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The significance of serum anti- rituximab (anti-CD20 monoclonal antibody) antibodies in treatment response of patients with B- cells malignancy

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ABSTRACT

Background: The chimeric anti-CD20 monoclonal antibody, rituximab, is an effective therapeutic agent used for treating patients with B- cell disorders, however there is a significant variation in therapy response among patients and relapse is common. Furthermore, it is unclear why a class of patients is initially not responding and other responding become refractory and later resistant to further treatment.

Aim: To study the significance of serum anti- rituximab antibodies in the patients initially responding to the treatment and became refractory and resistant to the treatment.

Patients and Methods: Serum samples were obtained from forty two patients with B- cells disorders after getting their consent, the serum samples were tested for the serum anti- rituximab antibodies by enzyme linked immunosorbant assay (ELISA). They received rituximab at initial dose of 375 mg/m^2 and a maintenance dose of 500 mg/m^2. The serum samples were obtained from each patient after subsequent doses of treatment.

Results: The patients' ages ranged between 20 -80 years. The response rate 50 days after treatment initiation was 40(95%). During the first year of treatment, the complete remission was found in 33(75%) of the patients and 9(21%) of the patients developed the non responsiveness to treatment. The serum level of anti- rituximab antibodies was detected among 15(36%) of the patients, only 2(22%) of them were among the 9 patients who developed the treatment non responsiveness and the other 13(39%) were among the complete remission patients with no significant difference statistically.

Conclusion: In conclusion, these results indicate that there was no significant association between the level of the serum anti rituximab antibodies and the response to rituximab treatment.

Recommendations: We recommend to include a lager sample size of patients receiving retuximab for treating diseases other than B cells disorders rheumatoid arthritis, SLE and multiple sclerosis patients.

Key words: Immunotherapy, Rituximab, B- cell malignancy, Chimeric antibodies

Introduction

Antibody-mediated immunotherapy has gained significant momentum since the first FDA-approved monoclonal antibody (mAb) in 1997, namely Rituximab which is a chimeric anti-CD20 mAb [1,2]. It is directed against cell surface membrane receptors,

CD20, expressed on mature B cells but not on pre-B cells or plasma cells [3]. The receptor CD20 is a tetramembrane spanning molecule of molecular weight 33–37 kDa and the gene is located on chromosome 11q12-q13.1

Subsequently, over 20 approved mAbs have been in use clinically for the treatment of various cancers and several non-cancer related diseases [4]. Further, the combination treatment of mAbs with chemotherapy, immunotherapy, proteaosome inhibitors and other inhibitors have resulted in synergistic anti-tumor activity with significant objective clinical responses. Despite their successful clinical use, the underlying mechanisms of rituximab *in vivo* activities remain elusive. Further, it is not clear why a subset of patients is initially unresponsive and many responding patients become refractory and resistant to further treatments; hence, the underlying mechanisms of resistance are not known, attempts have been made to develop model systems to investigate resistance to mAb therapy with the hope to apply the findings in both the generation of new therapeutics as well as their use as new prognostic biomarkers [5]. A major limitation of both conventional and targeted therapies is that a subset of patients does not initially respond to such therapies and another responding subset develops resistance to further treatments. Hence, many malignant cancers exhibit both intrinsic and acquired resistance. While the use of rituximab for treatment has been successful, however, a subset of patients has an innate resistance. The mechanisms of resistance *in vivo* are not clear. Several mechanisms have been reported including inhibition of ADCC by deposition of C3 activating fragments [6], polymorphism of the FcγRIIIa on cytotoxic cells [7,8], inhibition of CDC [9], loss of CD20 expression on the surface of subclones [10], overexpression of anti-apoptotic gene products (eg Bcl2) [11], CD20 mutations [12], shedding of CD20 Rituximab complexes [13] and the tumor micro-environment [14]. Development of the antibodies against the retuximab by the patient's immune system due to recurrent doses is not clearly reported, since the anti retuximab antibodies are neutralizing and then develop the treatment resistance. In the current study, we are aiming at estimating the significance of the anti retuximab antibodies in developing therapy resistance in patients with B cells disorders.

Patients and methods

Patients

The present study was conducted on patients suffering from B cells disorders, who were registered to be treated in the oncology-hematology unit in Azadi Teaching Hospital in Duhok city between January 2018 to November 2018. Forty two patients with B- cells disorders were recruited in the study, they were diagnosed at the oncology- hematology unit as having the B-cell hematologic malignancy, and all of the recruited patients have started the rituximab infusion empirical treatment as initial dose and then the maintenance dose. Their ages ranged between 20-80 years.

Methods

The patients were already diagnosed as B cells disorders patients based on serial complete blood count, peripheral blood smear examination, flourescent in situ hybridization, and bone marrow examination, at baseline. Sera samples were obtained from each patient after subsequent doses of retuximab treatment, the consent has been obtained from each patient.

The presence of anti- retuximab antibodies in the patients' sera was estimated by the ELISA as described by Arnold *et al*. (2004) [15] with some modifications. Briefly**,** Maxisorp plates (Nunc, Roskilde, Denmark) were coated with 50 ml retuximab (1 mg/ml) in 0·1 M NaHCO3 pH 9·5.After three washes with wash buffer [20 mMHEPES, 140 mM NaCl, 5 mM CaCl2, 0·1% (v/v) Tween 20, pH 7·4], wells were incubated with 200 ml blocking solution [skimmed milk, 0·1% (v/v) Tween-20, pH 7·4] overnight at 4°C and washes repeated. Patients'sera, were diluted 1 : 1 in serum dilution buffer [40 mMHEPES, 2 MNaCl, 10 mMCaCl2, pH 7·4] and 50 ml per well added. Following incubation wells were washed three times with wash buffer. Bound anti-retuximab antibodies were detected by horseradish peroxidase-conjugated monoclonal anti-human IgG antibodies (Sigma, Poole, UK) in wash buffer. Substrate was tetramethyl benzoate (Sigma). All samples were tested in duplicate. The negative control wells were used in the plate for comparison. The serum samples were considered positive for the anti- retuximab antibodies when color of the reaction changed.

