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Possible neglected transient (T) polyagglutination in critically ill patients with coronavirus disease-2019 (Covid-19)

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Abstract: T-activation polyagglutination can be caused by bacteria or viruses and has been associated with haemolytic anaemia. Coronavirus disease-19 (COVID-19) is also associated with haemolytic anaemia. The presented study aims to determine T activation polyagglutination in critically ill COVID-19 patients. Anti-T Arachis hypogaea lectin was incubated with the red blood cells of the COVID-19 patient and checked for agglutination. Thirty-four percent (34.3%) of COVID-19 patients in the intensive care unit (ICU) had potentially activated T cells and polyagglutinable red blood cells, as demonstrated by their cryptantigen exposure that caused agglutination. The study revealed a high prevalence of anti-T among ICU-admitted COVID-19 patients, suggesting that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) may cause transient T activation, polyagglutination in critically ill COVID-19 patients *in vitro* and possibly haemolysis *in vivo*.

Keywords: Polyagglutination, T activation, critically ill patients, Covid-19.

INTRODUCTION

Polyagglutination is defined as the nonspecific (not related to blood group) agglutination of red blood cells by nearly all allogeneic adult human sera but not by autologous or newborn sera. *In vitro* polyagglutination was first defined by HuEÌbener in 1925 and by Thomsen in 1927, but it was clarified and named T haemagglutination by Friedenreich in 1930 (Ramasethu J and Luban, 2001).

There are two types of polyagglutination: inherited, which includes Tn polyagglutination and HEMPAS (hereditary erythroblastic multinuclearity with positive acidified serum lysis test); and acquired, including most T-antigens (T, Th, Tk and Tx).

Acquired polyagglutinations are transient and triggered by bacteria, mostly *Clostridium perfringens* and *Streptococcus pneumoniae*, or viruses such as influenza (Jacquot *et al.*, 2021). These microorganisms produce enzymes, such as β-galactosidase or neuraminidase, which remove the terminal sialic acid residues on glycophorins (A, B and C) on red blood cell (RBC) membranes, resulting in T-antigen (Thomsen (T) cryptantigen) exposure (fig. 1). The exposed T antigen binds to normal human plasma that contains naturally occurring antibodies against the cryptantigens, causing agglutination *in vitro* and possible haemolysis of T-activated red blood cells *in vivo*.

T-activation is the most common type of acquired polyagglutination and has been associated with haemolysis in neonates and children (Jacquot *et al.*, 2021). The reported incidence of T-antigen activation has also been seen in hospitalised elderly patients (Rawlinson and Stratton, 1984).

Although most T activation is benign, causing transient T-antigen alterations that resolve after the clearance of the causative infection, it can cause problems in the laboratory. Numerous case reports have shown severe

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haemolysis after transfusions of plasma to T-activation patients (Moh-Klaren *et al.*, 2017).

As a result, authors advocate that patients diagnosed with T activation should only receive washed RBC and platelet products and plasma transfusion should be avoided when possible (Williams *et al.*, 1989).

Several laboratory agglutination tests can be used to diagnose and differentiate T-antigen activation using peanut (*Arachis hypogaea*), *Salvia sclarea* and soybean (*Glycine soja max*) lectins (table 1).

Several studies have recently shown that coronavirus disease-19 (COVID-19) is associated with haemolytic anaemia (Sahu *et al.*, 2021; Lazarian *et al.*, 2020; Jacobs and Eichbaum, 2021; Jawed *et al.*, 2020). However, the impact of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on red blood cell membranes has not been well investigated.

Moreover, the frequency of T-antigen activation in a hospitalised population is 7% in patients diagnosed with acquired immunodeficiency syndrome (AIDS) (Adams *et al.*, 1989) and 7.6% in patients with higher risk due to anaemia, malignancy and sepsis (Buskila *et al.*, 1987). However, only one study had investigated the possible role of T polyagglutination and severe complications with blood group types in COVID-19 infection (Dobie *et al.*, 2023). Whether SARS-CoV-2 causes T activation polyagglutination in hospitalised COVID-19 patients, leading to *in vivo* hemolysis or not. Therefore, our study aims to determine the T activation polyagglutination in critically ill COVID-19 patients.

