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Original Article

The Effect of Wharton's Jelly Mesenchymal Stem Cells in Colorectal Cancer

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ABSTRACT

Mesenchymal Stem Cells (MSCs) are self-renewing multipotent cells that can be isolated from various organs including Wharton's jelly of umbilical cord (WJMSCs). WJMSCs play a fundamental role in field of regenerative medicine and hold therapeutic potential for various diseases including gene therapy of different cancers. WJMSCs were evaluated for their potential behavior in cancer conditions by culturing them in the plasma obtained from colorectal cancer patients. The cancer plasma was tested LDH release, paracrine factors (p53, p38, BAX and BCl2) and oxidative stress. Cell viability, death and proliferation was assessed by crystal violet staining, LDH release and MTT assay respectively. Assessment of cancer plasma expressed high LDH release, low expression of pro-apoptotic gene BAX, high expression of survival gene BCl2 p38 kinase and tumor suppressor gene p53. In oxidative stress, GSH and SOD were increased in CRC. In CAT, it was highly expressed in CRC. Furthermore, increased level of MDA was expressed in cancers' plasma. It was observed that cells cultured in plasma of cancer demonstrate less viability, low proliferation rate, high LDH release, low expression of BAX and p38 kinase, whereas, high expression of BCL2 and p53. In oxidative stress, low GSH, SOD, CAT and high MDA was observed in CRC.When WJMSCs cultured in plasma from colorectal cancer patients these cells show reduced viability, proliferation and increased apoptosis. For the future, certain measures should be taken to improve viability of WJMSCs in cancer conditions.

Keywords: Wharton's Jelly, Mesenchymal Stem Cells, Colorectal Cancer

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Colorectal cancer (CRC) is the most prevalent and incident cancers worldwide and a main cause of cancer-related death in western countries (1). It is a very heterogeneous disease that is caused by the interaction of genetic and environmental factors and can be classified based on the importance of each of

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these factors (2) also CRC cases could be avoided with a healthy lifestyle (3). In colon epithelial cells, changes in genetic and epigenetic leads to malignant tumor originating in glandular epithelium (4) its survival rate is 5 years. It further include growth of cancer in the colon, appendix and rectum (5). Mostly, CRCs are arises from adenomatous polyps located in colon which can be removed before they become cancerous (6). Treatment such as radio and chemotherapy, are employed to CRC and a various researchers have identified prognosticative factors. Still, complete remedy of CRC has not been full filled in contempt to limitless efforts (7).

The common causes for CRC includes, less use of fruit and vegetables, excessive intake of red meat and saturated fat, alcohol intake, male gender, smoking, a sedentary life-style, tobacco, use of drugs, body mass index and being overweight. Age is also the main risk factor it is more frequent in 50 onwards and this risk is directly proportional age (8-10).

Colonoscopy is considered the standard tool for the diagnosis of colorectal pathologies and use of postmenopausal hormone have been linked with reduced CRC risk (11, 12). Treatment of CRC consists of complete surgical removal of primary lesions and lymph nodes (13). The mutations and epigenetic alterations in CRC usually involved in the progression

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are shown in Figure below. There are 5 stages of this disease. Stage 0 during formation of an early adenoma, stage I where the tumor is located in mucosal layer, stage II when it just penetrates the serosa followed by increasing angiogenesis, stage III when the cancer has spread to surrounding lymph nodes and finally stage IV with distal metastasis, the cancer has spread to other organs.

MSCs are a type of heterogeneous cells that have the ability to self-renew, cells isolated majorly from bone marrow and other post-natal tissues and are multipotent cells. They can be easily isolated, have potential to migrate towards the inflammatory sites and can be therapeutically used in clinical studies to target tumors (14).

MSCs derived from human umbilical cord is an extremely attractive source of SCS having great potentials in clinical applications and regenerative medicine. There are benefits to use UC stem cells because these cells are non-tumorigenic, highly proliferative, and less immunogenic and ethically approved (15). UC is an extremely rich source of SCs. WJMSCs have many potentials in comparison other SCs types (16). The UC comprises of two umbilical arteries and one umbilical vein, cord lining and Wharton's jelly (17). Stem cells obtained from UCB play a vital role as these cells gave a reduced rate of rejection after transplanting them into mismatched host and their potential to generate large amounts of homogeneous tissue or cells (18). WJMSCs hold a great therapeutic potential for tissue repair and regeneration in various *in vitro* studies of human diseases including cancer, heart problems, neurodegenerative disease, etc. (19). MSCs are attractive nominees for cell based therapies as till now human trials of MSCs show no inauspicious reactions towards MSC transplants (20). MSCs presents various approaches that makes them a perfect source of therapeutic agents in the field of regenerative medicine (21).

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The aim of study was to evaluation the potential of Wharton's jelly mesenchymal stem cells in colorectal cancer.

Materials and Methods

Sample collection

Blood samples of cancer patients were taken from INMOL (Institute of Nuclear Medicine and Oncology) hospital, Lahore. A total of 3-5 ml of blood sample was collected from each patient in heparinized vials. Plasma was isolated from these blood samples. Plasma samples were pooled of 3 patients and passed through syringe filter (0.22µm) before storing aliquots at -20˚C. Several tests were performed on blood from patients with CRC including hematology analysis, LDH assay, ELISA, catalase assay, estimation of oxidative stress, examination of glutathione, examination of sodium dodecyl sulphate, estimation of malondialdehyde.

