Comparative Evaluation of the Effect of DTT Treatment and Heat Inactivation on ABO Isoagglutinin Titers

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

Original Research Article	*Received: 10/01/2024* *Accepted: 16/03/2024* *Published: 27/03/2024*

ABSTRACT

Background and aims: DTT treatment and heat inactivation are the two common methods used for removing the interference of IgM antibodies which can affect the accuracy of IgG measurements. By comparing the effects of these treatments on ABO isoagglutinin titers using two different techniques of titration namely conventional test tube technique (CTT) and column agglutination technique (CAT), the study aims to determine which method is more effective or suitable for this purpose.

Materials and methods: Conducted between October 2019 to March 2020, this was a prospective, observational study which included A, B and O group donors. Each donor's serum was treated with DTT and heat. Titrations were performed by CTT and CAT both before and after treatment.

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Results: A total of 300 whole blood donors participated in this study; 100 each for group A, B and O. Anti-A and anti-B IgG titers of group O individuals were higher than corresponding titers of non-O individuals. Anti-A titers in group O individuals were slightly higher than anti-B titers, whereas anti-A and anti-B titers from non-O individuals were found to be similar. Heat treated titers were higher than DTT treated titers. Median IgG titers were higher than median IgM titers. Group O titers were higher than non-O titers.

Conclusion: DTT treatment was more effective than heat inactivation in terms of efficiently eliminating IgM activity. This study provides insights into the optimal methods for diminishing interference and obtaining accurate measurements of ABO IgG titers, which are crucial for various medical and research applications

1. INTRODUCTION

"ABO blood group determination is a fundamental component of pre-transfusion testing".[1] ABO isoagglutinins can cause serious complications if not taken in account during pre-transfusion testing leading to renal failure due to hemoglobinuria, disseminated intravascular coagulation (DIC), and ultimately death in severe cases.[2] "The presence of ABO isoagglutinins also plays a crucial role in the outcomes of ABO-incompatible solid organ and hematopoietic stem cell transplants".[3-6] "In the complications of ABO incompatible solid organ transplants like hyper acute graft rejection and those of ABO incompatible hematopoietic stem cell transplants like pure red cell aplasia and delayed engraftment, these ABO isoagglutinins are important".[7,8] Hence determining the concentration of these isoagglutinins is very crucial. The measurement of ABO isoagglutinins is typically done using a semi-quantitative method known as titration. Outcome of solid organ transplants and hematopoietic stem cell transplants largely depends on determining and monitoring the titres of ABO isoagglutinins..

"The immunoglobulins IgG and IgM antibodies have an important role in any transfusion service. In A and B blood group individuals, anti-A and anti-B are predominantly of IgM type while in O blood group individuals these are predominantly of IgG type. The IgM antibodies can mask the concentration of IgG antibodies during titration".[1] In order to accurately measure the concentration of IgG antibodies, particularly in situations like in O blood group individuals, where IgM antibodies may interfere with the measurement, it's essential to inactivate or eliminate the IgM antibodies beforehand which can be done by the use of heat inactivation and certain sulfhydryl reagents such as dithiothreitol (DTT) and 2-mercaptoethanol (ME).[9,10] These treatments have least effect on the IgG antibodies as they have much less labile disulphide bonds as compared to IgM antibodies.[11] However, they can be slightly affected.[12] In situations where IgM interference can be suspected, the use of IgM inactivating treatments has been recommended as routine part of pre-transfusion testing. [13,14]

"In cases where DTT may not be readily accessible due to its high cost, heat inactivation can be considered as a cheap and easy alternative to DTT in low resource settings. However, the effects of DTT and heat inactivation has not been compared for their effect on titres of ABO isoagglutinins" [13,14].

"While the conventional test tube technique (CTT) has been a longstanding method for titration and is often considered a standard, it does come with certain limitations such as Its laborious and time-consuming nature. Additionally, the manual handling involved in CTT can lead to technical errors, causing potential inaccuracies in the results. Moreover, variations in interpretation among different observers can introduce inter-observer variability, further complicating the reliability and consistency of the measurements. Automated immunohematology analysers offer high throughput, allowing for rapid processing of a large number of samples and their ease of use reduces the burden on laboratory personnel and minimizes the potential for human errors. Furthermore, they can contribute to standardization by reducing inter-observer and inter-laboratory variations compared to CTT" [13,14]. "Techniques employed by these automated analysers, such as column agglutination technology (CAT) and solid-phase red cell adherence (SPRCA) or hemagglutination (HA), offer several advantages, including

enhanced sensitivity, specificity, and reproducibility of test results. There are studies which compare results obtained using different methods of titration". [15-21] Many of these techniques conclude that the results of the age old CTT do not correlate with the results obtained by newer techniques. [1,22-24]

The present study aimed to compare the effect of DTT treatment and heat inactivation on ABO isoagglutinin titers performed by CAT and CTT.

2. MATERIALS AND METHODS

2.1 Settings and Design

This was a prospective, observational study which was conducted in the department of Transfusion Medicine at a tertiary healthcare center from October 2019 to March 2020. Each donor's serum was divided into two parts. One part was treated by DTT and other by heat. Anti-A and anti-B titres of treated serum samples were performed by CTT and CAT. All results were recorded and compared later.

2.2 Study Population

The study included consecutive donors with A, B, and O blood groups, regardless of their Rh factor, who met the eligibility criteria for blood donation according to the Drugs and Cosmetics Act, 1940, and the Standards for Blood Banks and Blood Transfusion Services. [25,26] Donors of blood group AB were not included in the study due to the absence of anti-A and/or anti-B isoagglutinins in their plasma. The study utilized pilot tubes collected during the blood donation process for titration. After conducting routine testing on the blood samples collected, such as blood typing and screening for infectious diseases, antibody titration was performed using the remaining sample. This titration was conducted either on the same day as the collection or on the following day. If tested on the next day, the sample was stored at 4°C. Donors who did not give consent to participate in the study, reactive for transfusion transmitted infections, positive for direct antiglobulin test or antibody screen were excluded from the study.

2.3 Methods of Titration

2.3.1 Conventional Test Tube Technique (CTT)

"Titration by CTT was performed according to a standardized and widely accepted approach as described in the American Association of Blood Banks (AABB) technical manual". [4] The titer endpoint in the titration process was determined by the reciprocal of the lowest dilution at which 1+ agglutination was observed with the naked eye. The reactions were recorded separately for IgM and IgG antibodies on a case reporting form.

2.3.2 Column Agglutinnation Technique (CAT)

* **For IgM titer determination:** Neutral Ortho BioVue System cassettes (Ortho Clinical Diagnostics, Raritan, New Jersey, USA) were used while.

* **For IgG titer determination:** Anti-IgG Monospecific Ortho BioVue System cassettes (Ortho Clinical Diagnostics, Raritan, New Jersey, USA) were used.