Neuraminidase removes the portions of the glycophorin A and B chains on the surface of the red blood cell that lead to the exposure of the T antigen. The T antigen renders the cells more susceptible to agglutination by naturally occurring anti-T antibodies found in normal human plasma.

MATERIALS AND METHODS

Materials

Phosphate-buffered saline with a pH of 7.0-7.5 and Anti-T Arachis hypogaea Lectin Peanut Agglutinin (Lectin PNA) from Peanut from EY Laboratories (San Mateo, CA). Polyspecific anti-human globulin anti-IgG/C3d was purchased from Bio-Rad Laboratories (Dubai, UAE).

COVID-19 patients' samples

Venous blood samples were collected from critically ill COVID-19 patients into ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes at the time of intensive care unit (ICU) admission between January 2021 and March 2021 at Baish General Hospital. The EDTA tubes were used for a complete blood count (CBC) analysis and anti-T detection. The study protocols were approved by the Jazan Health Ethics Committee, "Jazan IRB", Ministry of Health, Project No. 2053.

Hematologic parameters

Hematologic parameters were determined on EDTA samples collected from COVID-19 patients in the ICU using the Sysmex XN-1000 Haematology Analyser (Kobe, Japan).

Detecting polyagglutinable red blood cells

One to two drops of appropriately diluted anti-T Arachis hypogaea lectin were incubated with one drop of a 3-5% suspension of the patient's red blood cells. After a 15-minute incubation at room temperature, the tubes were centrifuged and macroscopically read for agglutination. Normal, healthy donor samples were used as a negative control.

The direct antiglobulin test

The direct antiglobulin test (DAT) was performed as previously described (Zantek *et al.*,2012).

Data analysis

The collected data were entered in Microsoft Excel software, and analysis was performed using GraphPad Prism version 8.0. The frequency of the collected data was tabulated. The hemoglobin (Hb) values were presented as mean ± standard deviation (SD).

RESULTS

In this study, samples were collected from 81 confirmed COVID-19 patients (46 males and 35 females) who were over 54 years old, in critical condition and admitted to the ICU. The samples were examined for direct anti-globulin testing (DAT) and T-activation polyagglutinable red blood cells.

Out of 81 patients, 11 (13.6%) had a positive DAT (polyspecific anti-human globulin anti-IgG/C3d), whereas 70 (84.4%) were DAT negative (fig. 2).

On the other hand, out of 70 patients examined with negative DAT, 24 (34.3%) had potentially T-activation polyagglutinable red blood cells, as shown by their cryptantigen exposure when tested with anti-T Arachis hypogaea lectin. Meanwhile, 46 (65.7%) tested negative for T-activation (fig. 3A). fig. 3B shows the representative images of positive (+) and negative (-) anti-T tests.

The haemoglobin concentration (Hb) of the patients was measured for the diagnosis of anaemia (table 2). Out of 46 male patients with COVID-19, 39 (84.8%) were anaemic (Hb<13.5g/dl), while 7 patients (15.2%) had a normal haemoglobin level.

Table 1: Role of lectin reactivity in diagnosis and classification of T-antigen activation subtypes

Lectin	Normal cells	Polyagglutinable RBCs				
		T	Th +	Tk +	Tx +	Tn +
Arachis hypogaea	0	+	+	+	+	0
Glycine max	0	+	0	0	0	+
Salvia sclarea	0	0	0	0	0	+
Dolichos biflorus	0	0	0	0	0	+
Vicia cretica	0	+	+	0	0	0

Abbreviations: 0 (no agglutination), + (agglutination), adopted from (2).

