Human Leukocyte Antigen (HLA) of DR type and its association with Children acute lymphoblastic Leukemia (ALL) in Basrah.

Thanaa Khaleel Ibrahim, Department of Biology, College of Science, University of Basrah, Basrah , Iraq. Email: thana.kaleel@yahoo.com; Mobile: +9647722050626; ORCID: http://orcid.org/0000-0003-0878-8114
Awatif Hamid Issa, Department of Pathological Analysis, College of Science, University of Basrah, Basrah , Iraq. Email: awatifhi@gmail.com; Mobile: +9647801059989; ORCID: http://orcid.org/0000-0001-8953-5308
Ahmed Abd Al Wahid Salman, Al-Sadder Teaching Hospital, Basra Health Directorate, Basrah , Iraq. Email: ahmedsalmann@yahoo.com; Mobile: 07707027659; ORCID: http://orcid.org/0000-0001-8537-0972

Correspondence author: Awatif Hamid Issa, Department of Pathological Analysis, College of Science, University of Basrah, Basrah , Iraq. Email: awatifhi@gmail.com; Mobile: +9647801059989
ORCID: http://orcid.org/0000-0001-8953-5308

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Abstract:
Background: Similar to other diseases, there are obvious correlation between human leukocyte antigen (HLA) and resistance to cancer.
Aim: To clarify the association between the alleles of HLA of DRB1 type with childhood acute lymphoblastic leukemia (ALL).
Patients and methods: This study was performed Basrah Specialized Hospital for children and Al-Sadder Teaching Hospital in Basrah Governorate during the period from October 2017 to march 2018. A total of 98 cases of newly diagnoses cases of acute leukemia were registered. Of them, 72 cases were diagnosed as ALL and 25 of them were enrolled in this study. Of the 25 cases, 15 were male and 10 were female and their age ranged between 1 to 12 years. Ten samples from apparently healthy individuals were considered as control group. This study included DNA extraction from 200µl whole blood. Molecular HLA-DRB1 typing for Basrah patients with acute lymphoblastic leukemia and controls using PCR-SSP (sequence-specific primers).
Results: The most common allele in DRB1 locus in Basrah patients and controls was HLA-DRB1* 07, which forms 18% (9/50) in patients and 50% (5/10) in controls, with a significant difference (P=0.015). Whereas HLA-DRB1*03 and *04 were more predominant alleles in patients (22% and 14% respectively) as compared to controls (15% and 0%, respectively), the difference not statistically significant (P>0.05). OR indicated an association between HLA-DRB1*03, *04, *11,*14 and *15 (OR= 1.598, 8.2, 3.02, 4.95 and 2.11 respectively) and susceptibility to ALL development in children. However, this finding need to be confirmed in a large scale study. HLA-DRB1*04 was more frequent in male patients with ALL compared with male controls, but the difference was not significant (10%, P=0. 3).

OR confirm an association between HLA-DRB1*03 and ALL in both gender (OR= 1.46 for male and 1.65 for female). HLA-DRB1*03 and *04 were more frequent in patients (22% for each) with age of >10 years than that in controls, but the difference was not significant. While HLA-DRB1*07 was with higher frequency in controls (55%) with age of > 10 years as compared to patients (27%), but not reach significant level. OR indicated as association between ALL and HLA-DRB1*03 (OR=2.28) and *04 (OR=5.8), but not significant.

Conclusion: This study indicated an inverse significant association between HLA-DRB1*07 and childhood Acute lymphoblastic leukemia (ALL) in all strata of analysis and may play be a protective loci against ALL in childhood. In contrast, HLA-DRB1 *03 and *04 were more common in patients than in control and OR (> 1) indicated a positive association which may reflect child susceptibility for the development of ALL. However, these findings need confirmation in a large scale study.

Keywords: Human leukocyte antigen-DRB1, childhood acute lymphoblastic leukemia(ALL), Polymorphism, PCR-SSP Marker.