The endpoint was determined based on the lowest dilution yielding 1+, 2+, and 3+ agglutination visible to the naked eye. Again, the reactions were recorded separately for IgM and IgG antibodies on a case reporting form, ensuring comprehensive documentation of the titration results for each donor.

2.3.3 DTT preparation

0.01M DTT was prepared by dissolving 0.154 grams of DTT in 100 ml of phosphate buffered saline (PBS) with a pH of 7.3, following the steps outlined in the AABB technical manual. [4]

2.3.4 DTT treatment of serum

In the method described in the AABB technical manual, serum was treated with 0.01M DTT.[4] Equal volumes of serum and 0.01M DTT solution were mixed. The mixture was then incubated for 30 to 45 minutes at 37°C, with mixing every five minutes. This incubation period allowed the DTT to effectively reduce any disulfide bonds present in the serum proteins. After incubation, the mixture was used to prepare serial dilutions. These dilutions were then subjected to both IgM and IgG antibody titration using CAT. A dilution control was prepared by mixing one volume of patient serum with one volume of PBS. This mixture served as a control to ensure that the dilution process did not reduce the reactivity of the antibodies present in the serum. The dilution control was then used separately for serial dilutions and titration.

2.3.5 Heat inactivation of plasma

By following the protocol outlined in the American Society for Histocompatibility and Immunogenetics (ASHI) Laboratory Manual, heat inactivation of plasma was performed. [27] Plasma samples were heated at 63°C for exactly 13 minutes using a pre-heated heat block. After heating, the tubes containing the plasma were removed from the heat block and subjected to centrifugation. Following centrifugation, the supernatant was removed and transferred into another labelled test tube. The supernatant containing the heat-inactivated serum was then used for titration.

2.4 Statistical Analysis

The data was entered in MS excel sheet (version 16.0, 2016, Microsoft Corporation, Washington, USA). Numerical values, percentage, mean and standard deviation were calculated and SPSS software (Version 25.0.0.0) was used for statistical analysis.The distribution of post DTT and post heat inactivation anti-A and anti-B IgG titres done by CTT and CAT was plotted on a box and whisker plot. Post-DTT and post-heat inactivation (post-HI) IgM and IgG median titres obtained by CTT and CAT were also calculated. Correlation between the treatments was tested using Spearman's rho for all samples. The strength of the correlation was calculated using the following guide for the absolute value of r_s:

    0.0-0.19 - very weak
    0.20-0.39 - weak
    0.40-0.59 - moderate
    0.60-0.79 - strong
    0.80-1.0 - very strong

3. RESULTS

A total of 300 whole blood donors participated in this study which included 100 donors each from group A, B and O. Out of them 79 % were males and 21% were females. Mean age for blood group A was 31.25±6.12 years, for blood group B was 32.59±7.62 years and for blood group O was 31.91±7.8 years.

A box and whisker plot illustrating the distribution of anti-A and anti-B IgG titers post HI treatment and post DTT treatment performed by CTT has been shown in Fig. 1. Anti-A and anti-B IgG titers of non-O individuals were lower than corresponding titers of O group individuals. Anti-B titers in group O individuals were slightly lower than anti-A titers. Anti-A and anti-B titers from non-O individuals were found to be similar. DTT treated titers were lower than heat-treated titers.

A comparison of median anti-A and anti-B titers after HI and DTT treatment performed by CAT and CTT has been shown in Fig. 2. Median heat-treated IgM titers were higher than median DTT treated IgM titers when performed by CTT and CAT. Median IgG titers were higher than median IgM titers. Group O titers were higher than non-O titers. Median IgG titers performed by CAT were lower than median IgM titers. Group O IgG heat treated titers were found to be lower than group O IgG DTT treated titers and group O IgG DTT treated titers were mostly similar to group O IgG heat treated titers. For non-O individuals, IgM and IgG titers post DTT treatment were lower than IgM and IgG titers post heat-treatment. A comparison of the frequency of distribution of IgG titers performed by CAT and CTT, both post HI and DTT treatment for A, B and O blood groups has been illustrated in Fig. 3. Results of anti-A and anti-B IgG titers performed by CTT were lower as compared to titers performed by CAT. The observation that the distribution of anti-A titers showed a shift to the left compared to anti-B titers suggested that the levels of anti-A antibodies were lower than those of anti-B antibodies in the tested samples. This implied that individuals within the sample population had a higher average concentration of anti-B antibodies compared to anti-A antibodies. Additionally, after treatment (both post HI and post DTT), the IgG titers for both anti-A and anti-B showed a further shift to the left. This indicated a reduction in the average concentration of IgG antibodies for both antigens post-treatment. The shift was more pronounced in the post-DTT treatment results, suggesting that DTT treatment may have a more significant impact on reducing IgG antibody levels compared to HI treatment.

Spearman's rho (r_s) was calculated to outline the correlation analysis between various antibody titration methods (CAT and CTT) before and after DTT treatment, specifically focusing on IgG titers for anti-A and anti-B antibodies in group O individuals, which has been depicted in Table 1. There was a strong to very strong correlation between the titration results obtained using CAT and CTT methods for both anti-A and anti-B IgG titers in group O individuals, both before and after DTT treatment. This indicated a high degree of agreement between the two methods. The correlation between paired samples (pCTT and pCAT) for both anti-A and anti-B IgG titers