Table 2: Haemoglobin (Hb) concentration for COVID-19 patients

	Male (46) Anaemic (n = 39)	Female (35) Anaemic (n = 31)
Hb(g/dl)	10.5 ± 2.2	9.0 ± 1.6

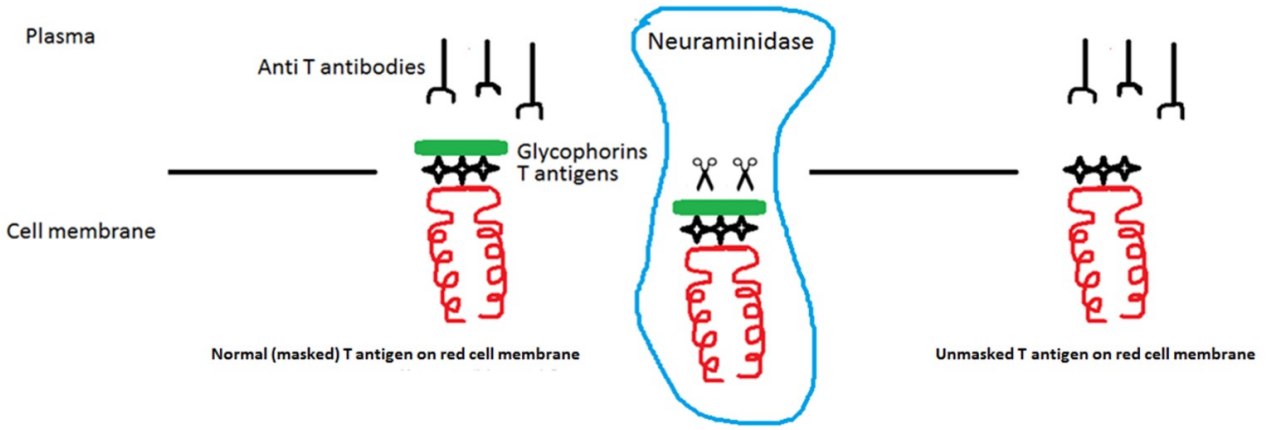

Fig. 1: Effect of neuraminidase on sialic acid residues that contribute to the negative charge on glycophorin A and B, exposing the T antigen.

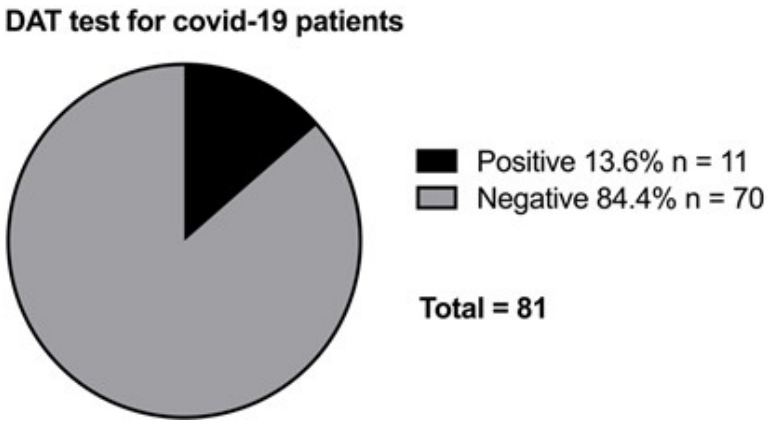

Fig. 2: Direct anti-globulin test (DAT) in COVID-19 ICU patients

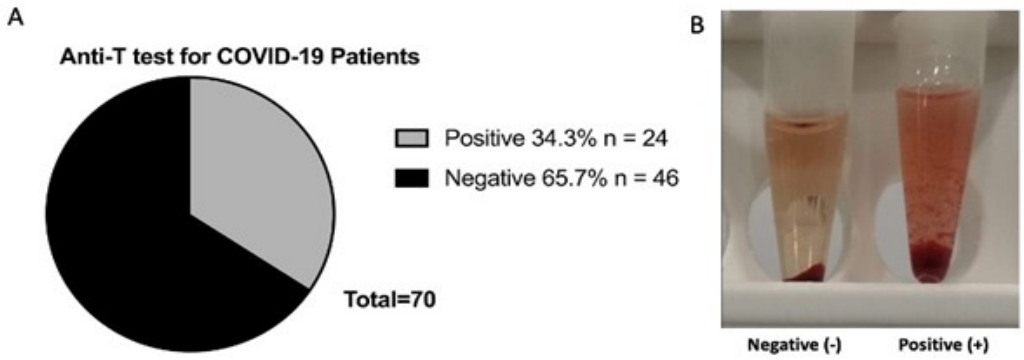

Fig. 3: T-activation polyagglutinable red blood cells in COVID-19 ICU patients. (A) Percentage of presence of T-cryptantigen in COVID-19 ICU patients. (B) Representative images of positive (+) and negative (-) anti-T test.

