Indian J Hematol Blood Transfus (Apr-June 2021) 37(2):210–219
https://doi.org/10.1007/s12288-020-01281-0

ORIGINAL ARTICLE

Association of Interleukin-1β-31C/T, -511T/C and -3954C/T Single Nucleotide Polymorphism and Their Blood Plasma Level in Acquired Aplastic Anemia

Saurabh Shukla[1] · Anil Kumar Tripathi[1] · Shailendra Prasad Verma[1] · Deependra Kumar Yadav[1] · R. K. Tripathi[2] · Shweta Maurya[1] · Nidhi Awasthi[3]

Received: 26 January 2020 / Accepted: 15 April 2020 / Published online: 26 April 2020
© Indian Society of Hematology and Blood Transfusion 2020

Abstract Aplastic anemia (AA) is an immune-mediated disorder in which hematopoietic stem and progenitor cells are targeted by a number of cellular and molecular pathways. This case control study aims to investigate the association of interleukin-1beta (*IL-1β*) gene polymorphisms, (*IL-1β-31, IL-1β-511* and *IL-1β-3954*) and their plasma levels with acquired AA. Genotyping was done by Restricted Fragment Length Polymorphism (PCR–RFLP) method and *IL-1β* plasma levels were evaluated in peripheral blood using ELISA. Increased level of *IL-1β* was reported to be significant in cases as compared to controls. The susceptibility of developing AA was higher in the cases for *IL-1β-3954* genotype. *IL-1β-511* genotype showed significant association with the severity groups of AA. No significant association was noticed in responder versus non-responder group. Plasma level of *IL-1β* gene was found to be significantly higher in severe and very-severe group of AA versus control group. Our findings suggest that *IL-1β* gene and its genotypes might be involved in the pathophysiology of AA and play a central role in the etiopathogenesis of AA.

Keywords Aplastic anemia · Interleukin · *IL-1β-31* · *IL-1β-511* · *IL-1β-3954* · Polymorphism · PCR–RFLP · ELISA

Abbreviations

AA	Aplastic anemia
IL	Interleukin
SNPs	Single nucleotide polymorphisms
RFLP	Restricted Fragment Length Polymorphism
EDTA	Ethylene-diamine-tetra-acetic-acid
SAA	Severe aplastic anemia
NSAA	Non-severe aplastic anemia
VSAA	Very-severe aplastic anemia
ELISA	Enzyme linked immunosorbent assay

✉ Anil Kumar Tripathi
aktkgmu@gmail.com

Saurabh Shukla
saurabh28189@gmail.com

Shailendra Prasad Verma
drspkgmu@rediffmail.com

Deependra Kumar Yadav
deependrakgmu@gmail.com

R. K. Tripathi
rajkamalt@yahoo.com

Shweta Maurya
tashu1191@gmail.com

Nidhi Awasthi
awasthivn75@gmail.com

[1] Department of Clinical Hematology, King George's Medical University, Chowk, Lucknow 226003, India

[2] Division of Toxicology, Central Drug Research Institute, Sitapur Road, Jankipuram Extension, Lucknow 226031, India

[3] Department of Pediatrics, King George's Medical University, Chowk, Lucknow 226003, India

Introduction

AA is an infrequent, life-threatening problem of haemopoiesis characterized by pancytopenia and hypocellular bone marrow in the absence of an abnormal infiltrate, with no increase in reticulin [1–3]. According to the modified Camitta classification criteria for diagnosis, AA is clinically classified into three types based on the severity of

the disease; severe aplastic anemia (SAA), non-severe aplastic anemia (NSAA) and very-severe aplastic anemia (VSAA) [4]. The incidences of AA vary geographically, and the precise incidence of AA in India is not well known due to lack of epidemiological studies. There is a biphasic age distribution with peaks between the ages of 15 and 25 years and a second smaller peak in incidence was noted after 60 years, with no significant difference in incidence between males and females [5].

The *IL-1*family comprises three related genes, namely *IL-1α, IL-1β and IL-Ra*, which encode the pro-inflammatory cytokine *IL-1α* and *IL-1β* and their receptor *IL-1R* [6, 7]. The *IL-1* plays a major role in inflammation-mediated autoimmune diseases. The *IL-1Ra* is a natural anti-inflammatory molecule that neutralizes the pro-inflammatory effect of *IL-1β,* and thus plays a role in maintaining homeostasis [8]. It might be possible that polymorphism in *IL-1β-511* and *IL-1β-3954* may lead to changes in the production of *IL-1β*, hence disturbing the homeostasis and making individuals more susceptible towards developing AA. *1L-1β* is a cytokine protein which is encoded by the *IL-1β* gene in humans and has a molecular weight of 17.5 kDa [9]. It is known to be a strong proinflammatory cytokine with multiple biological effects including cell proliferation, differentiation, and apoptosis [10]. It exists in three polymorphic forms *IL-1β-31C/T, IL-1β-511T/C and IL-1β-3954 C/T* [11, 12].

It is evident from various studies that cytokine genes are polymorphic in nature [13]. The promoter sequence is a potential source of polymorphism affecting gene expression. Several single nucleotide polymorphisms (SNPs) have been reported in the regulatory region of cytokine genes, and some of them were associated with altered gene expression [6]. Various studies from across the world have shown that genetic polymorphism plays an essential role in development of AA [14, 15]. However, genetic associations found in one population need not necessarily hold true in another population with a different ethnic background. Therefore, in the present study, we identified three isoforms of *IL* gene (*IL-1β-31 C/T, IL-1β-511T/C and IL-1β-3954 C/T*) and their occurrence, which might increase susceptibility towards developing AA [16].

