DIFFERENCES ON THE IL-6 CYTOKINE RESLEASE FROM CORD BLOOD DERIVED STEM CELLS AND PHERIPHERAL BLOOD MONONUCLEAR CELLS IN RESPONSE TO HUMAN ORAL PATHOGENS

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ABSTRACT

Acute phase protein synthesis and the production of neutrophils in the bone marrow was depend on the cytokine named as IL-6 which is a Powerful Pro-inflammatory cytokine. It also supports the growth of B cells and is antagonistic to regulatory T cells. The aim of the present study was to investigate the *S.oralis* mediated IL-6 production in cord blood derived human stem cells (CBSC) in comparison with peripheral blood mononuclear cells (PBMC). In this study, stem cells were isolated from the umbilical cord blood and mononuclear cells were isolated from pheripheral blood of a healthy donar. A virulent heat inactivated *Streptococcus oralis* bacterial culture was used to stimulate both cord blood derived human stem cells (CBSC) in comparison with peripheral blood mononuclear cells (PBMC). After stimulation the culture supernatant was collected and subjected to quantification of IL-6 release by ELISA. The results showed that the *Streptococcus oralis* induces the IL-6 cytokine release in PBMC cells than the cord blood derived human stem cells.

Key words Interleukin-6, *Streptococcus oralis*, ELISA, PBMC, CBSC.

1. INTRODUCTION

Stem cells are cells which will differentiate into other sorts of cells, and may also divide in self-renewal to supply more of an equivalent sort of stem cells. Telomerase is active in normal stem cells. Stem cells are classified into two types, They are: Embryonic stem cells, they're isolated from the inner cell mass of blastocysts within the stage of early embryonic development, and adult stem cells, which are found in various tissues of fully developed mammals. In adult organisms, stem cells and progenitor cells plays an role within the repairing mechanism of cells, replenishing adult tissues. during a developing embryo, stem cells can differentiate into many specialized cells—called as ectoderm, endoderm and mesoderm but it maintain the traditional turnover of regenerative organs, like blood, skin, or intestinal tissues.

In Human there are three types autologous adult stem cells: bone marrow, fat , and blood. Stem cells also can be isolated from duct blood just after birth. Of all somatic cell therapy types, autologous harvesting involves the smallest amount risk. Adult stem cells are mostly utilized in various medical therapies (e.g., bone marrow transplantation). we will grow the Stem cells artificially and transformed it into specialized cell types with characteristics of cells of varied tissues like muscles or nerves. By using vegetative cell nuclear transfer of dedifferenciation process the formative cell lines and autologous embryonic stem cells are generated in future.[1]

Cytokines are alittle proteins which plays a crucial role in cell signaling. they're petides and that they cannot enter into the cytoplasm through lipid bilayer. they're involved within the process of autocrine, Paracrine and endocrine as a task Immunomodulating agents. Cytokines are produced by immune cells like Macrophages, B and T cells, Mast cells, Endothelial cells like Fibroblasts, and Stromal cells. These are act through receptors, they're important within the regulation of immune responses and plays an major role in Maturation, growth and responsiveness of immune cells.

Nowadays many parents want to store their duct blood stem cells for future. From the cells differentiated from stem cells, Macrophages are vital . They play an vital role in Antigen Processing and Presentation. due to this role these cells are called as Antigen Presenting cells. The another important cells in system is Natural Killer cells. They displays a robust cytolytic function on tumor cells and infected cells. The term Lymphokine was proposed by Dudley Dumonde (1969). Cytokines also are developed into Protein therapeutics by using recombinant deoxyribonucleic acid technology.

S.oralisis may be a Gram positive bacterium which is grown characteristically as chains. These forms Pieris rapae colonies on a Wilkins-Chalgren agar plate. they're mostly found in mouth . they're under the family of the streptococcus mitis group. These are the group of opportunistic pathogens. Strains of S.oralis produce neuraminidase and an IgA prokase and can't bind alpha amylase.