Statistics

The association between treatment response and the level of serum anti-retuximab antibodies analysed using the χ^2 test.

Results:

A total of 42 B-cells malignancy patients were involved in the study, all of them were receiving retuximab for different doses and periods, 22(52%) of them were males with age range 20-80 years (mean 46 ±4 years) and 20(48%) females with age range 25-70 years (mean 42±4 years) as shown in table 1.

Table 1: Demographic characteristics of the patients

Patients	No.(%)	Age range (y)	Age mean (y±sd)
Males	22 (52)	20 - 80	46 ± 4
Females	20 (48)	25 - 70	42 ± 4
Total patients	42		

All of the patients were received rituximab as empirical regimen at initial does of 375 mg/m2 and followed by maintenance dose of 500mg.m^{-2}. The response rate of 50 days after treatment initiation was 40(95%) and the other 2 patients did not respond to the treatment. During the first year of treatment and a media follow up duration of 11

months , the complete remission was found in 33(75%) of the patients and 9(21%) of the patients developed the non responsiveness to treatment, table 2.

Table 2: Response rate of B cells malignancy patients to retuximab therapy.

Response rate 50 days after initial dose	Complete remission during the first year of treatment	Non responsiveness to treatment
40 (95%)	33 (75%)	9 (21%)
Total No. of patients	42	

The presence of serum anti- rituximab antibodies was measured by ELISA, the antibodies were detected in 15(36%) patients, the others gave negative results for the antibody presence, only 2(22%) of them were among the 9 patients who developed the treatment non responsiveness and the other 13(39%) were among the complete remission patients with no statistical significant difference ($p < 0.001$), as shown in table 3.

Table 3: Anti -retximab antibodies in the sera of the patients treated with subsequent doses of retuximab detected by ELISA.

	Treatment non responsiveness patients (%)	Complete remission patients (%)	Total (%)
No. of patients positive (+) for anti rituximab antibodies	2 (22%)	13 (39%)	15 (36%)
No. of patients Negative (-) for anti rituximab antibodies	7 (78)	20 (61)	27 (64)

Discussion:

This study described the possibility of the immune system to develop anti-rituximab antibodies in B cell disorders patients treated with rituximab which in turn establishes a mechanism of treatment resistance. Although rituximab efficacy in treating patients of B-cell hematologic malignancies is well studied, some patients are refractory to the initial dose of treatment, and others may experience relapse after they have responded to the initial dose. In the current study, we found that 21% of the B-

cell malignancy patients did not respond to the initial dose of treatment and 25% of them experienced relapse after initial response to the treatment. These results are comparable with those of a study conducted by Davis et al [16], they found that 30% of the patients were partially responding to rituximab treatment. On the other hand, our results are inconsistent with those obtained by McLaughlin et al [17] when they found that 52% of the patients did not respond to the treatment.

The exact mechanisms of the development of resistance to rituximab treatment in B- cells amlignancy remain unclear. However, given the reliance of rituximab on immune-effectors mechanisms for its antitumor efficacy, it has been hypothesized that the host immunologic environment may both play a role [18]. Rituximab is a human/murine chimeric, glycosylated immunoglobulin (Ig) G1-κ mAb containing murine light- and heavy-chain variable region sequences, and human kappa and human IgG1 constant region sequences, it has specific affinity for the B-lymphocyte transmembrane protein, CD20, which is expressed on normal B cells (excluding stem cells, pro-B cells, and plasma B cells) and on most malignant B cells [19]. Since the rituximab is a chimeric monoclonal antibody, the anitigenicity is proposed, which could induce the host immune system and the activation of the immune system may interfere with the efficacy of rituximab action and impairment of the treatment response. Based on the composition of the rituximab, patients treated with multiple doses of rituximab may develop antibodies against murine variable region sequence, named as "human antichimeric antibody" they have the potential to mount immunologic reactions when subsequently treated with other antibodies. We found in the current study that number of the patients who developed the human antichimeric antibody in their sera was 15/42(36%), only 2(22%) of them were among the treatment non responsiveness patients, and the other 13(39%) were completely remitted patients, and there was no statistical correlation between the resistance to treatment and the presence of anti rituximab antibodies in the patients' sera. Those results are in agreement with clinical trials in patients with B-cell malignancies, up to 2% of patients developed human antichimeric antibody, but no effect of this antibody on the efficacy or safety of rituximab has been demonstrated [20]. The non effectiveness of the anti-retuximab antibodies developed in by the immune system of the patients treated frequently could be due to the immunologic fact that those anti bodies are not necessarily targeting the variable region of the paratop of the antibody that in turn targets the B-cell CD20 transmembrane protein, so it might not interfere with mechanism of action of rituximab.

Conclusion

In conclusion, these results indicate that there was no significant association between the presince of the serum anti rituximab antibodies and the response to rituximab treatment in patient with B-cell malignancy.

Conflict of interests Nothing to declare.

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