Materials and Methods

Study Subjects

The present study involved two subject groups: cases and controls. The case group included 120 AA patients who were enrolled irrespective of their age and gender, at the Department of Clinical Hematology, King George's Medical University (KGMU) Lucknow, Uttar Pradesh, India. The control group included 120 healthy individuals not suffering from any disease during the same period. The control group included both the subjects from the general population and those from the hospital.

Data Collection

The data pertaining to all the AA patients includes various clinico-pathological parameters, demographic variables, and these were obtained and evaluated from the patient medical records, pathology reports, questionnaires and also from the personal interviews with the patients and their guardians (for those who were illiterate or unable to communicate). All the patients and their guardians were informed about the study and their willingness to participate in this study was documented using a predesigned questionnaire, and the same procedure was followed for the controls. All the procedures pertaining to the study subjects including sample procurement and the data collection were carried out in accordance with the ethical standards laid down by the Institutional Ethics Committee, KGMU, India [17].

Patients and Clinical Examination

The diagnosis, classification (severe AA, non-severe AA and very-severe AA) and the response assessment of AA were made according to the standard guidelines [4, 18, 19]. Samples were collected during March 2015 to March 2018 from 120 cases and equivalent number of controls after obtaining their written informed consent. For immuno suppressive therapy (IST) out of thetotal of 120 patients included for this study, (21) 17.50% received Anti-Thymocyte Globulin + cyclosporine (ATG + CsA) and 99 (82.5%) received cyclosporine only and follow up was conducted every month for 1 year.

Sample Preparation and Genotyping

Five ml of peripheral blood was collected by venipuncture, under aseptic condition, from each individual of the case and control group in ethylene-diamine-tetra-acetic-acid (EDTA) tubes (ADS Hitech Polymers) and stored at − 80 °C until further use [20]. The genomic DNA was extracted from the blood specimens using Qiagen Blood DNA Mini Kit, Hilden, Germany according to the manufacturers' instructions. The extracted DNA was stored at − 20 °C for further use. The qualitative analysis of the extracted genomic DNA samples was carried out by a UV–visible spectrophotometer (O.D. at 260 nm and 280 nm) and the quantitative analysis by agarose gel electrophoresis.

PCR amplification was carried out in a final volume of 20 µl (3 µl DNA, 10 µl Top Taq PCR Master Mix, 1 µl of forward and reverse primer and 5 µl distilled water). The PCR reaction was carried out in the DNA thermal cycler (Eppendorf® Mastercycler® Nexus Thermal Cyclers, Hamburg, Germany) [21]. The computerized thermal cycler was programmed for the following conditions:

IL-1β-31C/T polymorphism denaturation at 94 °C for 10 min, then 30 cycles at 94 °C for 30 s, 50.2 °C for 45 s, 72 °C for 45 s, and finally 72 °C for 10 min.

IL-1β-511T/C polymorphism annealing 49 °C for 45 s (rest all other conditions were similar)

IL-1β-3954C/T polymorphism annealing 54 °C for 45 s (rest all other conditions were similar).

Digestion of the Amplified Product by Specific Restriction Enzyme for Each Polymorphism

Amplified PCR product (10 µl) mixed with (1 µl) restriction enzyme (New England Biolabs, UK) was used in the reaction. The reaction mixture was incubated for 2 h at 37 °C. The digested products undergo gel electrophoresis in the range of 1.5–3%. Further, the separated fragments were stained with EtBr and visualized along with ladder using the molecular imager gel doc XR System (Bio-Rad, Hercules, CA). The details of restriction enzymes and their resulting base pair length are shown in Table 1.

Measurement of Plasma IL-1β

Venous blood was collected in commercially available EDTA tubes. Plasma was separated using a refrigerated centrifuge for 15 min at 2000 × *g*. Plasma of *IL-1β* level was determined by ELISA kit (Bioassay Technology Laboratory).

Data Analysis

Allele counting method was used to determine the allele and genotype frequencies. Odds ratio (OR) was determined and an assessment of confidence intervals (95%) was made. All these statistical calculations were carried out using software (Graph Pad Software ver. 3.05, San Diego, USA). p value found to be (< 0.05) was considered significant. To better define the association between the genotype and aplastic anemia, three genetic models (dominant, over-dominant, recessive) were analyzed. In the dominant model, wild type homozygous was compared with the pair of mutant homozygous and heterozygous. In genetic models, the over dominant model includes wild type homozygous plus heterozygous that was compared with homozygous mutant type. In the recessive model,

Table 1 Genotyping information of IL-1β-31 and IL-1β-511 and IL-1β-3954 single nucleotide polymorphisms

Gene SNP name	Primer sequence (5′–3′)	Restriction enzyme	Recognition sequence	Wild type fragment length	Variant (mutant) type fragment length	Heterozygous type fragment length
IL-1β-31	F:5′AGAAGCTTCCACCAATACTC-3′	AluI	5′-AG|CT-3′	239 bp (CC)	137 bp and 102 bp (TT)	239 bp, 137 bp and 102 bp (TC)
	R:5′AGCACCTAGTTGTAAGGAAG-3′		5′-TC|GA-3′			
IL-1β-511	F:5′TGGCATTGATCTGGTTCATC-3′	AvaI	5′C|YCGRG3′	305 bp (TT)	190 bp and 115 bp (CC)	305 bp, 190 bp and 115 bp (CT)
	R:5′GTTTAGGAATCTTCCCACTT-3′		5′- GRGCY|C -3′			
IL-1β-3954	F:5′AATTTTGCCACCTCGCCTCA-3′	TaqI	5′-T|CGA-3′	152 bp (CC)	88 bp (TT) and 64 bp	88 bp (TT) and 64 bp
	R:5′CGGAGCGTGCAGTTCAGTGAT-3′		5′-AGC|T-3′			

bp base pair

homozygous type mutant was compared with the pair of wild type homozygous and heterozygous.