Introduction

Childhood Acute lymphoblastic leukemia (ALL) is highly frequent blood cancer in pediatric patients, malignant white blood cells continuously proliferated leading to an excess of lymphoblast's in the peripheral blood and the bone marrow [1]. ALL is widespread among children, and the average age is between 3-7 years old [2]. ALL can be divided into 3 risk group according to treatment response: a standard –risk group (SRG), a moderate- risk group with adequate early treatment response, and a high- risk group (HRG) [3]. The common Symptoms of pediatric leukemia include anemia, extreme fatigue, weakness, feeling cold, pale skin, and shortness of breath. Pediatric leukemia symptoms are generally associated with decrease of white blood cell counts that cause infection, fevers, and effect on the immunity system [4]. Two factors, genetic and environmental, linked to stimulate ALL [5]. It was recorded that acute leukemias is about 30% of all cancer types. It causes death to people under age 35 years old [6].

The major histocompatibility complex (MHC) is a genetic region located on the short arm of chromosome six (6p21) [7]. MHC in human named human leucocyte antigen that has about 200 genes are highly polymorphic which are essential in regulating the immune response [8]. The variation in human leukocyte antigen is a critical factor of transplant rejection. [9]. HLA-DR is an MHC- class II cell surface receptor encoded via the HLA, composed of Alpha and βeta chains (heterodimer) are attached in the membrane [10]. The DR gene family includes DRA gene and 9 DRB genes (DRB1 - DRB9) [11-12].

HLA class II proteins are found only on active B cells and active antigen presenting cells (APC), which composed of two chain α and β chain are encoded within human leukocyte antigen and has two extra-cellular domains [13]. HLA is considered as a guide to the immune system. It has function main that play essential role in presenting antigen to cell named T lymphocyte [14]. The polymorphic HLA genes of the MHC palmed in various disorder such as autoimmune disease, cancer and infectious disease [15].

Studies suggested that HLA associated with many diseases, in addition to the correlation of these proteins with susceptibility, resistance and genetic evolution of human [16]. This developed our knowledge in the relationship between HLA alleles and diseases such as childhood acute lymphoblastic leukemia [17]. HLA may contribute to the risk of developing leukemia not only via presentation of the viral peptide or other antigen particular to cytotoxic cell (CD8) but also through escape from host immune surveillance mechanisms [18-19]. Several studies have demonstrated a link between HLA class II and lymphocytic leukemia such as DR5, DR3, and DR4 [20].

Aim: To clarify the association between the alleles of HLA of DRB1 type with childhood acute lymphoblastic leukemia (ALL).

Materials and Methods

Patients and Controls

The study was conducted in Al Basra Specialized Hospital for children and Al-Sadder Teaching Hospital in Basra Governorate during the period from October 2017 to march 2018. EDTA whole blood were collected from 25 patients and 10 control. Twenty-five patients were diagnosed and registered as acute lymphoblastic from them 10 female and 1 male aged between 1 to 12 years. The diagnosis of ALL was done by the hematology specialist in Hospital which based on blood film and bone marrow examination.

DNA Extraction:

The human DNA were purified by using QIAamp® DNA Mini and Blood Mini Kit; Qiagen, Germany, then we measured the concentration of the genomic DNA by using Nano drop (Nano photometer). The DNA purity were A260/280= 1.8-2.0, then stored at Deep freeze -20 ºc for use in PCR.

Amplification HLA-DRB1

HLA-DRB1 type was performed according to SSP technique (sequence specific primers) by using protrans HLA-DRB1* kit (Germany, 2016). The cycling program involved Initial denaturation at 94 ºc for 2 min followed by 10 cyclers of Denaturation at 94 ºc for 15 sec, Annealing & Extension at65 ºc for 60 Sec and followed by 20 cyclers of Denaturation at 94 ºc for 15 sec, Annealing at 61 ºc for 50 sec, and Extension at 72 ºc for 30 ºc. The electrophoresis using 1.5 gm agarose gel were done, After PCR, DNA bands were detected and examined under UV trans illuminator, then photographed by Darke hood. Clarification was achieved by using the start score software program we depended for analysis of bands. This program is used to determine HLA type.

Statistical Analysis:

Data analysis done by using SPSS program. The results of the investigated groups for the selected HLA alleles were analyzed using the Chi-square with Yates correction. For Odd ratio we used Fishers exact test, 95% confidence intervals were estimated according to Bland Altman limit, 1991. $P<0.05$ was considered to indicate statistically significant differences.

Results

During the collection of samples from the Specialized Child Hospital, 98 cases of leukemia were newly registered, 72 of them were diagnosed as acute lymphoblastic leukemia (ALL) cases and 26 cases as (AML).