showed moderate to strong correlation values, suggesting a reasonable agreement between pre- and post-treatment results obtained from the same method. There was a very strong correlation between CAT and CTT methods for both anti-A and anti-B IgG titers, indicating consistent results between the two titration methods across all conditions. The correlation between CTT and pCTT for anti-A and anti-B IgG titers was weak to moderate, suggesting some variability in the results obtained from the same method before and after DTT treatment. The correlation between pre- and post-treatment results (pCAT) and the original CAT or CTT results was very weak to weak for both anti-A and anti-B IgG titers, indicating some inconsistency or variability between the initial and post-treatment results.
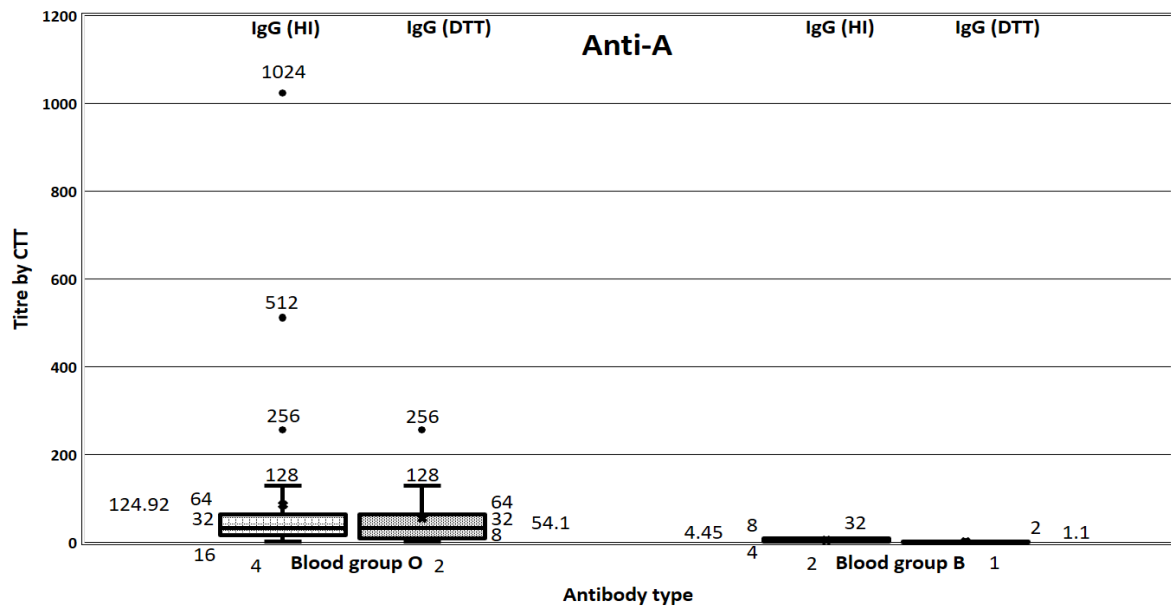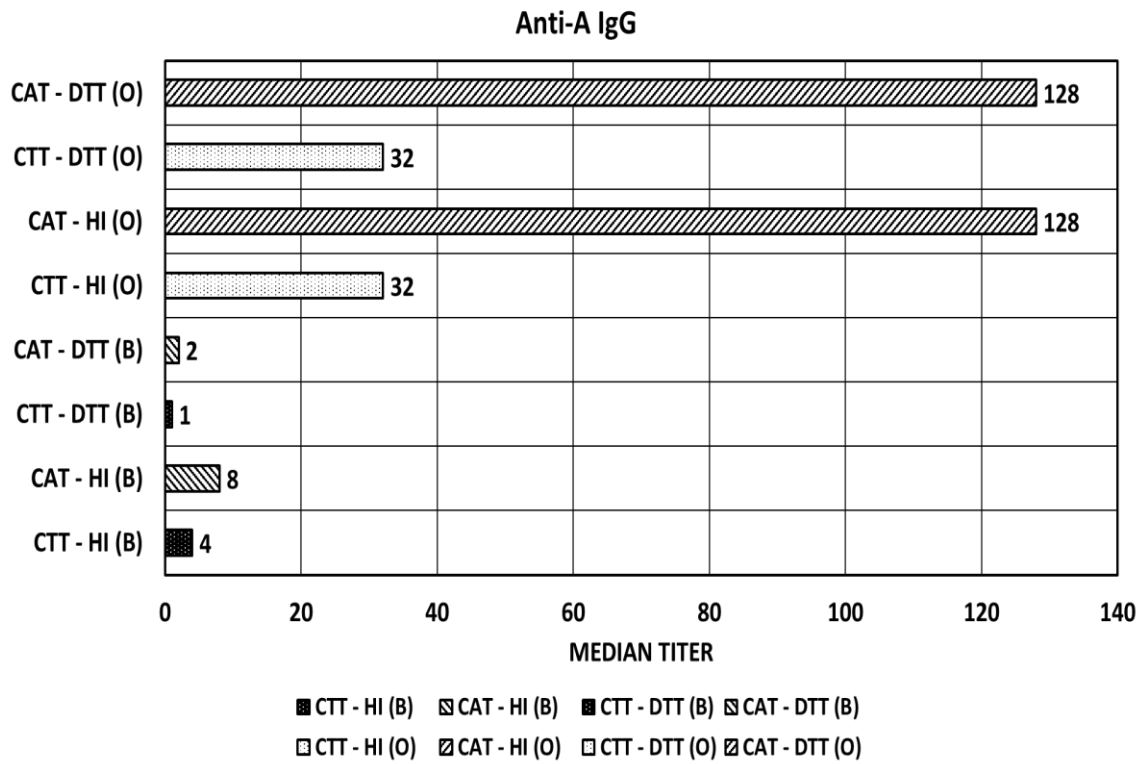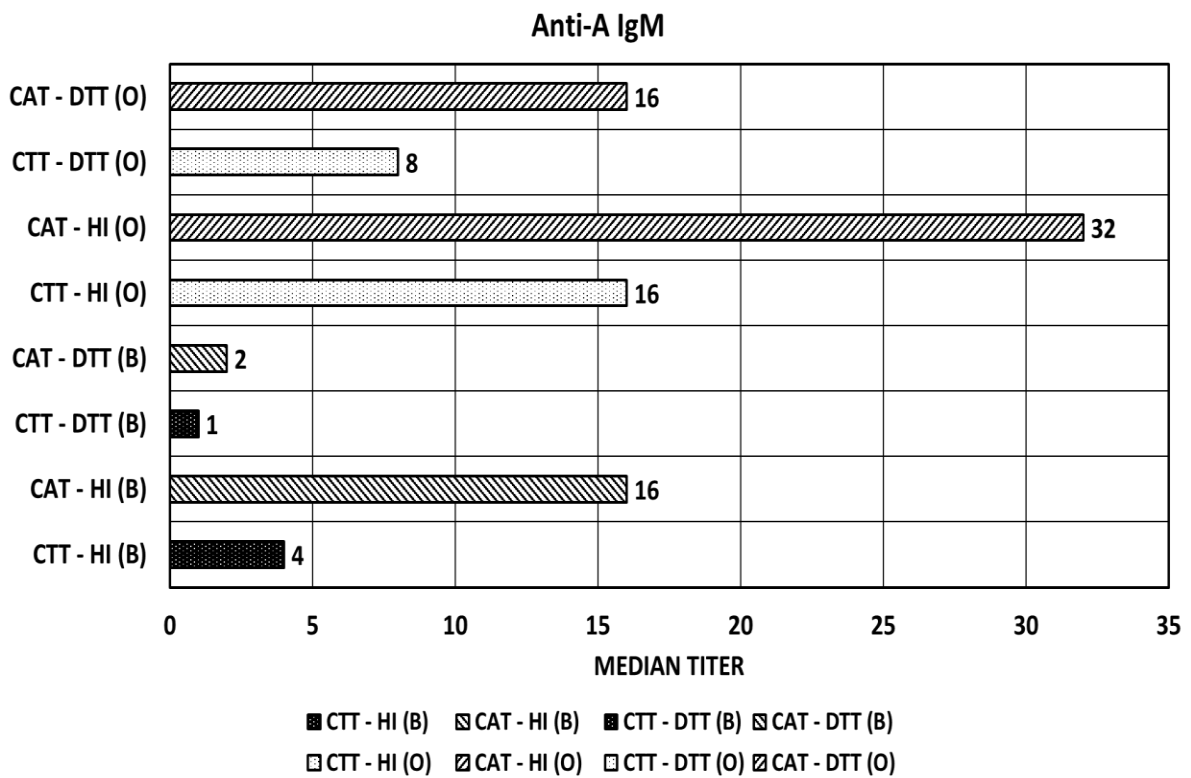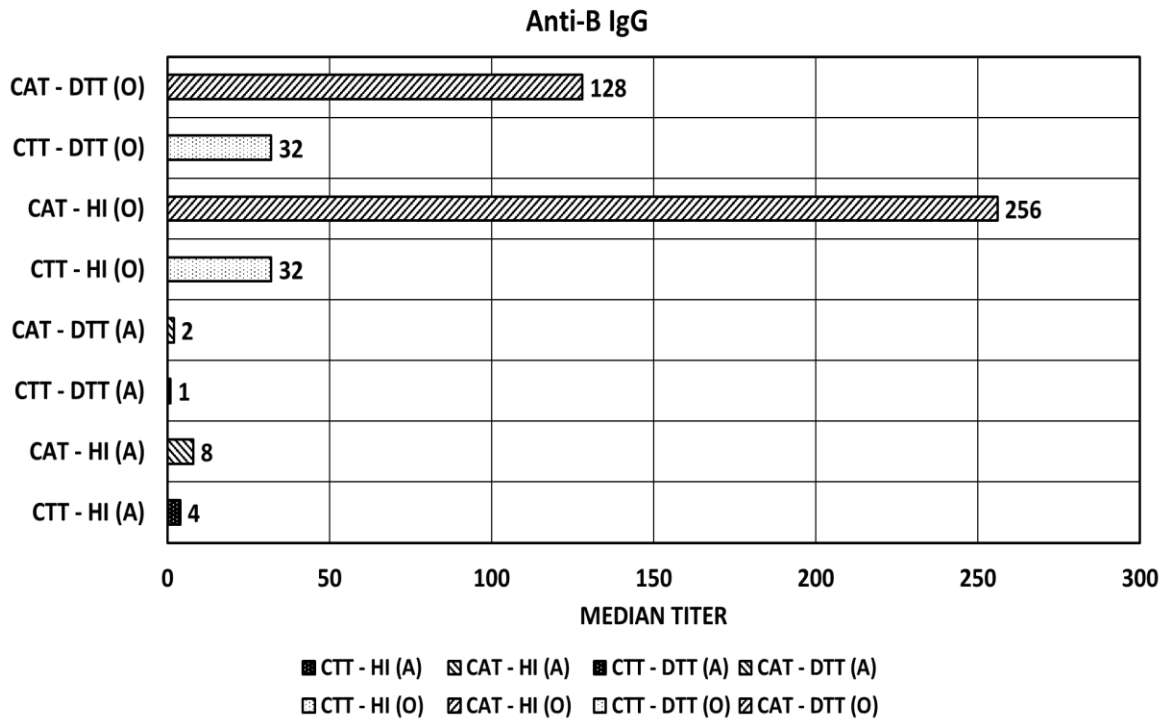