Results

In this study 240 participants (patients-120 and healthy control-120) were enrolled. The male to female ratio in AA patients was 70:30 and control was 51:49. The percentage of AA patients with severe, non-severe and very-severe was 44.2, 46.7 and 9.1 respectively. Demographic details of aplastic anemia patients and those of healthy controls are given in (Table 2).

Occurrence of *IL-1β-31*, *IL-1β-511 and IL-1β-3954* Polymorphisms and their Susceptibility to Developing AA

*IL-1β-31*polymorphismshowedhigher susceptibility to develop AA in over-dominant model (CC + TC vs TT), but was not significantly associated in any of the model with AA. The genotypic variants *IL-1β-511*did not show any significant susceptibility, although high occurrence was noticed inmutant variant (CC) and recessive model (CC vs TT + CT) of *IL-1β-511* was found in AA cases (Table 3). The occurrence of homozygous (TT) variant of *IL-1β-3954* had significantly higher susceptibility to developing AA when compared with the healthy controls (OR = 2.35, 95% CI 1.10–5.00, $p = 0.03$) (Table 3). Furthermore, the occurrence of mutant allele of *IL-1β-3954* showed a higher risk of developing AA. Table 3 depicts the genetic models of polymorphisms out of which a *IL-1β-3954* showed significant protective association in the over dominant model (CC + CT vs TT), (OR = 0.39, 95% CI 0.19–0.81, $p = 0.01$) (Table 3). However, recessive model (TT vs CC + CT) was also found to be significantly associated with the disease (OR = 2.5, 95% CI 1.22–5.11, $p = 0.01$).

Occurrence of *IL-1β-31, IL-1β-511 and IL-1β-3954* Polymorphisms in Non-severe, Severe and Very-Severe AA

The mutant variant of*IL-1β-31*showed higher risk with the severity index of SAA and NSAA patients when compared with those of controls (Table 4). The frequency of homozygous mutant (TT) and heterozygous (CT) variant of *IL-1β-511* showed significant association with the NSAA (OR = 0.31, 95% CI 0.12–0.76, $p = 0.018$and OR = 0.88, 95% CI 0.43–1.82, $p = 0.002$) (Table 4). The recessive model (TT vs CC + CT) of *IL-1β-511*showed significant correlation with disease severity in SAA and VSAA patients compared with healthy controls (OR = 3.01, 95% CI 1.31–6.87, $p = 0.007$ and OR = 4.7, 95% CI 1.21–18.27, $p = 0.03$) (Table 4). The heterozygous variant (CT) of *IL-1β-3954* showed a higher risk association with VSAA patients, but was not found significant (Table 4).

Occurrence Of *IL-1β-31, IL-1β-511 And IL-1β-3954* Polymorphisms With Response To Immunosuppressive Therapy

No significant association was found between *IL-1β-31, IL-1β-511 and IL-1β-3954,* with response to IST in any of the groups (Table 5).

Table 2 Demographic detail of acquired aplastic anemia patients and healthy control subjects:

Characteristics	Patients (n = 120)	Controls (n = 120)
Age, mean years ± SD	29.13 ± (16.4)	27.92 ± (8.9)
Gender		
Male (%)	83 (69.2%)	62 (51.7%)
Female (%)	37 (30.8%)	58 (48.3%)
Patients classification on the basis disease severity		
Severity		
Severe (%)	53 (44.2%)	0 (0)
Non-severe (%)	56 (46.7%)	0 (0)
Very severe (%)	11 (9.1%)	0 (0)
Patients categorization on the basis of response to immunosuppressive therapy		
Response to immunosuppressive therapy		
Responder (complete + partial) (%)	63 (52.5)	0 (0)
Non-responder (%)	57 (47.5)	0 (0)

Table 3 Genotype and allele frequencies of the IL-1β-31, IL-1β-511 and IL-1β-3954 cytokine gene among acquired aplastic anemia cases and controls and their risk associations with acquired aplastic anemia

Gene polymorphism	Patients (%) n = 120	Controls (%) n = 120	*p* value	OR (95% CI)
IL-1β-31 genotype				
CC (Wild)	85 (70.8)	80 (66.7)	–	Reference
TT (Mutant)	15 (12.5)	25 (20.8)	0.15	0.56 (0.27–1.14)
TC (Hetero)	20 (16.7)	15 (12.5)	0.67	1.25 (0.60–2.62)
Dominant	85 (70.8)	80 (66.7)	–	Reference
CC versus TT + TC	35 (29.2)	40 (33.3)	0.57	1.21 (0.70–2.09)
Over dominant	100 (83.3)	105 (87.5)	–	Reference
CC + TC versus TT	20 (16.7)	15 (12.5)	0.36	0.71 (0.34–1.47)
Recessive Model	105 (87.5)	95 (79.2)	–	Reference
TT versus CC + TC	15 (12.5)	25 (20.8)	0.08	1.84 (0.91–3.70)
C (Wild)	190 (79.1)	175 (72.9)	–	Reference
T (Mutant)	50 (20.9)	65 (27.1)	0.13	0.70 (0.46–1.08)
IL-1β-511 genotype				
TT (Wild)	60 (50.0)	58 (48.2)	–	Reference
CC (Mutant)	24 (20.0)	13 (10.8)	0.19	1.78 (0.83–3.83)
CT (Hetero)	36 (30.0)	49 (40.8)	0.29	0.71 (0.40–1.24)
Dominant	60 (50.0)	58 (48.2)	–	Reference
TT versus CC + CT	60 (50.0)	62 (51.8)	0.89	1.06 (0.64–1.77)
Over dominant	84 (70.0)	71 (59.2)	–	Reference
TT + CT versus CC	36 (30.0)	49 (40.8)	0.07	1.61 (0.94–2.47)
Recessive Model	96 (80.0)	107 (89.2)	–	Reference
CC versus TT + CT	24 (20.0)	13 (10.8)	**0.04***	0.48 (0.23–1.00)
Allele frequency				
T (Wild)	156 (65.0)	165 (68.8)	–	Reference
C (Mutant)	84 (35.0)	75 (31.2)	0.43	1.18 (0.80–1.73)
IL-1β-3954 genotype				
CC (Wild)	54 (45.0)	59 (49.2)	–	Reference
TT (Mutant)	28 (23.3)	13 (10.8)	**0.03***	2.35 (1.10–5.00)
CT (Hetero)	38 (31.7)	48 (40.0)	0.71	0.86 (0.49–1.52)
Dominant	54 (45.0)	59 (49.2)	–	Reference
CC versus TT + CT	66 (55.0)	61 (50.8)	0.60	0.84 (0.50–1.40)
Over dominant	82 (68.3)	72 (60.0)	–	Reference
CC + CT versus TT	38 (31.7)	48 (40.0)	0.22	1.43 (0.83–2.44)
Recessive Model	92 (76.7)	107 (89.2)	–	Reference
TT versus CC + CT	28 (23.3)	13 (10.8)	**0.01***	0.39 (0.19–0.81)
Allele frequency				
C (Wild)	146 (60.8)	166 (69.1)	–	Reference
T (Mutant)	94 (39.2)	74 (30.9)	0.06	1.44 (0.99–2.10)

