

IMMUNOMODULATORY EFFECT IN COVID-19 PATIENTS AFTER HUMAN CORD BLOOD TRANSFUSION

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ABSTRACT

Introduction: Arthritis related chronic pain is very common among elderly people. Joint pain was also exacerbated in few from moderate to severe RT-PCR positive COVID-19 patients. Whole human cord blood progenitor cells and cord blood plasma may help in immunomodulation for treating the disease.

Material Methods: It was a prospective, academic, none to minimum risk experimental study. COVID-19, RT-PCR positive 12 anaemic patients were studied. In this study 6 patients were transfused cord blood and 6 patients were treated haematinics for correction of anaemia along with outcome of symptoms like chronic pain in the joints. Both male and female participants were 18-70 years of age. The study was conducted at Department of RMTS, STM, Kolkata vide memo no. CREC-STM/2020-AS-27 dated 18/08/2020 & Department of Biochemistry & Biophysics, Kalyani university, Nadia. Cord blood was collected and transfused in sterile procedure according to ABO compatibility, from Govt. run COVID-19 hospital of West Bengal. All patients were followed up for consecutive 4 months.

Result: Statistically all data analysed by SPSS, IBM,20 and Data Tab. Wilcoxon Signed Rank Test (Non-Parametric) used to compare the variables of chronic arthritic pain in between control and experimental groups. There was a statistically significant $p \leq 0.05$ median decrease in arthritic pain, when patients accepted the treatment of cord blood for treatment of anemia (0.024) compared to control group (0.102).

Conclusion: Cord blood plasma and progenitor cells have immunomodulatory properties apart from symptomatic improvement of anaemia in COVID-19 patients.

Keywords: Umbilical Cord blood, RT-PCR, COVID-19

INTRODUCTION

Discovery of the secret of life ‘DNA’ by Sir James Watson and Crick, the cellular, molecular, and developmental biology have been truly revolutionized. Pharmacological drug molecules alter or slow the course of diseases by affecting the disease producing cells or a group of cells. Unfortunately, most of the drug molecules unable to cure or control of many diseases. This has resulted to search an alternative that can use alone or in combination for controlling human or animal diseases (Nakano et al., 1999).

The concept of stem cells was first described by Theodor Boveri and Valentin Haeckel, they used the term stem cell to describe cells committed for self-renewal and give rise to the germline (Andreas et al., 2011)

The umbilical cord blood is an important supplier of nutrient and protective in nature, that helps in organ development in a developing foetus with the important nutrition elements and oxygen required for proper foetal development (Bajada et al., 2008). Apart from the foetal development, umbilical cord blood has enumerable therapeutic applications. First therapeutic application was reported in 1972 by clinicians in the United States to treat a case of Lymphoblastic Leukaemia (Bigham et al., 2012). Apart from that it was regularly used for transplantation either in haematological malignancy or bone marrow failure after chemotherapy (Dan et al., 2019, Potten et al., 1997). Umbilical cord blood contains different tissue group of cells (Bacakoya et al., 2018)

that have additional potentiality in treatment of other pathological disorders and medical applications such as regenerative medicine and tissue engineering (Henry *et al.*, 2004, Hamed *et al.*, 2012).

Corona virus disease 2019 (COVID-19) is a communicable disease causative agent for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Incubation period of corona virus 1–14 days, infected persons commonly present with symptoms of fever, cough, fatigue, breathing difficulties, anaemia, cytopenia and loss of taste and smell (Ende *et al.*, 1972). Some of the patients felt pain of all joints which was chronic in nature. Majority of COVID-19 patients are asymptomatic or present with mild symptoms. Some COVID-19 patients experience symptoms of pain long after their COVID-19 polymerase chain reaction test (RT-PCR) turn negative; this is commonly referred to as “post-COVID-19 syndrome” or “long COVID.” (Gluckman *et al.*, 1989).

Freshly collected human umbilical cord blood (HUCB) contains an average of 70-80 mL of blood at term. HUCB is rich in foetal haemoglobin (HbF) that has the potential to carry 60 % more oxygen than adult haemoglobin (Hb). Apart from that UCB also has a higher platelet concentration of 750000 against 250000 per micro litre of adult blood and a WBC count of 24,000 against 6500-10,500 cells/micro litre in adult blood. (Slatter *et al.*, 2006) HUCB has also been found to contain an array of anti-inflammatory cytokines like IL-10, IL-4, etc. (Bhattacharya *et al.*, 2009). UCB is a rich source of haematopoietic stem cells and progenitor cells (Watt *et al.*, 2005). Progenitor cells have immunomodulatory effect, hyperactive Th17 and decrease Treg cells play an important role in joint destruction, which may initiate chronic pain in elderly persons.