Based on the structural characteristics of Protein they're classified into chemokine family. These chemokines are small and having the relative molecular mass of 8 to 10KDa. Chemokines share their Gene and aminoacid Sequence. in order that they are 20-50% unique in their form. For creating their 3 – dimensional structure they possess a aminoalkanoic acid .

B cells and T cells are plays an major role in Humoral and cell mediated immunity. B cells are produced at the location of bone marrow with the induction of antigen. When the Macrophages or Dendritic cells encounter the antigen they pass the signal to B cells or T cells to stimulate the immune reaction . The B cells mature into Plasma cells which produce Antibodies against that specific Antigens. The T cells release the Cytotoxic or killer T cells to encounter the antigen. this sort of activation of T and B cells are regulated by the Signalling molecules like Cytokines.

Cytokines plays an important role in signaling. It also helps for the interaction of Antigen and Antibody. during this study we are getting to understand the difference between the cytokines released from PBMC and CBSC.

MATERIALS AND METHODS

Streptococcus oralis (MTCC No. 2696) was purchased from MTCC, Chandigarh, India. The human IL-6 ELISA kit was purchased from Invitrogen, USA. Nutrient agar medium, were purchased from Himedia, India. RPMI medium, Stempro medium, Fetal Bovine Serum (FBS), Trypsin EDTA and Penicillin/Streptomycin antibiotic solution were from Gibco (USA), 1X PBS was from Himedia, (India). 96 well tissue culture plate and wash beaker were from Tarson (India). Test tubes, glass beakers, conical flasks and petri-plates were from Borosil India. Spirit lamp, double distilled water and test samples.

NUTRIENT AGAR MEDIUM

The medium was prepared by dissolving 2.8 g of the commercially available Nutrient Agar Medium (HiMedia) in 100ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.

NUTRIENT BROTH

Nutrient broth was prepared by dissolving 2.8 g of commercially available nutrient medium (HiMedia) in 100ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes.

CULTURE AND HEAT INACTIVATION OF BACTERIAL CELLS

The bacterial culture *Streptococcus oralis* (MTCC. No: 2696) was obtained from MTCC, Chandihar. Culture was inoculated into 10 ml of LB broth and incubated overnight at 37°C. Serial dilutions were made and the concentration of bacteria was adjusted to 10^4 CFU/ml. The bacterial culture was heat-inactivated at 75°C for 45 minutes. The culture was plated overnight to check for the nil growth of colonies.

Fig : 3. Culture of *S.oralis*

Fig:4. OD value of *S.oralis* culture after an incubation of 45 hrs.