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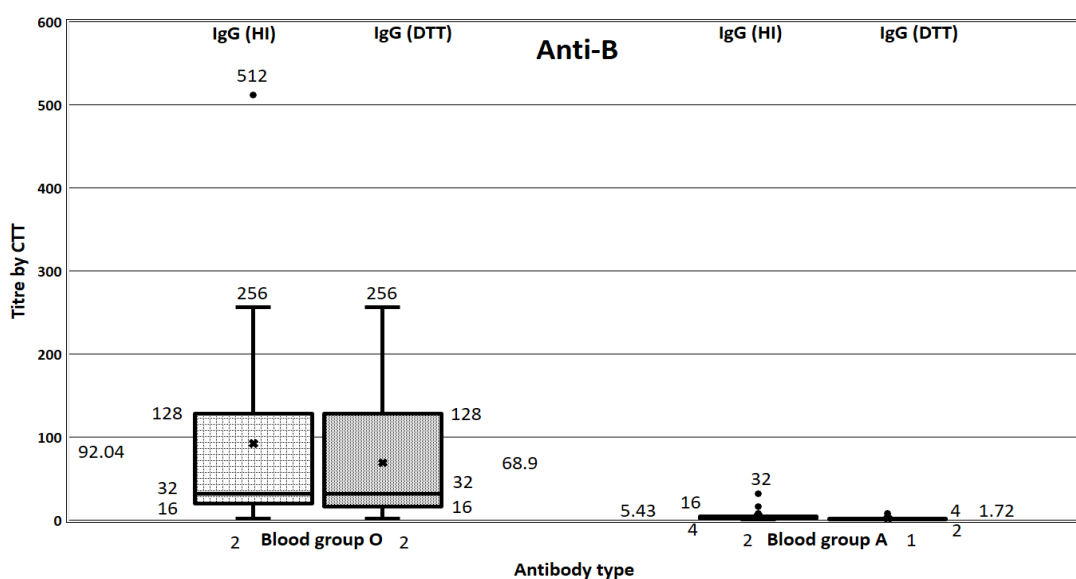
[b]

Fig. 1. Distribution of anti-A and anti-B IgG titers post DTT and post HI treatment performed by CTT [a] Anti-A titers [b] Anti-B

Anti-A IgG

[a]

Anti-A IgM

[b]

Anti-B IgG

[c]