Molecular and genetic study:

Determination of HLA typing:

HLA typing were identified by SSP method and the figures 1 and 2 show the band of amplified DNA in the agarose gel.

Patients characteristics:

A total of 72 cases newly diagnosed cases of ALL were registered in children Specialty Hospital in Basrah, 25 of them were enrolled in this study during the period from October 2017 to march 2018. Of them 15, were males and 10 were females. The male to female ratio was 1.5:1; at the time of diagnosis, patients mean age was 6.368 ±4.1081years (range,1-12 years). The total WBC count in 96% of the patients was less than 50,000/mm3 and 4% had a total WBC count ≥50,000/mm3.

Allele frequency shows that the HLA-DRB1*03,* 04, and *07 were the most frequent alleles in the patients (22%, 18%, and 14% respectively). HLA-DRB1*07 allele was more common in patients and controls, but it was significantly higher in controls (P<0.015). Although other alleles were more frequent in patients, however, the difference was not significant (P>0.05), Table 1. OR indicated an association between HLA-DRB1*03, *04, *11,*14 and *15 (OR= 1.598, 8.2, 3.02, 4.95 and 2.11 respectively) and susceptibility to ALL development in children.

Distribution of HLA allele in male Acute lymphoblastic leukemia.

This study shows that HLA-DRB1*07 was more common in male patients and male control and the frequency was diverted to controls (P=0.0382). While HLA-DRB1*03 and HLA-DRB1*04 alleles were with higher frequency in male patients as compared to male controls (14% versus 10%), but the differences were not statistically significant, Table 2.

Distribution of HLA Allele in Female Acute lymphoblastic leukemia.

In our study, the frequency differences between female patients and female control was not significant (P >0.05). Additionally, HLA-DR*03, HLA-DRB1*08, and*09 were more frequent in patients with ALL compared to the controls. Distribution of HLA allele in female ALL are shown in Table 3.

Distribution of HLA Allele in ALL more than 10 year

The frequency of HLA-DRB1*03 and HLA-DRB1*04 were more frequent in patients (22% for each) than in controls (11% and 0% respectively) in those with age of > 10 years. While HLA-DRB1*07 alleles were more frequent in controls (55%) than in patients (27%). However, these differences not statistically significant, Table 4.

Discussion

The present study shows that the more frequent allele in DRB1 locus in pooled sample of patients and controls in Basra was HLA-DRB1* 07 with a significant difference between patients and control groups. Additionally, OR confirm an inverse association between this DRB1 locus and development of ALL in children in this study cohort. Thus HLA-DRB1 *07 locus may play a role in children susceptibility to ALL development. However, this finding need to be confirmed in a large scale study. While HLA-DRB1*03, *04, *09, *014, and *015 alleles were more frequent in patients as compared to controls, but the difference was not statistically significant. None significant difference may be attributed to small sample size. OR (≥1.5) suggests that ALL in children was with positive

association with HLA-DRB 1 *03, *04, *14 and *15 alleles. Gender not influenced the frequency distribution of the tested HLA-DR loci, however, this finding must be confirmed in a large study population. Age of children of more than 10 years was associated with HLA-DRB1 *03 and *04 loci as demonstrated in frequency distribution and OR estimation.

In 2013, Urayama *et al* [20] demonstrated the correlation between DR*07 allele with acute lymphoblastic leukemia (ALL), that goes with our finding. On the other hand, the present study shows that HLA-DRB1*03 and *04 were higher among ALL patients as compared to control group (22%, 14%, respectively), but the difference not statistically significant (P>0.05). In some studies such as a study performed on patients with ALL in Turkey shows that the HLA-DRB1*03 and HLA-DRB1*04 alleles were significantly higher in patients with ALL (P=0.003, P=0.002, respectively), and this study also demonstrated that the HLA-DRB1*03, *04 alleles may play a predisposing role in patients with ALL [3]. Another study done on Moroccan patients confirmed that HLA-DRB1*03 and HLA-DRB1*04 alleles were more frequent in patients compared to that control [21]. Yari *et al*; (2008) carried a study in Iran on patients with ALL and they found an association between ALL and frequency of HLA-DRB1*04 (16%, P=0.188), may be considered a susceptible allele for children with ALL [22]. Dorak *et al*; (1999) reported that the frequency of the HLA-DRB1*04 was significantly higher in patients with ALL (P=0.0005).