Significant associations at the 0.05 significance level are bold

OR odds ratio, *CI* confidence interval

*Statistically significant susceptible genotype

Plasma Concentration of *IL-1β* in AA and in Controls

IL-1β level was elevated in blood plasma of 80.95% (34/42) of AA patients (3.37 ± 0.56 pg/ml) as compared to ($p < 0.001$) control subjects. Maximum increase in *IL-1β* concentration was observed in very-severe AA patients with a mean of 84.17 ± 2.1 pg/ml. Significant increase in *IL-1β* concentration was also observed in severe AA patients which was 35.73 ± 2.4 pg/ml. In non-severe AA patients, mean *IL-1β* concentration was 10.4 ± 0.62 pg/ml (Fig. 1).

Table 4 Genotype distribution of the IL-1β-31, IL-1β-511 and IL-1β-3954 cytokine gene polymorphism among severe, non-severe and very-severe type of aplastic anemia versus control

Genotype	Severe (n = 53)	Non-severe (n = 56)	Very-severe (n = 11)	Control (n = 120)	Severe patients and controls		Non- severe patients and controls		Very-severe and controls	
					p value	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)
IL-1β-31										
CC (Wild)	39 (73.6)	41 (73.2)	5 (45.5)	80 (66.7)	–	Reference	–	Reference	–	Reference
TT (Mutant)	6 (11.3)	7 (12.5)	2 (18.2)	25 (20.8)	0.21	2.03 (0.77–5.35)	0.27	1.83 (0.73–4.58)	0.77	0.78 (0.14–4.27)
TC (Hetero)	8 (15.1)	8 (14.3)	4 (36.3)	15 (12.5)	0.46	0.91 (0.35–2.34)	0.39	0.96 (0.37–2.45)	0.33	0.23 (0.05–0.97)
Dominant	39 (73.6)	41 (73.2)	5 (45.5)	80 (66.7)	–	Reference	–	Reference	–	Reference
CC versus TT + TC	14 (26.4)	15 (26.8)	6 (54.5)	40 (33.3)	0.36	1.39 (0.67–2.85)	0.38	1.36 (0.67–2.76)	0.15	0.41 (0.11–1.44)
Over-dominant	45 (84.9)	48 (85.7)	7 (63.6)	105 (87.5)	–	Reference	–	Reference	–	Reference
CC + TC versus TT	8 (15.1)	8 (14.3)	4 (36.4)	15 (12.5)	0.69	0.80 (0.31–2.03)	0.74	0.85 (0.34–2.15)	0.05	0.25 (0.06–0.95)
Recessive	47 (88.7)	49 (87.5)	9 (81.8)	95 (79.2)	–	Reference	–	Reference	–	Reference
TT versus CC + TC	6 (11.3)	7 (12.5)	2 (18.2)	25 (20.8)	0.13	2.06 (0.79–5.37)	0.18	1.84 (0.74–4.56)	0.99	1.18 (0.24–5.83)
C (Wild)	86 (81.1)	90 (80.3)	14 (63.7)	175 (72.9)	–	Reference	–	Reference	–	Reference
T (Mutant)	20 (18.9)	22 (19.7)	8 (36.3)	65 (27.1)	0.13	1.63 (0.93–2.87)	0.16	1.53 (0.87–2.62)	0.49	0.65 (0.26–1.62)
IL-1β-511										
CC (Wild)	34 (64.1)	21 (37.5)	5 (45.5)	58 (48.2)	–	Reference	–	Reference	–	Reference
TT (Mutant)	5 (9.4)	15 (26.8)	4 (36.3)	13 (10.8)	0.64	1.52 (0.49–4.64)	**0.018***	0.31 (0.12–0.76)	0.16	0.28 (0.06–1.19)
CT (Hetero)	14 (26.5)	20 (35.7)	2 (18.2)	49 (40.9)	0.07	2.05 (0.98–4.25)	**0.002***	0.88 (0.43–1.82)	0.62	2.11 (0.39–11.37)
Dominant	34 (64.1)	21 (37.5)	5 (45.5)	58 (48.2)	–	Reference	–	Reference	–	Reference
CC versus TT + CT	19 (35.8)	35 (62.5)	6 (54.5)	62 (51.8)	0.05	1.91 (0.98–3.72)	0.17	0.64 (0.33–1.22)	1.00	0.89 (0.25–3.07)
Over-dominant	39 (73.6)	36 (64.3)	9 (81.8)	71 (59.1)	–	Reference	–	Reference	–	Reference
CC + CT versus TT	14 (26.4)	20 (35.7)	2 (18.2)	49 (40.8)	0.06	1.93 (0.94–3.91)	0.51	1.24 (0.64–2.39)	0.20	3.10 (0.64–15.0)
Recessive	48 (90.6)	41 (73.2)	7 (63.6)	107 (89.2)	–	Reference	–	Reference	–	Reference
TT versus CC + CT	5 (9.4)	15 (26.8)	4 (36.4)	13 (10.8)	0.78	1.16 (0.39–3.45)	**0.007***	0.33 (0.14–0.75)	**0.03***	0.21 (0.05–0.82)
C	82 (77.3)	62 (55.3)	12 (54.6)	165 (68.8)	–	Reference	–	Reference	–	Reference
T	24 (22.7)	50 (44.7)	10 (45.4)	75 (31.2)	0.13	1.55 (0.91–2.64)	**0.02***	0.56 (0.35–0.89)	0.20	0.54 (0.22–1.31)
IL-1β-3954										
CC (Wild)	26 (49.1)	22 (39.3)	6 (54.5)	54 (49.2)	–	Reference	–	Reference	–	Reference
TT (Mutant)	11 (20.7)	14 (25.0)	3 (27.3)	28 (23.3)	0.79	1.22 (0.52–2.83)	0.77	0.81 (0.36–1.83)	0.96	1.03 (0.24–4.46)
CT (Hetero)	16 (30.2)	20 (35.7)	2 (18.2)	38 (40.0)	0.81	0.84 (0.38–1.82)	0.20	0.57 (0.26–1.21)	0.89	1.55 (0.29–6.21)
Dominant	26 (49.1)	22 (39.3)	6 (54.5)	54 (49.2)	–	Reference	–	Reference	–	Reference
CC versus TT + CT	27 (50.9)	34 (60.7)	5 (45.5)	66 (55.0)	0.62	1.17 (0.61–2.24)	0.47	0.79 (0.41–1.50)	0.54	1.46 (0.42–5.07)
Over-dominant	37 (69.8)	36 (64.3)	9 (81.8)	82 (68.3)	–	Reference	–	Reference	–	Reference
CC + CT versus TT	16 (30.9)	20 (35.7)	2 (18.2)	38 (23.7)	0.84	1.07 (0.53–2.16)	0.59	0.83 (0.42–1.62)	0.50	2.08 (0.42–10.12)
Recessive	42 (79.2)	42 (75.0)	8 (72.7)	92 (76.7)	–	Reference	–	Reference	–	Reference