Anti-B IgM
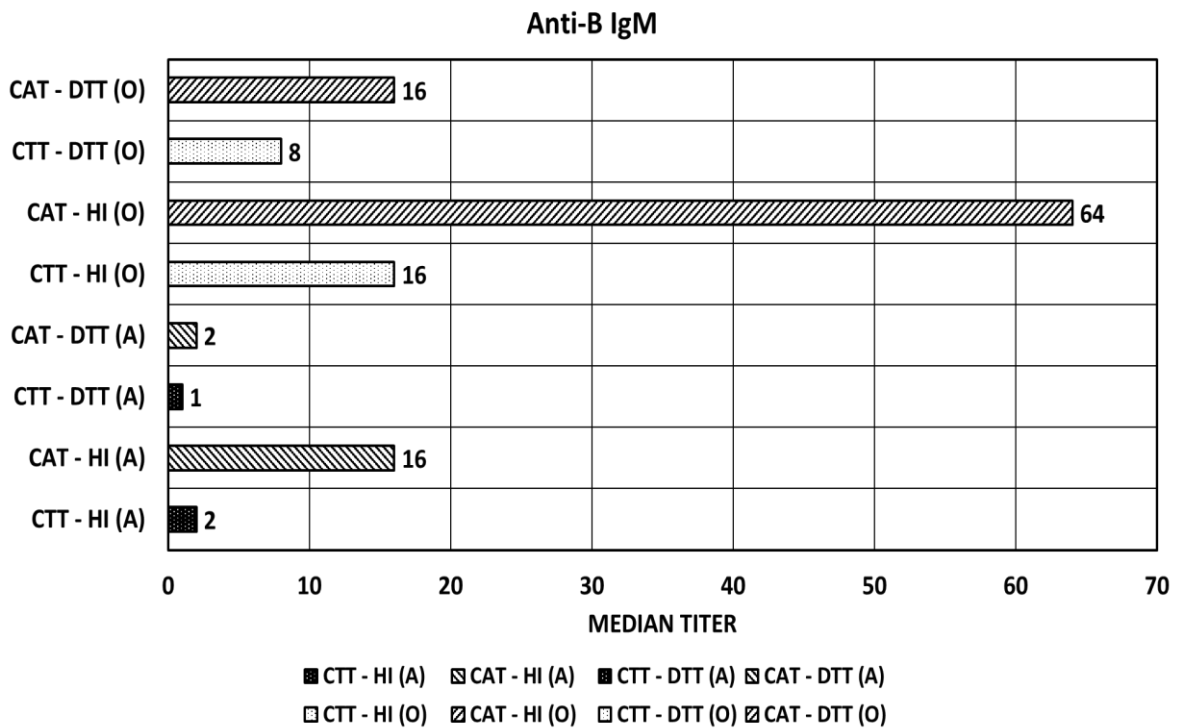

[d]