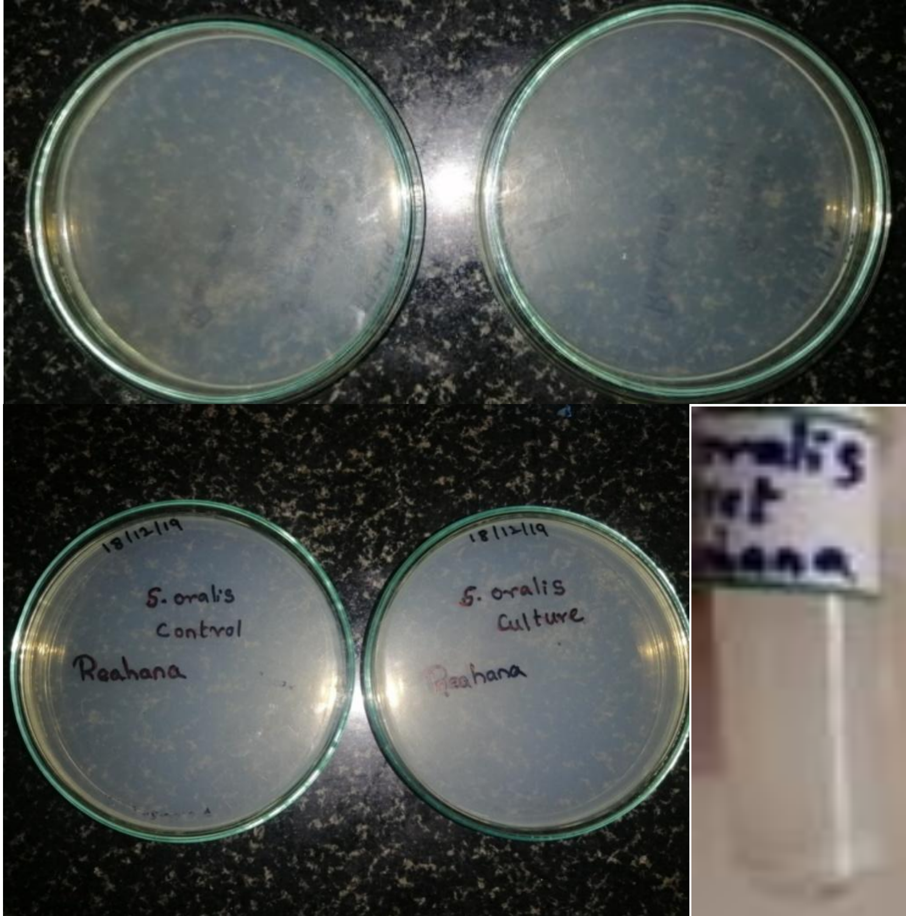


Fig : 5 Heat killed *S.oralis* culture after 24 hrs of incubation.

ISOLATION OF PERIPHERAL MONONUCLEAR CELLS

Human peripheral blood was collected from the healthy volunteers in 3.8 % sodium citrate-treated tubes in accordance with established institutional guidelines. Briefly, mononuclear cells were isolated by layering peripheral blood onto Lymphocyte Separating Medium (LSM). The leukocytes enriched buffy coat contains the mononuclear cells were collected and the presence of erythrocytes was removed by lysing with 1% RBC lysis buffer. The total number of cells present in the cell suspension was calculated using hemocytometer.

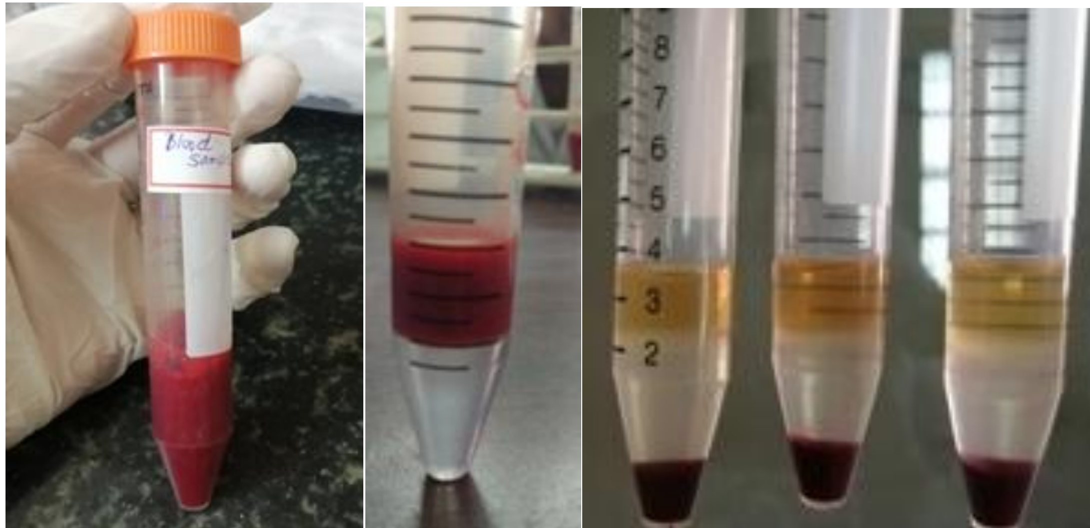


Fig: 6. Isolation of PBMC cells and its cell harvesting process

ACTIVATION OF MONONUCLEAR CELLS WITH HEAT KILLED *S. ORALIS*

The mononuclear cells were plated at a density of $1x10^6$/ml in a RPMI medium and stimulated with different numbers of heat inactivated *S.Oralis* incubated for 24hr at 37°C in Co2 incubator.