Fernandes *et al* (2010) carried a study in South-eastern Brazil on 15 patients with ALL and they found frequent significant association between ALL and HLA-DRB1*03 allele (P=0.0086), may be considered a predisposing allele for childhood ALL. While the frequency of HLA-DRB1*04 were significantly decreased in Brazilian patients (P=0.02), this allele could be considered a protective allele for childhood ALL and their finding was not goes with our finding [23,24]. In contrast, the present study findings suggest that HLA-DRB1*04 and *03 alleles were a predisposing allele for ALL development, while HLA-DRB1*07 was protective allele as confirmed by OR estimation. On the other hand, Zhou and Zhao, (2005) they found that the HLA–DRB1*03 allele were higher decreased in Hans patients (P<0.05), and they suggest that this allele may be resistance for leukemia [25].

This study shows that HLA-DRB1*07 allele was significant lower in male patients (P=0.0382) than in controls. In addition, HLA-DRB1*03 and HLA-DRB1*04 alleles were with higher frequency in male patients with childhood ALL compared to male control, but the difference was not statistically significant (P>0.05). However, other studies reported a significant differences between male patients and male controls [23, 26]. Dorak *et al*; (1999) in U.K on patients with children ALL and they found that the patients with HLA-DRB1*04 were a significantly higher in male compared with male control group (P<0.005; OR=2.9; 95% CI = 1.6 to 5.4) [23]. On the other hand, Ozdilli *et al* (2010)[26] carried out a study in Istanbul on patients with childhood acute lymphoblastic leukemia and they found that HLA-DRB1*04 allele could be a risk factor for childhood (ALL), the frequency of the HLA-DRB1*04 allele was significantly higher in male ALL patients as compared with control (P=0.004) [26]. Also Urayama *et al* (2013)

carried out a study in Turkey and they found that HLA-DRB1*04 allele were higher in male patients with ALL [20].

In the present study there was no significant difference between female patients and female control for the HLA-DRB1*03, *08, and *09 frequency (P>0.05). However, the frequency of the HLA-DRB1*03, *08, and *09 alleles were higher in female patients than in controls and this not agreed with other study [5]. For example, A study done in Egypt on patients with childhood ALL which demonstrated that HLA-DRB1*04 allele were significantly more frequent in female compared to female control group (P=0.03) [5].

In this study, in children with age of more than 10 years, HLA-DRB1*04 and HLA-DRB1*03 were more frequent in patients compared to controls, but the differences were not statistically significant. However, Elansary *et al* (2015) reported significant difference between patients and control for the frequency of HLA-DRB1*04 according to the age of <10 years compared to age >10 years (P =0.03) [5]. In children with age of > 10 years of age, the frequency distribution of HLA-DRB1*07 was more frequent in controls as compared to patients with ALL.

Conclusion

This study indicated an inverse significant association between HLA-DRB1*07 and childhood Acute lymphoblastic leukemia (ALL) in all strata of analysis and may play be a protective loci against ALL in childhood. In contrast, HLA-DRB1 *03 and *04 were more common in patients than in control and OR (> 1) indicated a positive association which may reflect child susceptibility for the development of ALL. However, these findings need confirmation in a large scale study.

Table 1: Distribution of HLA allele.

Allele	Case		Control		OR	95% C.I.	X^2	P
	No	%	No	%				
*01	2	4	1	5	0.791	0.067-9.25	0.432	0.51
*03	11	22	3	15	1.598	0.394-6.47	0.109	0.74
*04	7	14	0	0	8.2	0.45-149	2.2	0.13
*07	9	18	10	50	0.26	0.086-0.83	5.86	0.015
*08	5	10	1	5	1.21	0.118-12.4	0.04	0.84
*09	6	12	1	5	1.24	0.048-31.78	0.19	0.66
*10	0	0	1	5	0.073	0,003-1.59	0.22	0.63
*11	1	2	0	0	3.02	0.14- 61.17	0.4	0.52
*12	0	0	1	5	0.38	0.023- 6.59	0.22	0.63
*13	1	2	0	0	1.24	0.048-31.78	0.4	0.52
*14	4	8	0	0	4.95	0.261- 93.9	0.53	0.46
*15	2	4	0	0	2.11	0.09- 45.98	0.013	0.9
*16	2	4	2	10	0.18	0.015- 2.15	0.16	0.68
Total	50	100	20	100				

