T allele was found compared to with patients with CC genotype (p= 0.039), the difference in genotypic distribution between the controls and patients was not significant when all 3 genotypes were compared.

A study by Kim SY et al(3) concluded that TGF- 1 polymorphism may determine the progression of joint destruction in RA. However, the progression of radiographic severity, which was defined by a modified Sharp score plotted against disease duration, was significantly faster in the carrier of T allele at position -509 (p= 0.048) rather than +869 which was investigated by us.

A study by DL Mattey et al(4) concluded that TGF- 1 T869C gene polymorphism is associated with disease outcome in RA. It also concluded that carriage of the T allele (putatively associated with decreased TGF- 1 production) was associated with increased inflammatory activity and poor functional outcome, while increasing T allele dose was associated with worse survival. Similarities between the study design by DL Mattey et al and that of our study was that both studies used HAQ and ESR as study variables. DL Mattey et al noted that in the case of HAQ, patients with CT genotype had a significantly higher mean HAQ score than patients with CC genotype (p= 0.02). Even though there was no significant difference between CT and TT genotypes, overall those with a T allele (CT or TT genotype) had a significantly higher HAQ score than those lacking the T allele (p= 0.04). These findings do have some similarities in our study where in the case of HAQ- DI, patients with TT genotype had a higher percentage of HAQ- DI scores of at least 2 and above compared to genotype CC (31.3% versus 27.8%). Also noted was that patients with CT genotype had a lower percentage of HAQ scores of at least 2 and above compared to genotype CC (23.1% versus 27.8%).

In the case of ESR, DL Mattey et al noted that there were no significant differences between individual TGF- 1 genotypes for 5 years mean area under the curve ESR levels (MAUC ESR). However, comparison between patients with a T allele and genotype CC patients showed a weakly significant difference in MAUC ESR levels (p= 0.05). Our study showed that genotype CC had the highest percentage of ESR levels of 40 mm/hour and above. However, as in the study by Suguira et al(2), DL Mattey et al suggested or concluded rather than confirmed that the T allele was associated with increased inflammation in RA as there was no data of their own to show TGF- 1 concentrations by genotype. The study also involved only northern European white patients as opposed to our study which involved a more diverse ethnic population. Finally, in contrast to our

study which only recruited patients for approximately 8 months, DL Mattey et al recruited patients over 5 years. We also were able to use ESR only in our study as opposed to DL Mattey et al who was able to utilize MAUC ESR which was more reliable in assessing the patient's inflammatory status.

Another important point to be pointed out is that with the more widespread use of DMARDs now as compared to in the past, the incidence of severe disease and henceforth severity of the joints due to RA has reduced markedly. Therefore, a patient might have genetic predisposition to a more severe disabling disease but due to the treatment given would be phenotypically better compared to those who were not properly treated. This could also explain as to why we could not find an association between TGF- 1 nucleotide T869C polymorphism with severity in RA patients.

CLINICAL IMPLICATIONS

As the TGF- 1 gene is known to participate in both pro- and anti- inflammatory processes, further studies with more time, resources and financial support need to be conducted to determine the relationship between polymorphism in the signal sequence at position +869 (T869C) of the TGF- 1 gene (genotype TT and CT) and severity in RA, especially to determine whether pro- or anti- inflammatory properties dominate this gene.

Determining this could have an important impact on the type of treatment that would be offered to the RA patient.

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