After incubation, the culture supernatants were collected by centrifugation and stored frozen at −20 ◦C, until analysis.

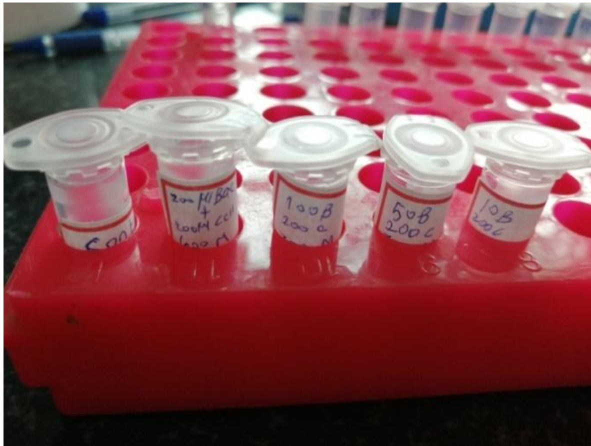


Fig :7. PBMC cell suspension treated with different concentration of heat killed *S.oralis*.

ISOLATION OF HUMAN CORD BLOOD DERIVED STEM CELLS

Human cord blood was collected during delivery in 3.8 % sodium citrate-treated tubes in accordance with established institutional guidelines. Briefly, stem cells were isolated by layering peripheral blood onto Lymphocyte Separating Medium (LSM). The buffy coat contains the mononuclear cells and stem cells were collected and the presence of erythrocytes was removed by lysing with 1% RBC lysis buffer. The total number of cells present in the cell suspension was calculated using hemocytometer.

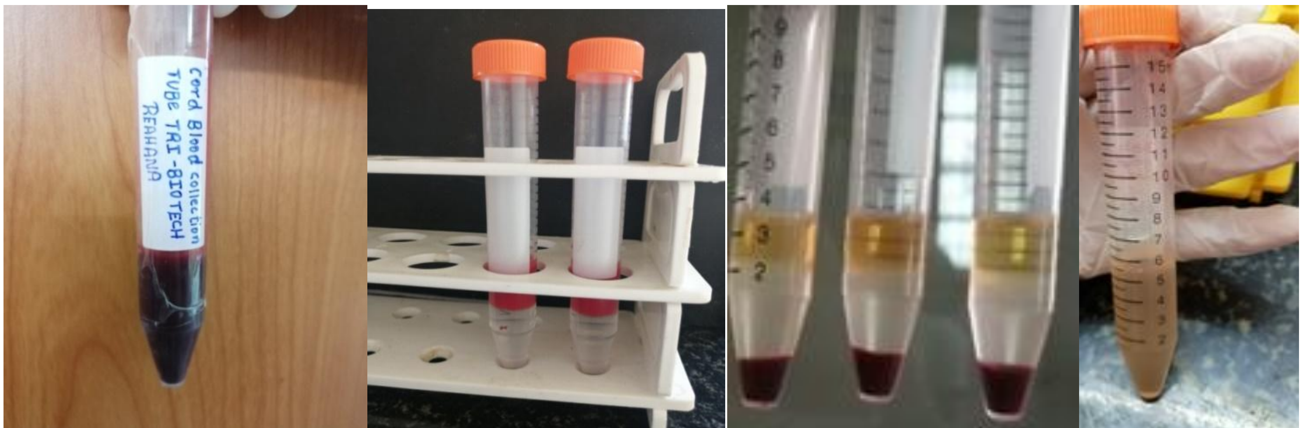


Fig: 8. Collection and isolation of CBSC cells.