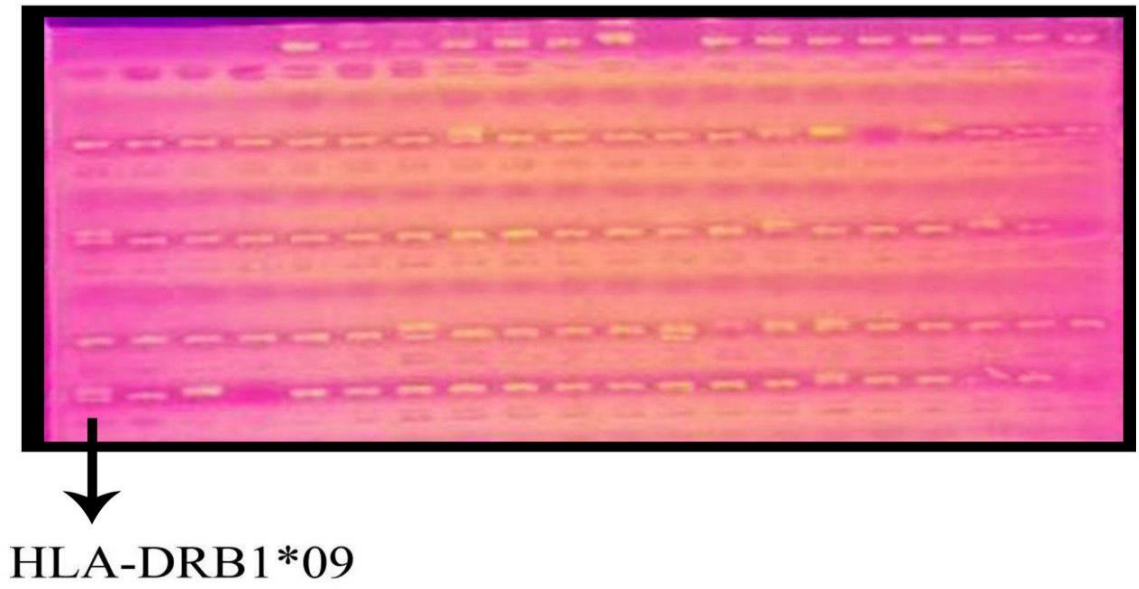

Figure 1: Demonstration of HLA type ex. HLA-DRB1*09

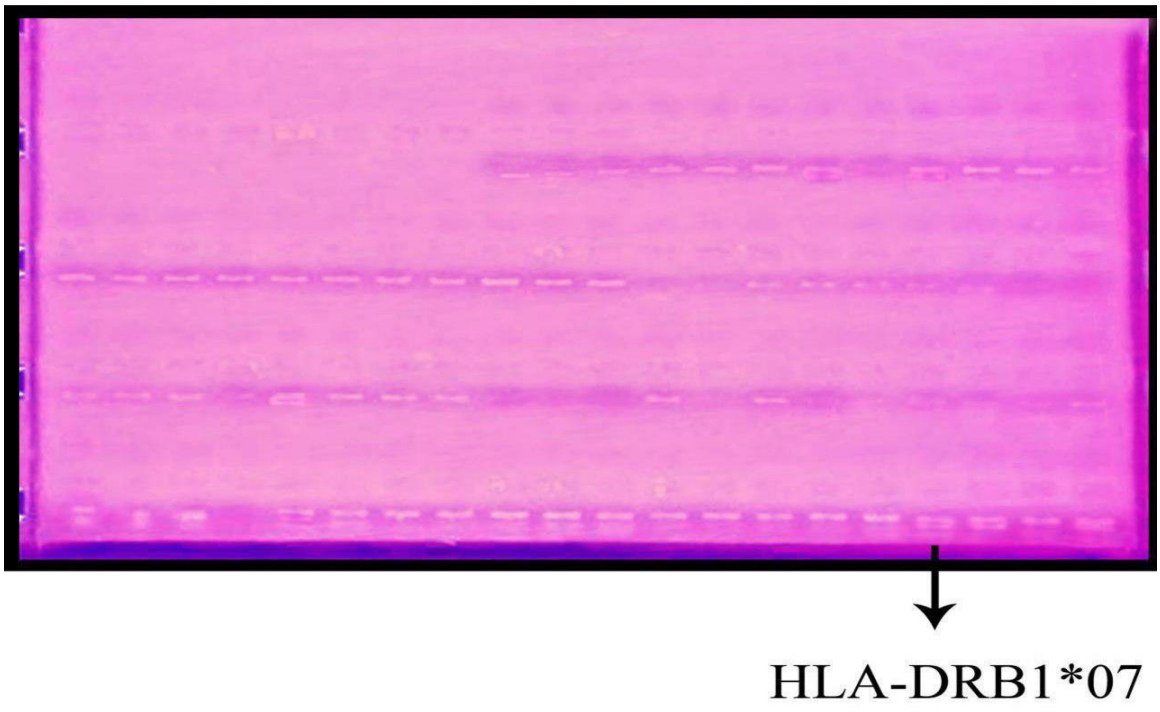

Figure 2: Demonstration of HLA typing ex. HLA-DRB1*07

Table 2: Distribution of HLA allele in male Acute Lymphoblastic Leukemia.

Allele	Case		Control		OR	95% C.I.	X^2	P
	No	%	No	%				
01	1	2	0	0	1.24	0.049-31.78	0.4	0.5
03	7	14	2	10	1.46	0.199 -3.31	0.03	0.9
04	5	10	0	0	-	-	0.9	0.3
07	7	14	8	40	0.244	0.955-1.444	4.295	0.0382
08	1	2	1	5	0.38	2.4 -3.2	0.4	0.4
09	2	4	1	5	0.8	1.6 -3.25	0.03	0.8
10	0	0	1	5	-	-	0.2	0.6
11	0	0	0	0	-	-	-	
12	0	0	0	0	-	--	-	
13	1	2	0	0	1.24	0.049-31.781	0.4	0.5
14	3	6	0	0	-	-	0.2	0.6
15	1	2	0	0	1.24	0.049- 31.781	0.4	0.5
16	2	4	1	5	0.79	1.6-3.2	0.03	0.4
Total	30	60	14	70				

Table 3: Distribution of HLA allele in female Acute lymphoblastic leukemia.

Allele	Case		Control		OR	95% C.I.	Chi-square	P
	No	%	No	%				
01	1	2	0	0	1.24	0.0486 to 31.781	0.4	0.5
03	4	8	1	5	1.65	0.6 -3.9	0.19	0.6
04	2	4	0	0	-	-	0.013	0.9