Fig. 2. Comparison of median anti-A and anti-B titers before and after DTT and HI treatment performed by CTT and CAT with 1+ strength as end point [a] Median anti-A IgG titers [b] Median anti-A IgM titers [c] Median anti-B IgG titers [d] Median anti-B IgM titers
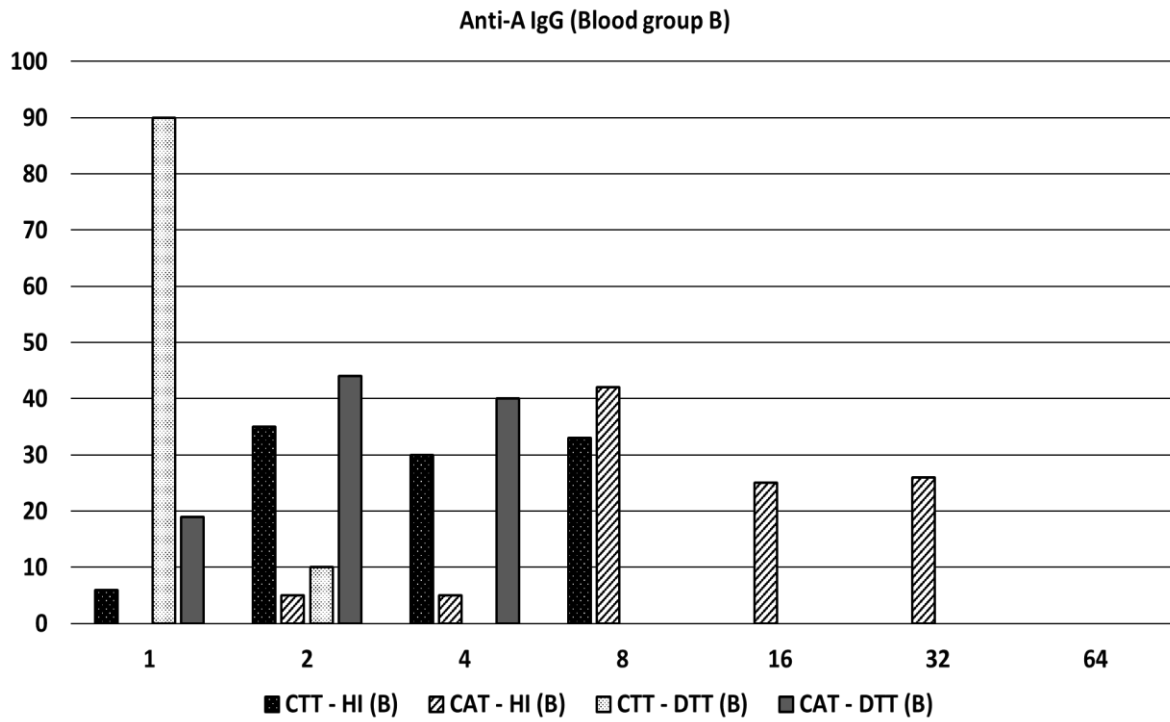
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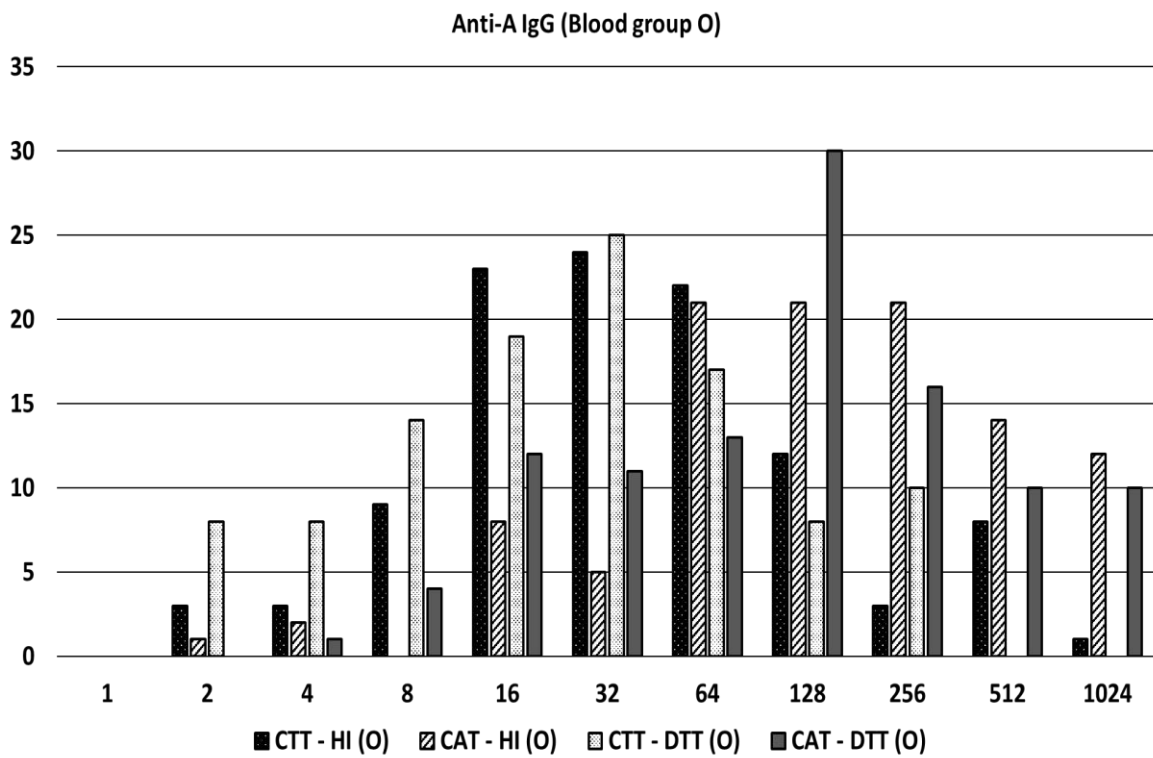
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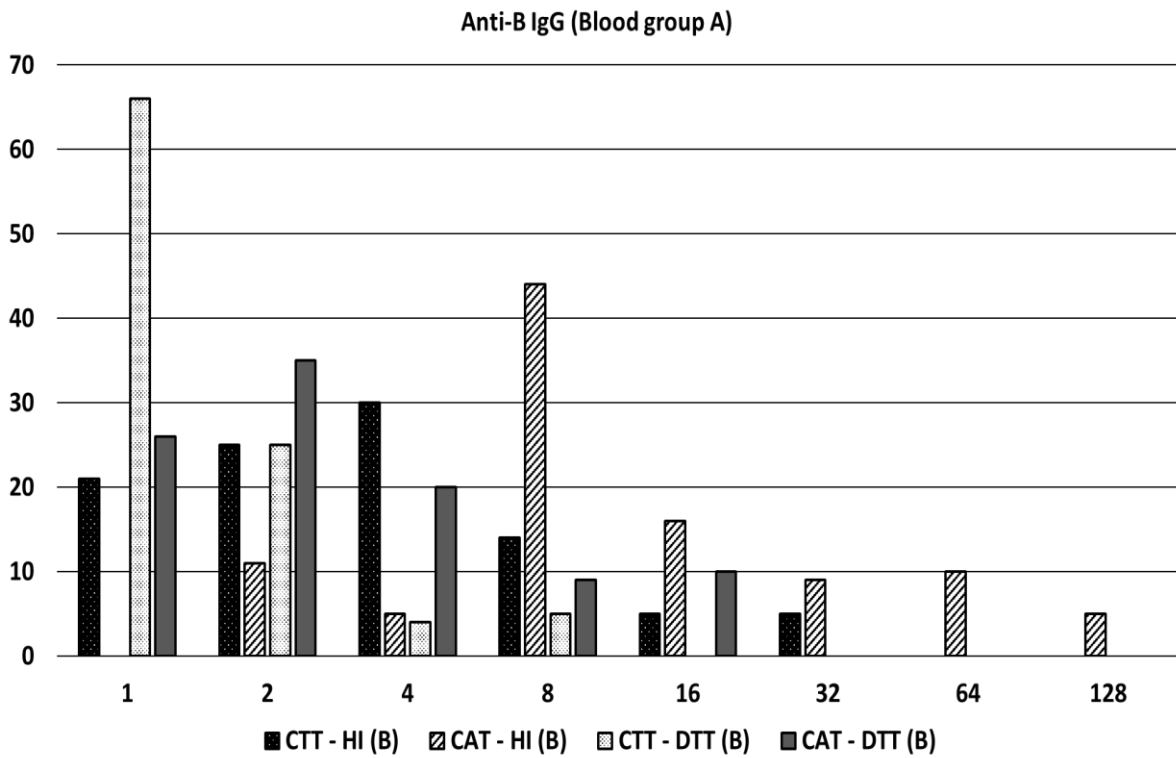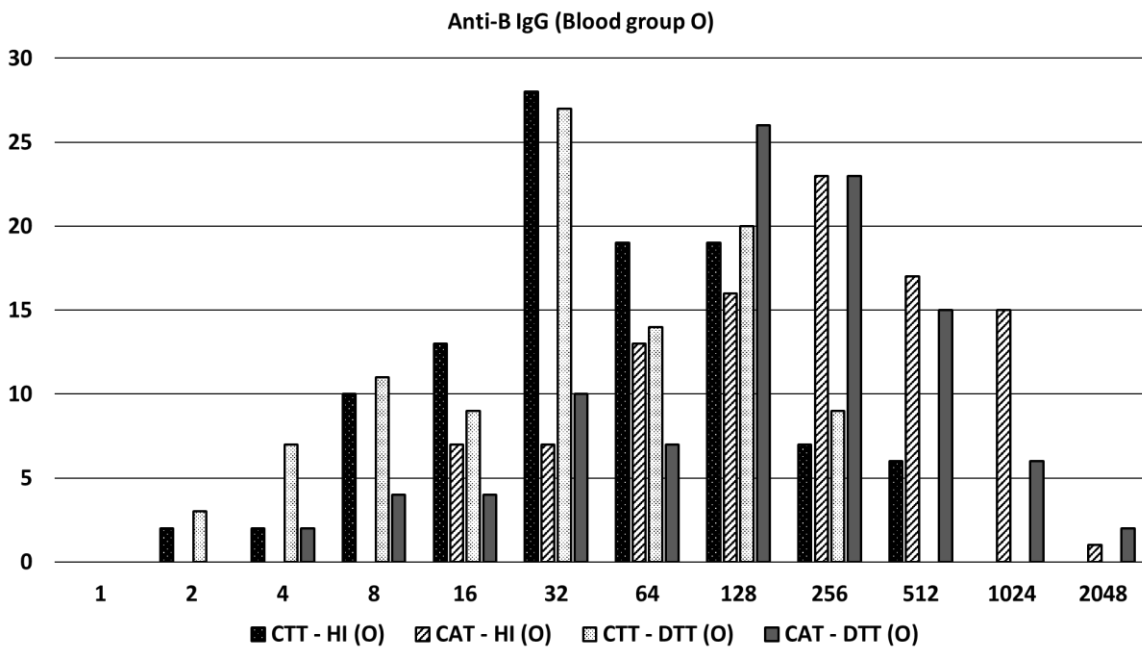
[c]

[d]

Fig. 3. Comparison of frequency of distribution of IgG titers (pre and post DTT and HI treatment) performed by CTT and CAT [a] Anti-A (blood group B) [b] Anti-A (blood group O) [c] Anti-B (blood group A) [d] Anti-B (blood group O)

Table 1. The correlation of pre DTT treatment (CTT, CAT) and post DTT treatment (pCTT, pCAT) results obtained by CTT and CAT measuring IgG antibodies for anti-A and anti-B (Blood group A,B,O)