ACTIVATION OF STEM CELLS WITH HEAT KILLED *S. ORALIS*

The stem cells were plated into a 24 well plate at a density of $1x10^6$/ml in a stempro medium and stimulated with different numbers of heat inactivated *S.Oralis* incubated for 24hr at 37°C in Co2 incubator. After incubation, the culture supernatants were collected by centrifugation and stored frozen at −20 ◦C, until analysis.

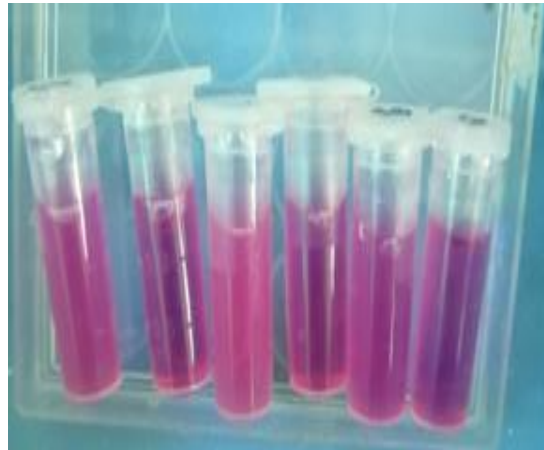


Fig : 9. Stimulation of CBSC cells with heat inactivated *S. oralis*

MEASUREMENT OF IL-6 BY ELISA

Inflammatory cytokine IL-6 levels were quantified from the PBMC and CBSC cells supernatant using sandwich ELISA as described in the manufacturer's protocols. The reaction was read at 450 nm in a Microplate reader, Thermo Scientific, USA.

Fig. 10. Quantification of IL-6 cytokine release using in CBMC and PBMC cells on 96 well plate.

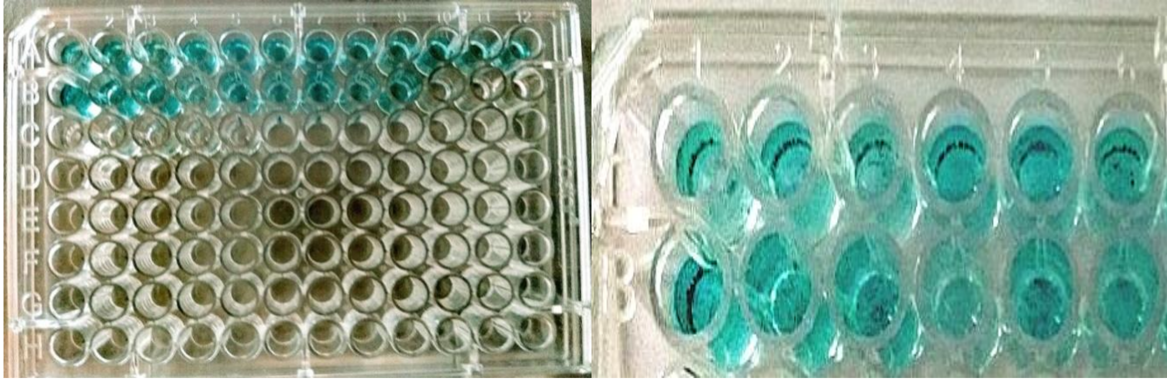


Fig. 11. Development of ELISA plate with streptavidine HRP enzyme and TMB solution

ELISA,14/2/20,19:6:50,

Row	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12
A	0.246	0.210	0.242	0.163	0.267	0.314	0.331	0.280	0.236	0.261	0.229	0.271
B	0.263	0.223	0.282	0.116	0.255	0.180	0.237	0.203	0.272	0.090	0.087	0.086
C	0.070	0.067	0.065	0.063	0.065	0.035	0.071	0.036	0.034	0.036	0.036	0.046
D	0.037	0.041	0.034	0.189	0.039	0.044	0.038	0.045	0.040	0.043	0.044	0.058
E	0.039	0.055	0.042	0.045	0.046	0.042	0.048	0.043	0.058	0.044	0.040	0.041
F	0.038	0.046	0.054	0.043	0.040	0.040	0.038	0.046	0.038	0.044	0.039	0.047
G	0.036	0.042	0.039	0.039	0.037	0.036	0.035	0.038	0.035	0.036	0.039	0.037
H	0.046	0.054	0.049	0.044	0.061	0.058	0.046	0.046	0.059	0.059	0.042	0.042