Table 4 continued

Genotype	Severe (n = 53)	Non-severe (n = 56)	Very-severe (n = 11)	Control (n = 120)	Severe patients and controls		Non- severe patients and controls		Very-severe and controls	
					p value	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)
TT versus CC + CT	11 (20.8)	14 (25.0)	3 (27.3)	28 (17.5)	0.70	1.16 (0.52–2.55)	0.80	0.91 (0.43–1.91)	0.72	0.81 (0.20–3.26)
C	68 (64.1)	64 (57.1)	14 (63.7)	146 (69.1)	–	Reference	–	Reference	–	Reference
T	38 (35.9)	48 (42.9)	8 (36.3)	94 (30.9)	0.64	1.15 (0.71–1.85)	0.58	0.85 (0.54–1.35)	0.97	1.12 (0.45–2.79)

Significant associations at the 0.05 significance level are bold

OR odds ratio, *CI* confidence interval

*Statistically significant susceptible genotype

Discussion

The pathogenesis of AA is very complex [22]. The pathogenesis of AA includes abnormal cellular immunity, gradual destruction of the hematopoietic stem cells, and hematopoietic failure, which can lead to a reduction in all blood cells [23, 24]. Recent studies have shown that cytokine gene polymorphisms enhance the susceptibility of the patients to develop AA [15, 25]. Scanty data is available on the role of cytokine gene polymorphisms in causation of AA in the Indian population. In this study, we identified the association of *IL-1β*polymorphism with susceptibility and severity of AA, and also the effect of IST on AA. We also determined the expression of blood plasma level in AA patients (Fig. 2).

The pathophysiology of *IL-1β*involves several pathways which lead to a wide range of immunological and inflammatory effects [26]. The main role of *IL-1β* cytokine is to manage proinflammatory reactions in response to tissue injury. It stimulates and initiates other cytokines via accessory cells. Thus, it is the chief intermediate of innate immune reactions [27].

Different cellular signaling pathways may operate in response to varying levels of *IL-1β* leading to genotoxic damage, cell apoptosis or cell growth [28]. *IL-1β* represents the primary activator of early cytokines that facilitate the migration of leukocytes from blood vessels into the tissues [29]. A significant number of AA patients had increased levels of *IL-1β* in their blood plasma and these levels were significantly higher in patients in severe and non-severe group as compared to controls. This is the first study from India carried out by us showing elevated *IL-1β* levels in the blood of AA patients.