Antibody	Blood group	Comparing methods	Spearman's rho	P-value	Strength of correlation	Association	Direction of correlation
IgG							
Anti-A	B	CTT – pCTT	0.32	<0.05	Weak	Significant	Negative
Anti-A	B	CTT – CAT	0.88	<0.05	Very strong	Significant	Positive
Anti-A	B	CTT – pCAT	0.08	>0.05	Very weak	Not significant	Positive
Anti-A	B	CAT – pCAT	0.04	>0.05	Very weak	Not significant	Positive
Anti-A	B	CAT - pCTT	0.54	<0.05	Moderate	Significant	Positive
Anti-A	B	pCAT - pCTT	0.49	<0.05	Moderate	Significant	Positive
Anti-A	O	CTT – pCTT	0.77	<0.05	Strong	Significant	Positive
Anti-A	O	CTT – CAT	0.78	<0.05	Strong	Significant	Positive
Anti-A	O	CTT – pCAT	0.61	<0.05	Strong	Significant	Positive
Anti-A	O	CAT – pCAT	0.76	<0.05	Strong	Significant	Positive
Anti-A	O	CAT - pCTT	0.77	<0.05	Strong	Significant	Positive
Anti-A	O	pCAT - pCTT	0.86	<0.05	Very strong	Significant	Positive
Anti-B	A	CTT – pCTT	0.58	<0.05	Moderate	Significant	Positive
Anti-B	A	CTT – CAT	0.97	<0.05	Very strong	Significant	Positive
Anti-B	A	CTT – pCAT	0.31	<0.05	Weak	Significant	Positive
Anti-B	A	CAT – pCAT	0.35	<0.05	Weak	Significant	Positive
Anti-B	A	CAT - pCTT	0.62	<0.05	Strong	Significant	Positive
Anti-B	A	pCAT - pCTT	0.76	<0.05	Strong	Significant	Positive
Anti-B	O	CTT – pCTT	0.74	<0.05	Strong	Significant	Positive
Anti-B	O	CTT – CAT	0.77	<0.05	Strong	Significant	Positive
Anti-B	O	CTT – pCAT	0.74	<0.05	Strong	Significant	Positive
Anti-B	O	CAT – pCAT	0.68	<0.05	Strong	Significant	Positive
Anti-B	O	CAT - pCTT	0.67	<0.05	Strong	Significant	Positive
Anti-B	O	pCAT - pCTT	0.82	<0.05	Very strong	Significant	Positive

Table 2. The correlation of pre heat inactivation (CTT, CAT) and post heat inactivation (pCTT, pCAT) results obtained by CTT and CAT measuring IgG antibodies for anti-A and anti-B (Blood group A,B,O)

Antibody	Blood group	Comparing methods	Spearman's rho	P-value	Strength of correlation	Association	Direction of correlation
IgG							
Anti-A	B	CTT – pCTT	0.32	<0.05	Weak	Significant	Negative
Anti-A	B	CTT – CAT	0.57	<0.05	Moderate	Significant	Positive
Anti-A	B	CTT – pCAT	0.18	<0.05	Very weak	Significant	Positive
Anti-A	B	CAT – pCAT	0.31	<0.05	Weak	Significant	Positive
Anti-A	B	CAT - pCTT	0.48	<0.05	Moderate	Significant	Positive
Anti-A	B	pCAT - pCTT	0.46	<0.05	Moderate	Significant	Positive
Anti-A	O	CTT – pCTT	0.76	<0.05	Strong	Significant	Positive
Anti-A	O	CTT – CAT	0.58	<0.05	Moderate	Significant	Positive
Anti-A	O	CTT – pCAT	0.59	<0.05	Moderate	Significant	Positive
Anti-A	O	CAT – pCAT	0.76	<0.05	Strong	Significant	Positive
Anti-A	O	CAT - pCTT	0.77	<0.05	Strong	Significant	Positive
Anti-A	O	pCAT - pCTT	0.78	<0.05	Strong	Significant	Positive
Anti-B	A	CTT – pCTT	0.52	<0.05	Moderate	Significant	Positive
Anti-B	A	CTT – CAT	0.73	<0.05	Strong	Significant	Positive
Anti-B	A	CTT – pCAT	0.29	<0.05	Weak	Significant	Positive
Anti-B	A	CAT – pCAT	0.32	<0.05	Weak	Significant	Positive
Anti-B	A	CAT - pCTT	0.58	<0.05	Moderate	Significant	Positive
Anti-B	A	pCAT - pCTT	0.74	<0.05	Strong	Significant	Positive
Anti-B	O	CTT – pCTT	0.71	<0.05	Strong	Significant	Positive
Anti-B	O	CTT – CAT	0.72	<0.05	Strong	Significant	Positive
Anti-B	O	CTT – pCAT	0.77	<0.05	Strong	Significant	Positive
Anti-B	O	CAT – pCAT	0.58	<0.05	Moderate	Significant	Positive
Anti-B	O	CAT - pCTT	0.59	<0.05	Moderate	Significant	Positive
Anti-B	O	pCAT - pCTT	0.76	<0.05	Strong	Significant	Positive

As shown in Table 2, Spearman's rho (r_s) was calculated to analyse the correlation between CAT and CTT post HI treatment for IgG titers of anti-A and anti-B antibodies in group O individuals. There was a strong correlation between the titration results obtained using CAT and CTT methods for both anti-A and anti-B IgG titers in group O individuals after HI treatment. This indicated a high degree of agreement between the two methods post HI treatment. The correlation between paired samples (pCTT and pCAT) for both anti-A and anti-B IgG titers showed moderate to strong correlation values after HI treatment. This suggested a reasonable agreement between pre- and post-treatment results obtained from the same method. The correlation between CTT and pCTT for anti-A and anti-B IgG titers was weak to moderate post HI treatment, indicating some variability in the results obtained from the same method before and after HI treatment. The correlation between pre- and post-treatment results (pCAT) and the original CAT or CTT results was weak for both anti-A and anti-B IgG titers after HI treatment, indicating some inconsistency or variability between the initial and post-treatment results.