07	2	4	2	10	0.37	1.6-2.4	0.166	0.6
08	4	8	0	0			0.4	0.5
09	4	8	0	0			0.4	0.5
10	0	0	1	5	-	-	0.2	0.6
11	1	2	0	0	1.24	0.0486 to 31.781	0.4	0.5
12	0	0	1	5				
13	0	0	0	0				
14	1	2	0	0	1.24	0.0486 to 31.781	0.4	0.5
15	1	2	0	0	1.24	0.0486 to 31.781	0.4	0.5
16	0	0	1	5	-	-	0.2	0.6
Total	20	40	6	30				

Table 4: Distribution of HLA allele in ALL more than 10 year.

Allele	Case		Control		OR	95% C.I.	Chi-square	P
	No	%	No	%				
*01	0	0	1	11	0.15	0.005 to 4.161	0.13	0.72
*03	4	22	1	11	2.28	0.216 to 24.141	0.03	0.86
*04	4	22	0	0	5.8	0.283 to 122.55	0.97	0.33
*07	5	27	5	55	0.31	0.058 to 1.636	0.97	0.32
*08	0	0	0	0	-	-	-	-
*09	1	5	1	11	0.47	0.026 to 8.522	0.27	0.6
*10	0	0	1	11	0.15	0.005 to 4.161	0.13	0.72
*11	1	5	0	0	1.6	0.06 to 44.009	0.52	0.47
12	0	0	0	0	-	-	-	-
13	1	5	0	0	1.6	0.06 to 44.009	0.52	0.47
14	0	0	0	0	-	-	-	-
15	1	5	0	0	1.6	0.06 to 44.009	0.52	0.47
16	1	5	0	0	1.6	0.06 to 44.009	0.52	0.47
Total	18	100	9	100				

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