The results of *IL-1β-31*showed higher susceptibility in over-dominant model (Table 3). It also indicates that the mutant variant and over-dominant genetic model was higher in SAA and NSAA, but not significantly when compared with those of controls (Table 4). The homozygous mutant variant of *IL-1β-511* and its recessive model showed higher trend towards susceptibility, although they were not found to be significantly associated (Table 3). The results of *IL-1β-511* showed a relationship between polymorphism in recessive model of *IL-1β-511* with severity of NSAA and VSAA patients (Table 4). The findings of this study also show that there is an association between polymorphism in homozygous mutant (TT) variant of *IL-1β-3954* and susceptibility to developing AA (Table 3). Our results are in disagreement with the earlier studies where cytokines were found to be associated with other diseases and not with aplastic anemia, and this might probably be due to ethnic and geographical differences between the studied populations [30, 31]. To the best of our

Table 5 IL-1β-31, IL-1β-511 and IL-1β-3954 genotype distribution in responder (complete + partial responder) and non-responder

Gene polymorphism	Complete + partial responder (n = 63)	Non-responder (n = 57)	*p* value	OR (95% CI)
IL-1B-31 genotype				
CC (Wild)	45 (71.5)	40 (70.1)	–	Reference
TT (Mutant)	7 (11.1)	8 (14.1)	0.86	1.28 (0.42–3.86)
TC (Hetero)	11 (17.4)	9 (15.8)	0.86	0.92 (0.34–2.44)
Allele frequency				
C (Wild)	101 (80.1)	89 (78.1)	–	Reference
T (Mutant)	25 (19.9)	25 (21.9)	0.81	1.13 (0.60–2.11)
Dominant	45 (71.5)	40 (70.1)	–	Reference
CC versus TT + TC	18 (28.6)	17 (29.9)	0.88	1.06 (0.48–2.33)
Over dominant	52 (82.5)	48 (84.2)	–	Reference
CC + TC versus TT	11 (17.5)	9 (15.8)	0.80	0.88 (0.33–2.32)
Recessive	56 (88.9)	49 (85.9)	–	Reference
TT versus CC + TC	7 (11.1)	8 (14.1)	0.62	1.30 (0.44–3.86)
IL-1B-511 genotype				
TT (Wild)	32 (50.8)	28 (49.1)	–	Reference
CC (Mutant)	14 (22.2)	10 (17.6)	0.86	0.81 (0.31–2.12)
CT (Hetero)	17 (27.0)	19 (33.3)	0.71	1.27 (0.55–2.92)
Allele frequency				
T (Wild)	81 (64.3)	75 (65.8)	–	Reference
C (Mutant)	45 (35.7)	39 (34.2)	0.91	0.93 (0.55–1.59)
Dominant	32 (50.8)	28 (49.1)	–	Reference
TT versus CC + CT	31 (49.2)	29 (50.9)	0.85	1.06 (0.52–2.19)
Over-dominant	46 (73.0)	38 (66.7)	–	Reference
TT + CT versus CC	17 (27.0)	19 (33.3)	0.44	1.35 (0.61–2.96)
Recessive	49 (77.8)	47 (82.5)	–	Reference
CC versus TT + CT	14 (22.2)	10 (17.5)	0.52	0.74 (0.30–1.84)
IL 1β 3954 genotype				
CC (Wild)	32 (50.8)	22 (38.6)	–	Reference
TT (Mutant)	11 (17.5)	17 (29.8)	0.13	2.24 (0.88–5.71)
CT (Hetero)	20 (31.7)	18 (31.6)	0.67	1.30 (0.56–3.02)
Allele frequency				
C (Wild)	84 (66.7)	62 (54.3)	–	Reference
T (Mutant)	42 (33.3)	52 (45.7)	0.06	1.67 (0.99–2.82)
Dominant	32 (50.8)	22 (38.6)	–	Reference
CC versus TT + CT	31 (49.2)	35 (62.5)	0.17	1.64 (0.79– 3.39)
Over dominant	43 (68.2)	39 (68.4)	–	Reference
CC + CT versus TT	20 (31.8)	18 (31.6)	0.98	0.99 (0.45–2.14)
Recessive	52 (82.5)	40 (70.2)	–	Reference
TT versus CC + CT	11 (17.5)	17 (29.8)	0.10	2.00 (0.84–4.76)

OR odds ratio, *CI* confidence interval

knowledge, this is the first study of *IL-1β-3954*polymorphism in cases of AA in India. Further, our findings are also in consonance with the earlier study, where polymorphism in the *IL-1β-3954* was found to be associated in chronic periodontitis [32].

Our studies have thus shown that *IL-1β-511* and *IL-1β-3954* polymorphisms might play a role in the pathogenesis of AA. The limitation of our study is that the sample size was small; for better results, a larger sample size and different ethnic population are required. We have also demonstrated that elevated levels of *IL-1β* in plasma of AA