4. DISCUSSION

"ABO isoagglutinins, play a significant role in ABO incompatible transplants, including solid organ and hematopoietic stem cell transplants". [28-30] "Accurate measurement of ABO isoagglutinin concentrations is essential for optimizing patient outcomes in transplantation and transfusion medicine by facilitating risk assessment, treatment planning, and monitoring of immune responses. In comparison to blood groups A and B, IgG in present in more amount in blood group O individuals". [1] The findings from the present study highlight an important consideration in ABO incompatible transplant management. When comparing titers of IgG measured by both CAT and CTT, it is observed that IgG titers are higher than IgM titers in the samples. This discrepancy suggests that the presence of IgM antibodies in the samples could potentially cause an overestimation of IgG titers. This overestimation can have significant implications for the management of ABO incompatible transplant recipients. Without the use of heat inactivation or DTT, there is estimation of total antibody titer which includes both IgM and IgG. Comparing titers of IgG and IgM measured by CAT and CTT, it was noted that for reactions with a strength of 1+, both IgG and IgM titers measured by CAT were higher

compared to CTT. IgG antibodies are particularly relevant in the context of graft outcomes, especially in ABO incompatible transplantation. To accurately assess the levels of IgG antibodies, it's crucial to minimize the interference from IgM antibodies, as they can potentially overestimate IgG titers. Methods such as DTT treatment of plasma or heat inactivation are recommended to inactivate IgM antibodies. [13,14] Both these methods have been known to inactivates IgM antibodies and their effect on IgG antibodies is minimal. [11,14] Steinberg and Cook first mentioned the technique of heating plasma for removing IgM antibodies in 1981. [31] Several studies have investigated the impact of IgM antibodies on crossmatch results in solid organ transplantation indicating that IgM antibodies can potentially lead to false-positive results in complement-dependent cytotoxicity (CDC) crossmatches, and the use of heat inactivation techniques has been shown to mitigate the effect of IgM antibodies, leading to negative crossmatch results.[32,33] However, its specific impact on ABO isoagglutinin titers hasn't been extensively studied in comparison to sulfhydryl reagents like 2-mercaptoethanol (2-ME) or dithiothreitol (DTT).

Conventionally, heat inactivation of plasma is typically performed at 56°C for 30 minutes. This process is commonly employed to inhibit complement activity in the plasma. [34,35] The study by Hasekura et al demonstrated that heating plasma at 70°C for 10 minutes resulted in a significant decline in both IgM and IgG titers of anti-A and anti-B antibodies suggesting that heat treatment effectively reduces the levels of ABO isoagglutinins in the plasma sample. [36] The study by Riley et al. provides evidence that heating plasma at 63°C for 10 minutes can ameliorate the effect of IgM antibodies, leading to a reduction in false-positive CDC crossmatch results. [33] In the present study, heat inactivation was conducted at 63°C for 13 minutes, following the method described in the ASHI guidelines. [27]

CAT is known for its high sensitivity and is widely recommended for various immunohematological investigations. However, antibody level determination has not been explored well. [20,37] Differences were observed between antibody titer results obtained by CAT and CTT, both pre- and post-treatments. CTT results were found to be lower in general in comparison to CAT results. Median anti-A and anti-B IgG titer results showed a one-to-two folds difference between CAT and

CTT and titers reported by CAT were higher. Antibody titer results measured using heat inactivated and DTT-treated and untreated samples was compared. Anti-A and anti-B titers were reduced after heat inactivation as well as DTT treatment, both by CAT as well as CTT. Median IgM and IgG titers showed a decrease with both DTT and heat treatments. However, the reduction was more pronounced with DTT treatment. Heat inactivation was less effective in reducing titers throughout all categories in comparison to DTT treatment. Heat inactivation resulted in a one-fold decrease in median IgM titers, whereas post-DTT treatment showed a two-to-three-fold decrease.

On comparing the results of 50 samples, Nayak et al concluded a poor agreement between IgG titers performed by CTT and CAT. [38] The study by Matsuura et al. on 10 individuals with blood group O, comparing antibody titration by CTT and automated CAT simultaneously, yielded several important findings which included the concordance rate of 45% between CTT and automated CAT and the correlation between CTT and automated CAT was described as weak. They recommended the use of DTT-treated plasma for automated titer estimation by CAT in order to define the cut-off value in antibody titration. [39] In the present study, when correlation was calculated, strong correlation was observed for anti-A and anti-B IgG titer results, between CAT and CTT, for both HI and DTT. treatment. A strong to very strong correlation was observed between CAT and CTT for IgG measurement of anti-A and anti-B antibodies in group O individuals, both before and after DTT treatment, while this correlation before and after heat treatment was strong.

"On 60 O blood group individuals, Park et al. conducted a study comparing titer estimation methods for anti-A and anti-B antibodies, specifically comparing CAT and CTT, both with and without DTT treatment". [40] "They found that the median titers obtained by CAT were higher than those obtained by CTT, and even after DTT treatment, the titers remained higher with CAT compared to post-DTT-CAT titers. Based on these findings, they concluded that CAT results, both with and without DTT treatment, were more sensitive than CTT for individuals with blood type O". [41] Shim et al. compared "three methods of antibody titration using 40 samples and discovered that median IgG and IgM titers were higher when using CAT compared to the other methods they tested".

"Additionally, they noted that the agreement between the methods was better for IgM titers compared to IgG titers". [42] In our study, separate median titers were determined for IgG and IgM for anti-A and anti-B which has been illustrated in Fig. 2. Median titers obtained by CTT were generally lower than those obtained by CAT, particularly after DTT treatment. This suggests that CAT may be more sensitive in detecting and measuring median titers of anti-A and anti-B antibodies, especially following DTT treatment. Based on the distribution of titers depicted in Fig. 3, several deductions can be made such as IgG antibody titers tend to be higher when determined using CAT compared to CTT; DTT or heat treatment of plasma can lead to a decrease in titers; and anti-B titers are generally higher than anti-A titers.

Heat inactivation was found to have less efficiency in inactivation of IgM antibodies compared to DTT treatment. This implies that DTT treatment may be more reliable when complete inactivation of IgM antibodies is necessary. Heat inactivation is noted for its simplicity and cost-effectiveness. It consumes less time and is a relatively straightforward process, making it easier to incorporate into laboratory workflows. Additionally, the equipment needed for heat inactivation is generally more accessible and less expensive compared to DTT treatment. Heat inactivation may be particularly advantageous in resource-constrained settings where access to expensive reagents like DTT is limited. Since the equipment needed for heat inactivation is more readily available, laboratories in such settings can still perform antibody titration effectively without relying on costly reagents. Strength of this study was large sample size. This sample includes 100 individuals each from blood groups A, B, and O and application of two different methods, CAT and CTT, to evaluate the impact of heat on antibody titers. This approach allows for a comprehensive examination of how heat treatment influences antibody titers across different blood groups and using different titration methods. By including a large sample size and multiple blood groups, this study provides valuable insights into the practical implications of heat treatment on antibody titration, which can inform laboratory practices and protocols in transfusion services.

Certainly, the inability to assess the clinical impact of titration performed before and after heat inactivation of plasma is an important limitation of this study.

5. CONCLUSION

While DTT treatment may offer greater efficiency in IgM inactivation, heat inactivation presents advantages in terms of simplicity, cost-effectiveness, and accessibility, especially in resource-constrained settings. These considerations can help laboratories make informed decisions about which method to employ based on their specific needs and constraints.

CONSENT AND ETHICAL APPROVAL

Written consent was obtained from all the donors who agreed to participate and the study was carried out after the approval of institutional review board and institutional ethics committee. (Jaypee Hospital, MOM Institutional Ethics Committee_DNB, approved on 08/09/2018).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/114390