For the female group, 31 (88.5%) out of 35 COVID-19 patients examined were anaemic (Hb < 12.0 g/dl), while 4 (11.5%) patients had normal haemoglobin.

DISCUSSION

T-activation polyagglutination is a phenomenon that involves the exposure of a concealed receptor (cryptantigen) on the RBC membrane called T-antigen (Thomsen antigen). This leads to the agglutination of unmasked T-antigens by antibodies against T-antigens present naturally in adult human sera. Cryptantigens can be exposed due to several conditions, including infections, anaemias, malignancies and diseases of unknown aetiology (Beck, 1983).

The predominance of T-activation polyagglutination in hospitalised patients is not very high, with a range between 7-7.6% and is associated with different conditions (10,11). The study of the incidence of T-activation polyagglutination in hospitalised patients with COVID-19 has not been done yet.

In the presented study, the investigations were directed at 81 COVID-19 patients in the ICU due to the progression of the disease. A total of 81 samples from healthy blood donors were used as a control group and none of them showed T-antigen polyagglutinable RBCs. A DAT was done to exclude DAT-positive drug/immune haemolytic anaemia. Only 11 patients were excluded because their DAT was positive, whereas 70 patients were included in the examinations.

The data showed that 34.3% of COVID-19 patients included in the study had T-activation polyagglutination, as shown by their reaction to anti-T Arachis hypogaea lectin (fig. 2). It has been previously shown that microbial agents, including Escherichia coli, Vibrio cholera, Pneumococcus, Streptococcus, Staphylococcus, Clostridia and influenza viruses, induce polyagglutination *in vitro* (Klein and Anstee, 2014).

Therefore, the result may suggest the role of coronavirus 2 (CoV-2) infection in exposing T-antigen on RBC membranes, causing them to agglutinate by anti-T *in vitro* and potentially leading to haemolysis *in vivo*. However, other comorbidities associated with CoV-2 infection must not be neglected, as certain disorders are associated with ACE-2 receptor expression, which allows the entry of the virus into the host cells.

A structure of terminal *N*-acetylneuraminic acid (NeuAc or sialic acid), D-galactose, *N*-acetyl-D-galactosamine (GalNac) and serine/threonine normally covers and masks the T-antigen on the RBC membranes. However, the T-antigen can be unmasked due to the removal of the sialic acid terminal by neuraminidase produced by microorganisms (Issitt and Anstee, 2018), causing the exposed galactose residues to become the receptor for anti-T (Roseff, 2017). Interestingly, a study showed that samples from critically ill COVID-19 patients revealed overexpression of neuraminidase (Formiga *etal.*, 2020), which may cause cleavage of sialic acid and result in T activation and polyagglutination *in vitro*.

Although SARS-CoV-2 resembles influenza viruses that use sialic acid, no evidence is yet available for an association between CoV-2 and cleavage of the terminal sialic acid. It has been shown that SARS-CoV-2 uses hemagglutinin-esterase (HE), which acts as a classical glycan-binding lectin and receptor-degrading enzyme, to enter host cells (Kim, 2020). However, it is still unknown whether this leads to the uncovering of T-antigens and causes polyagglutination.

CONCLUSION

T-activation polyagglutination is induced by microorganisms' neuraminidase, which cleaves the sialic acid on RBC membranes, resulting in the exposure of the T-antigen. T-antigens bind with naturally occurring anti-T antibodies found in the plasma, resulting in agglutination *in vitro*. Our results showed a high

prevalence of anti-T in COVID-19 patients, suggesting that SARS-CoV-2 causes transient T activation, polyagglutination in critically ill COVID-19 patients *in vitro* and possibly haemolysis *in vivo*.

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