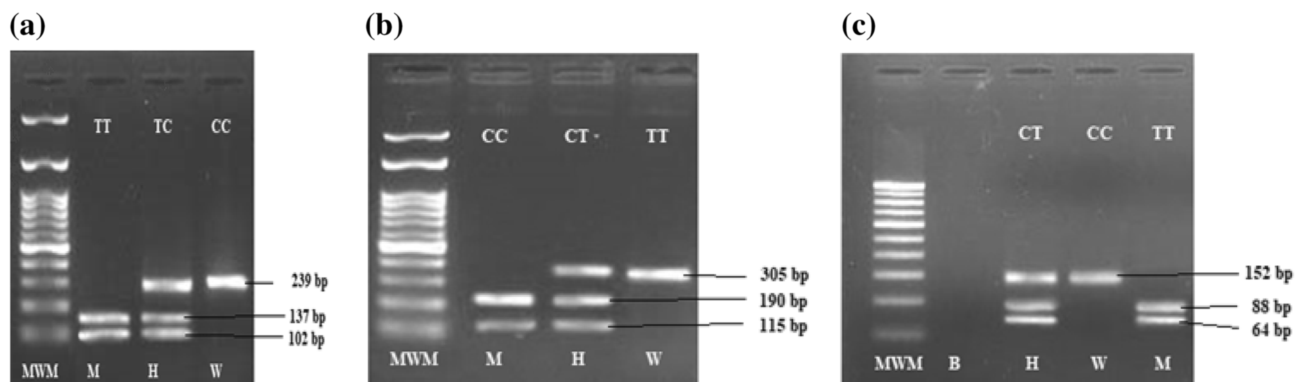

Fig. 1 **a** IL-1β-31T/C polymorphism; CC: wild homozygous (239 bp), TC: heterozygous (239 bp, 137 bp and 102 bp), TT: mutant (137 bp and 102 bp) MWL, molecular weight ladder (100 bp ladder). **b** IL-1β-511 C/T polymorphism; TT: wild homozygous (305 bp), CT: heterozygous (305 bp, 190 bp and 115 bp), CC: mutant (190 bp and 115 bp) MWL, molecular weight ladder (100 bp ladder). **c** IL-1β+3954 C/T polymorphism; CC: wild homozygous (152 bp), CT: heterozygous (152 bp, 88 bp and 64 bp), TT: mutant (88 bp and 64 bp) MWL, molecular weight ladder (50 bp ladder)

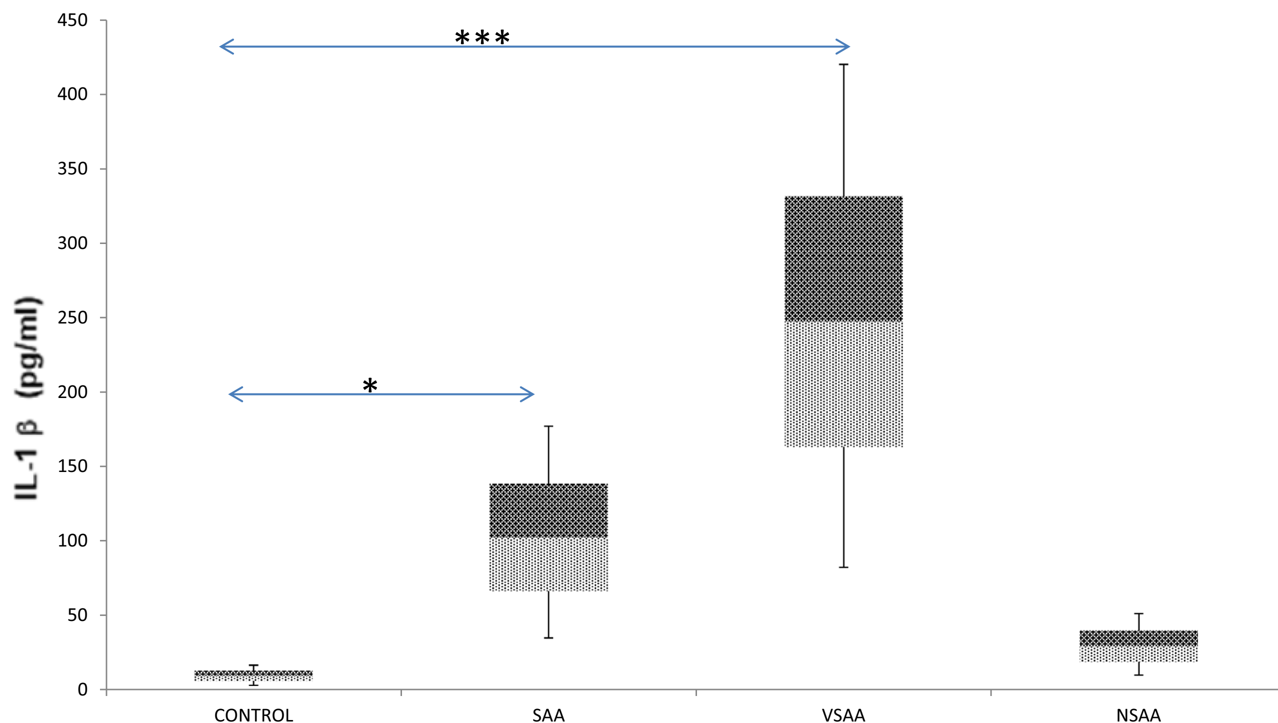

Fig. 2 Box plot showing blood plasma levels of IL-1β in healthy controls and AA patients. The box includes observations from the 25th to 75th percentile, and the horizontal line within the box represents the median value. The upper and lower lines outside the box represent the highest and lowest values of NSAA, SAA and VSAA respectively. Significant increase in IL-1β concentration were observed in SAA and VSAA patients with respect to contol

patients show a direct correlation with severity of the disease, and thus play an important role in development of AA.

Acknowledgements This study was financially supported by Indian Council of Medical Research (ICMR), New Delhi (56/19/2012-HAE-BMS to Saurabh Shukla).

Author Contributions Conceptualization: SS, AKT. Funding acquisition: SS, AKT. Investigation: SS, AKT. Methodology: SS, AKT. Project administration: AKT, RKT. Resources: SS, AKT, RKT, SPV. Software: SS, DY, SM, NA. Validation: SS, AKT, NA. Visualization: SS, AKT, SPV, RKT, DY, SM. Writing-review and editing: SS, AKT, SPV, NA.

Compliance with Ethical Standards

Conflict of interest The authors report no conflicts of interest in this work.