MATERIALS AND METHODS

The present study was conducted in the Department of Regenerative Medicine and Translational Science (RMTS), Carmichael Hospital, School of Tropical Medicine (STM) and Hospital, Kolkata, West Bengal in the year 2020 to 2021. The patients admitted in the Department of RMTS, STM during the period 2020-2021 were the subjects of present study (IEC ref No: CREC-STM/2020-AS-27 dated 18-08-2020). The criteria for selecting subjects for the study were 18-70 years old, both male and female volunteers, patients having haemoglobin levels less than equal to 10g/dL and post COVID patients voluntary participating in study. While the patients suffering from COVID-19 pneumonia & severe anaemic patients requiring urgent blood transfusion, not adhering to the study protocol, missing follow up visits, decision made by the expert clinical team. Anaemic patients due to unknown causes, haemoglobinopathies and systemic disease and patients having the presence of other chronic ailments e.g CKD, COPD, hypertension, malignancy were excluded from the study. The control group comprised of 6 Post Covid-19 patients with anaemia who had been treated with haematinics and other standard therapy as decided by the clinical expert team in the department. The experimental group consisted of 6 post Covid-19 patients with anaemia who had been transfused with freshly collected human umbilical cord blood (UCB). The study was conducted up-to 4 months from the day 0 or baseline study day.

Collection of cord blood

The collection of Umbilical Cord Blood was done immediately as soon the baby was born (Follow the SOP of cord blood bank; license No-DL-2MB/SLA/CLAA/WB). Prior to collection phlebotomist had confirmed the identity of donor. After delivery of baby and just after transection of the cord between clamps the phlebotomist cleaned the cord site with 70% IPA or 70% ethanol following the standard sterile blood collection protocol. Before taking out the UCB collection expiry date of the bag, the content and leakage were checked. During collection the needle was inserted into the umbilical cord at already cleaned site and bag was kept a bit lower than the puncture level so that blood flows freely into it in favour of gravity. Gentle shaking was done to prevent coagulation and proper mixing of anticoagulant. As soon as blood stopped tickling the clamp was placed above the puncture site. Clamps were placed subsequently at least for 3 times. UCB containing bag was wrapped with a clean wipe placed in the aluminium foil bag and placed it in room temperature with consent form and collection booklet duly filled up.

Application of cord blood in anaemic patients:

The collected bags were stored at room temperature. Three sample from the collected blood was taken for blood group (Rh and ABO) and matching, cross reactivity, HIV (1 and 2), hepatitis B and C, VDRL, malaria as per standard blood transfusion protocol (Denner *et al.*, 2007, Suvvari *et al.*, 2019) In case of any contamination or confusion, the culture was put aside for identification of the pathogen, if any, through appropriate protocol (Suvvari *et al.*, 2021, McNanley *et al.*, 2008), and the blood bag that matched the sample was marked unfit for transfusion. Post Covid healed patient with a haemoglobin count <- 8 g/dL was confirmed through hemogram tests were admitted and transfused with cord blood on day care basis. The patients were observed carefully for the initial 1 h or so in cases of any transfusion related reactions. The follow up studies was conducted by the group of medical officers and post graduate PhD students at the Dept. Follow up studies will be included complete hemogram, serum ferritin, along with severity scoring index (Broxmeyer *et al.*, 1989) Table- 1, and oxygen saturation.

RESULTS AND DISCUSSION

A total of 12 patients volunteered for our study. Amongst them only 6 patients agreed to receive Umbilical Cord Blood transfusion. 6 patients were given hematinic or other medications. Each patient from each group was thoroughly examined for over 120 days as well as the data from their blood reports and clinical symptoms were collected. Prior to the clinical symptoms variables were assessed and analyzed for comparison between the groups using the standard software excel SPSS, IBM 20 and DataTab for the part of our descriptive statistics and frequencies.

Wilcoxon Signed Rank Test (Non-Parametric) used to compare the variables of chronic pain related to arthritis in between study and control groups. There was significant median difference between follow-up and initial study groups with respect to chronic pain 0.05 level of significance with p values of 0.024. The median difference of chronic pain related to arthritis in study group and control groups was 1 and 0.25. There was a statistically significant median decrease in chronic pain related to arthritis when patients accepted the treatment as compared to the initial stage of the treatment (Table-1). (Figure 1-4).

Hyperactive Th 17 cells are differentiation from CD4+ T cells, proliferation of Th cell subset depends on the expression of specific transcription factors. Retinoic acid receptor-related orphan nuclear receptor- γ t (ROR γ t) and fork head or winged helix transcription 3 (Foxp3) are positive transcriptional regulators of differentiation between Th17 cells and Treg cells (Kotake *et al.*, 2017, Kondo *et al.*, 2018, Tada *et al.*, 2016). UCB induces of Foxp3 is upregulated, which regulates the Treg cell differentiation and releases anti-inflammatory factors such as transforming growth factor- β (TGF- β). During inflammatory environment, ROR γ t expression increases, which may promotes Th17 cell differentiation, production of many pro-inflammatory factors such as interleukin-17 (IL-17), and an induces inflammatory response. tissues and have high proliferation, multi-differentiation, and immunoregulatory. Cord blood stem cells can inhibit the proliferation of immune cells and the secretion of inflammatory factors (Ma D *et al.*, 2019, Tyndall *et al.*, 2014).