Fig : 12. IL-6 ELISA result

S.No	Concentration of *S. oralis*	PBMC	CBSC
1.	Control	58.895	47.502
3.	24,000 Cells/ml	79.013	87.740
4.	12,000 Cells/ml	78.044	83.134
5.	6,000 Cells/ml	73.723	73.438
6.	1,200 Cells/ml	70.287	63.015

RESULTS

ISOLATION OF PBMC AND CBSC CELLS

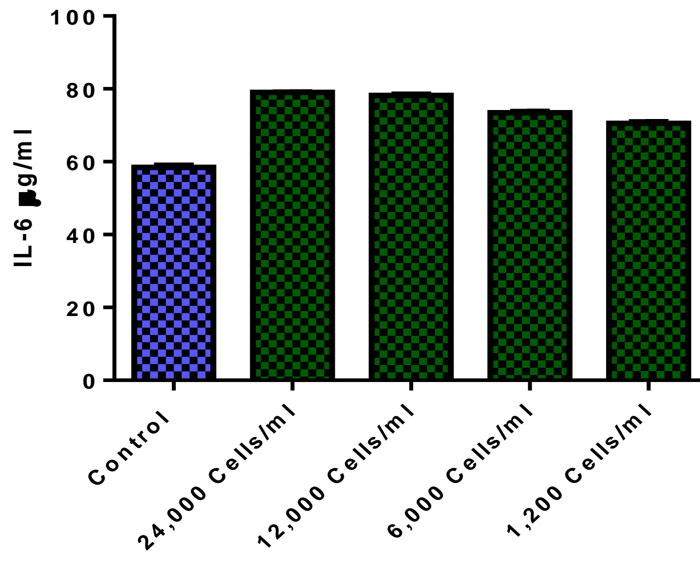
The PBMC cells were isolated from the peripheral blood of a healthy donor. Similarly, the cord blood derived stem cells were isolated from the umbilical cord blood. The isolated cells were counted using hemocytometer and seeded on to the 24 well tissue culture plate at the density of $1x10^6$ cells/ml.

1.1 DETERMINATION OF IL-6 RELEASE FROM PBMC AND CBSC CELLS

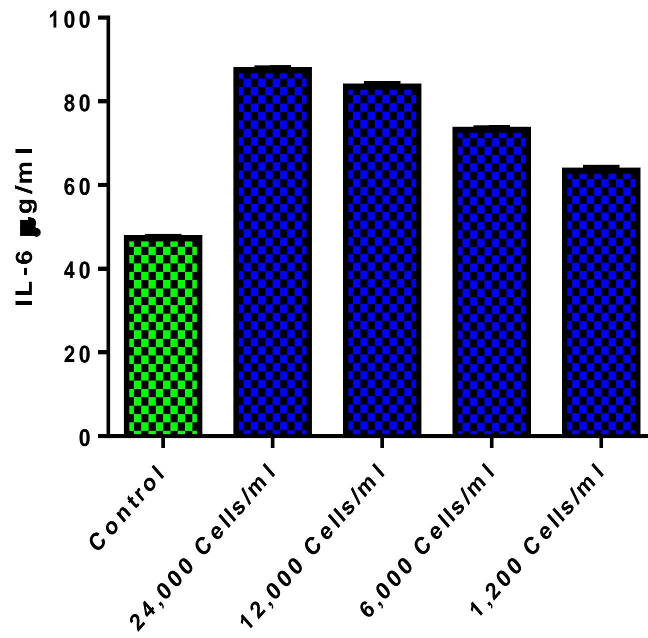
The differences in the IL-6 cytokine release from PBMC and CBMC cells in response to *S. oralis* were quantified by the human IL-6 ELISA kit. The results showed that, both PBMC and CBSC cells produce IL-6 cytokines, which are enhanced by the stimulation of *S. oralis*. Compare to mononuclear cells (70.013 µg/ml),

stem cells (87.740 µg/ml) showed higher release of IL-6 cytokine upon stimulation with 24000 cells/ml of *S. oralis*.