References

1. Issaragrisil S, Kaufman DW, Anderson T et al (2006) The epidemiology of aplastic anemia in Thailand. Blood 107(4):1299–1307
2. Dezern AE, Brodsky RA (2011) Clinical management of aplastic anemia. Expert Rev Hematol 4(2):221–230
3. Young NS, Calado RT, Scheinberg P (2006) Current concepts in the pathophysiology and treatment of aplastic anemia. Blood 108(8):2509–2519
4. Marsh JC et al (2009) Guidelines for the diagnosis and management of aplastic anaemia. Br J Haematol 147(1):43–705
5. Melinkeri SR (2015) Epidemiology, pathogenesis and diagnosis of aplastic anaemia. J Assoc Physicians India 63(3 Suppl):8–12
6. Banday MZ, Mir AH, Sameer AS, Chowdri NA, Haq E (2017) Interleukin-1β (IL-1β) -31C/T and -511T/C promoter single nucleotide polymorphism in colorectal cancer in ethnic Kashmiri population—a case control study. Meta Gene 12:94–103
7. Borthwick LA (2016) The IL-1 cytokine family and its role in inflammation and fibrosis in the lung. Semin Immunopathol 38(4):517–534
8. de Mooij CEM, Netea MG, van der Velden W, Blijlevens NMA (2017) Targeting the interleukin-1 pathway in patients with hematological disorders. Blood 129(24):3155–3164
9. Lopez-Castejon G, Brough D (2011) Understanding the mechanism of IL-1beta secretion. Cytokine Growth Factor Rev 22(4):189–195
10. Chen L, Deng H, Li Y, Cui H, Wang X, Fang J, Zhao L (2017) Inflammatory responses and inflammation-associated diseases in organs. Oncotarget 9(6):7204–7218
11. Chao TH, Yu HN, Huang CC, Liu WS, Tsai YW, Wu WT (2010) Association of interleukin-1 beta (-511C/T) polymorphisms with osteoporosis in postmenopausal women. Ann Saudi Med 30(6):437–441
12. Fang Y, Xie H, Lin Z (3954C) Association between IL-1beta + 3954C/T polymorphism and myocardial infarction risk: a meta-analysis. Medicine 97(30):e11645
13. Moreira ST, Silva GF, de Moraes CF et al (2016) Influence of cytokine and cytokine receptor gene polymorphisms on the degree of liver damage in patients with chronic hepatitis C. Meta Gene 9:90–96
14. Liang XH, Rong L, He G, He H, Lin S, Yang Y, Xue Y (2017) Fang Y (2017) Polymorphisms of the TGF-β1 gene and the risk of acquired aplastic anemia in a Chinese population. Ann Hematol 96(3):339–344
15. Zayed RA, Abdel-Hamid SM, El-Lithy H (2016) The association of cytokine genes polymorphisms and susceptibility to aplastic anemia in Egyptian patients. Hematology 21(2):106–112
16. Taylor SL, Renshaw BR, Garka KE, Smith DE, Sims JE (2002) Genomic organization of the interleukin-1 locus. Genomics 79(5):726–733
17. Tripathi P, Kumar A, Ahmad R, Balapure AK, Vishwakerma AL, Singh RK (2008) DNA aneuploidy study for early detection of chromosomal abnormality in patients with aplastic anemia: prognostic and therapeutic implications. In vivo 22:837–884
18. Camitta BM, Rappeport JM, Parkman R et al (1975) Selection of patients for bone marrow transplantation in severe aplastic anemia. Blood 45:355–363
19. Bacigalupo A, Hows J, Gluckman E et al (1988) Bone marrow transplantation (BMT) versus immunosuppression for the treatment of severe aplastic anaemia (SAA): a report of the EBMT SAA working party. Br J Haematol 70:177–182
20. Gupta D, Gupta V, Singh V et al (2014) Study of polymorphisms in CX3CR1, PLEKHA1 and VEGF genes as risk factors for age-related macular degeneration in Indian patients. Arch Med Res 45(6):489–494
21. Yadav DK, Tripathi AK, Gupta D et al (2017) Interleukin-1B (IL-1B-31 and IL-1B-511) and interleukin-1 receptor antagonist (IL-1Ra) gene polymorphisms in primary immune thrombocytopenia. Blood Res 52(4):264–269
22. Zeng Y, Katsanis E (2015) The complex pathophysiology of acquired aplastic anaemia. Clin Exp Immunol 180(3):361–370
23. Young NS (2018) Aplastic anemia. N Engl J Med 379(17):1643–1656
24. Danielle MT, Winkler T (2016) Nontransplant therapy for bone marrow failure. Hematol Am Soc Hematol Educ Program 1:83–89
25. Gidvani V, Ramkissoon S, Sloand EM, Young NS (2007) Cytokine gene polymorphisms in acquired bone marrow failure. Am J Hematol 82(8):721–724
26. Elkordy M, Crump M, Vredenburgh JJ et al (1997) A phase I trial of recombinant human interleukin-1b (OCT-43) following high-dose chemotherapy and autologous bone marrow transplantation and WP Peters. Bone Marrow Transplant 19:315–322
27. Cullinan MP, Westerman B, Hamlet SM, Palmer JE, Faddy MJ, Lang NP, Seymour GJ (2008) A longitudinal study of interleukin-1 gene polymorphisms and periodontal disease in a general adult population. J Clin Periodontol 28:1137–1144
28. Platanias LC, Vogelzang NJ (1990) Interleukin-l: biology, pathophysiology, and clinical prospects. Am J Med 89:621–629
29. Hemmati et al (2017) Inflammatory signaling pathways in pre-leukemic and leukemic stem cells. Front Oncol 7:265
30. Shete AR, Joseph R, Vijayan NN, Srinivas L, Banerjee M (2010) Association of single nucleotide gene polymorphism at interleukin-1beta +3954, −511, and −31 in chronic periodontitis and aggressive periodontitis in Dravidian ethnicity. J Periodontol 81(1):62–69
31. Amirisetty R, Patel RP, Das S, Saraf J, Jyothy A, Munshi A (2015) Interleukin 1beta (+3954, −511 and −31) polymorphism in chronic periodontitis patients from North India. Acta Odontol Scand 73(5):343–347
32. Masamatti SS, Kumar A, Baron TK, Mehta DS, Bhat K (2012) Evaluation of interleukin-1B (+3954) gene polymorphism in patients with chronic and aggressive periodontitis: a genetic association study. Contemp Clin Dent 3(2):144–149

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.