Human umbilical cord MSCs (hUCMSCs) have many advantages, such as a wide source, easy access to materials, strong proliferation ability, low immunogenicity, and great differentiation potential. They are most likely to become pluripotent stem cells.

cells with clinical application prospects.

There was significant median difference between Hb follow up and Hb initial Study groups at 0.05 level of significance as the p-value of 0.046 is less than 0.05. There was statistically significant median increase in Hb (8.89 gm/dl.) when patient accepted the treatment compared to initial stage of the treatment (8.20 gm / dl.). The median increases by an amount of 0.92 gm /dl. in Hb with the initial versus the follow up after giving umbilical cord blood treatment.

Table 1

Serial No	Symptoms	Control group (n-6)			Experimental group (n-6)			Statistics
		Initial mean	Follow up mean	Statistical significance	Initial mean	Follow up mean	significance	
1.	Chronic pain related to arthritis	1.50	1.08	0.102	2.00	0.75	0.024	<0.05

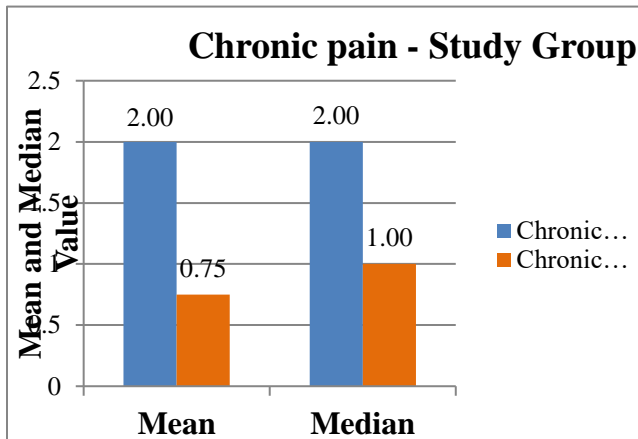


Fig. 1: Graph showing the mean and Median values of Chronic pain Initial and Chronic pain Follow up of Study Group

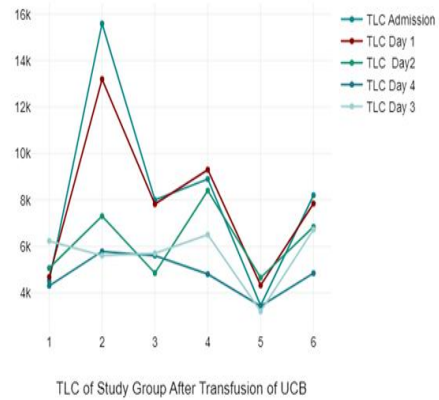


Figure: 2 TLC in transfused patients

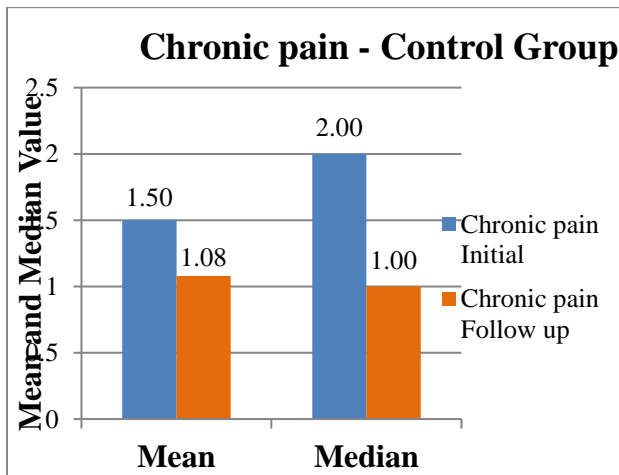


Fig. 3: Graph showing the mean and median values of Chronic pain Initial and Chronic pain Follow up of Control Group

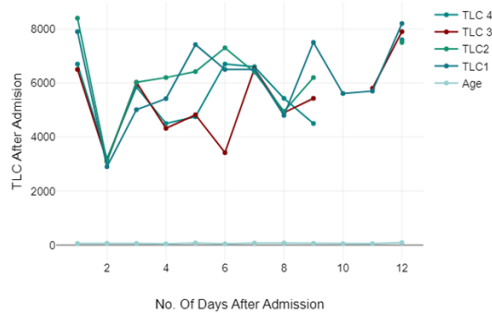


Fig. 4: Line Chart of TLC of Control group

ACKNOWLEDGEMENT

I acknowledge Mr. Binoy Bhattacharya & Mr. Apurba Sarkar for their constant supports for statistical analysis and constant supports.

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