Whereas, PBMC cells (58.99 µg/ml) baseline IL-6 cytokine level was found to be higher than the CBSC cells (47.502). Taken together, these results showed that both PBMC and CBSC cells produce IL-6 cytokines, but differential expression of IL-6 levels in response to human oral pathogen *S.oralis* infection.



Diff. Conc *S. Oralis* and PBMC 1 x 10^6 cells/ml



Diff. Coc. *S.oralis* and 1X10^6 cells/ml of CBSC

DISCUSSION

IL-6 may be a soluble mediator with a pleiotropic effect on inflammation, immune reaction , and hematopoiesis.The present study shows that the cellular difference between cord blood derived stem cells and pheripheral mononuclear cells in response to pathogens, it helps to review the differences within the release of IL6 between PBMC and CBSC cells. Human oral pathogen S.oralis plays a serious role in oral infection. Herein his study, heat killed *S.oralis* pathogen wont to induce both PBMC and CBMC cells for the discharge of IL-6 cytokine. Since the study primarily addressed the discharge of IL6 cytokine in both the cells. within the previous study, the precise release of preformed IL-6 from peripheral blood mononuclear cells (PBMC) after 20 minutes incubation with 0.15–0.5 μM of pure drugs was measured in two groups of drug-allergy suspected donors and respective controls. IL-6, TNF-alpha, IL-2, IL-4, IFN-gamma are measured from cell supernatants by ELISA or by cytometric bead assay. perform IL-6 release assays with cytostatics emerging from the patients' history but keeping in mind that no published data are available yet. A possible candidate might be azathioprin [155]. In those early experiments during which exogeneous IL-6 was introduced to humans, induction of both IL-1Rα that bound IL-1β and circulating TNF receptors was shown [156]. From earlier experiments of PBMCs in drug hypersensitive patients. a basic release of 100–300 pgxml-1 TNF-α was evident at 24 hrs [157]. There are not any data available for the interval between 0–60 min. The finding of fetal neutrophils into duct (funisitis) and within the chorionic plate vessels has been considered the last stage of inflammatory response related to [158] a worse neonatal outcome [159]–[161]. Our study ratifies the very fact that a high fetal inflammatory response mediated by duct IL-6 was observed in newborns. The results showed that, both PBMC and CBSC cells produce IL-6 cytokines, which are enhanced by the stimulation of *S. oralis*. Compare to mononuclear cells (70.013 μg/ml), stem cells (87.740 μg/ml) showed higher release of IL-6 cytokine upon stimulation with 24000 cells/ml of *S. oralis*. Whereas, PBMC cells (58.99 μg/ml) baseline IL-6 cytokine level was found to be above the CBSC cells (47.502). In my study both the PBMC and CBMC cells are treated with different concentration heat inactivated *S.oralis* and therefore the CBSC cells release more IL6 than PBMC cells hence PBMC cells contains more immune reaction of Interleukin 6 as a base level. IL6 acts as both pro-inflammatory and anti inflammatory myokine.

CONCLUSION

The novel study was done by using PBMC and CBSC cells for the identification of differences in the release of IL6 in response to human oral pathogen *S.oralis*. Hence the umbilical cord derived stem cells plays a major role in IL-6 release. Though both PBMC and CBSC cells produce IL-6 cytokines, but showed the differences in the expression levels of IL-6 cytokines in response to a human oral pathogen (*S.oralis*). In the final negotiation, the study will helps to understand the differential IL-6 expression in various against the human oral pathogen.

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