Furthermore, Human umbilical cords from normal full term caesarian sections without HCV, HBV and HIV infection were obtained from Social Security Hospital, Lahore. A 1 inch piece of human umbilical cord was taken in a 50 ml tube containing PBS (Phosphate Buffer Saline) with 100U/ml penicillin and 100µg/ml streptomycin.

LDH assay

LDH is an enzyme used to measure cell membrane integrity. Normal and different cancer plasma were compared to evaluate LDH release. Cytotoxicity level was assessed by the lactate dehydrogenase assay (LDH) assay kit according to manufacturer's instructions. Briefly, reagent was prepared by mixing R1 and R2 in a ratio of 4:1. 5 µL plasma and 95 µL reagent was added in single well of a microtiter plate. Absorbance was measured at a wavelength of 340 nm**.**

ELISA

Solid phase sandwich ELISA was performed for BCL-2, BAX, p53 and p38 (Invitrogen, USA). A microtiter plate (Corning, USA) was coated with primary antibodies i.e. mouse anti BCL-2, BAX and p53 and rabbit anti p38 and incubated for 48 hours at 4°C. After washing thrice with TBS, 100µL serum samples were loaded and incubated for 18 hours. The wells were then washed three times, blocked for 1 hour with 10% BSA leading to overnight incubation with secondary antibody i.e. HRP conjugated goat anti rabbit for p38 and HRP conjugated goat anti mouse for BCL-2, BAX and p53. After washing equal volume of chromogenic solution (TMB) and 0.1mM HCL was added, to stop the reaction. Using a microtiter plate reader, read the plate at the appropriate wavelength for the substrate at 450nm keeping 650nm as reference value.

Estimation of glutathione

100µl of plasma was taken, homogenate was prepared in 100µl (10%) TCA and centrifuged at 1000 rpm for 10 minutes at 25˚C. In a microtiter plate, 40µl the supernatant was added with 150µl disodium phosphate buffer and 25µl DTNB. Incubated this plate for 15 minutes and absorbance was taken at 412 nm.

Estimation of sodium dodecyl sulphate

50µl of plasma was taken, homogenate was prepared in 50µl (50%) TCA and centrifuged at 13,000 rpm for 10 minutes at 25˚C. 15µl of the

supernatant was added to 120µl sodium pyrophosphate buffer (52mM) pH=8.3, 12µl phenazine methosulphate, 36µl NBT and reaction was started by addition of 24µl (NADH). After incubation at 37˚C for 90 seconds, the reaction was stopped by the addition of 12 µl of glacial acetic acid. The reaction mixture was stirred vigorously with 400µl of n-Butanol. The mixture was incubated for 10 minutes and then was centrifuged at 2000 rpm for 5 minutes at 25˚C. 200µl of butanol layer was taken in microtiter plate. Absorbance was observed at 560 nm.

Catalase assay

40µl plasma was taken, homogenate was prepared in 360µl phosphate buffer (10mM) pH=7.4, centrifuged at 13,000 rpm for 10 minutes at 25˚C. 21µl supernatant and 180µl phosphate buffer (10mM) pH=7 were added in an eppendorf (1.5ml) tube. Reaction was started by addition of freshly prepared 75µl H_2O_2 (0.2M). 360µl potassium dichromate acetic acid reagent (5%) leading to incubation for 10 minutes in boiling water. 150µl of the mixture was added in a microtiter plate and absorbance was taken at 530 nm.

Estimation of Malondialdehyde

The levels of MDA were measured by using thiobarbituric acid reactive substances. 40 µl of plasma was taken and a homogenate was prepared in 360µl of 10 mM phosphate buffer (pH 7.4) and centrifuged at 13,000 rpm for 10 minutes at 25˚C. Concentration of MDA, a metabolite of polyunsaturated fatty acids, was assessed as a marker for lipid peroxidation. 15µl of the supernatant was added to 15µl SDS (8.1%), 96µl TBA (0.8%), 96µl Acetic Acid (20%) and 18µl distilled water and incubated at 90˚C for 60 minutes. Afterwards 60µl distilled water, 300µl mixture of n-Butanol and pyridine with the ratio of 15:1 was added and the mixture was shaken vigorously and centrifuged at 4000rpm at 25 °C for 10 minutes. The thiobarbituric acid-reactive substances in the n-butanol layer 125µl were taken in a microtiter plate. Absorbance was taken at 532 nm.

Isolation and culturing of WJMSCS

Samples were transferred to the laboratory and washed twice with PBS. The entire process was done in Class II BioSafety Cabinet under sterile conditions. Small piece of cord was minced thoroughly into very fine pieces. The pieces were transferred to a 25cm^2 culture flasks (Sigma Aldrich, USA) with Dulbecco's modified Eagle's medium with low glucose (DMEM LG) and 10% Human Plasma, penicillin (100 units/ml) and streptomycin (100 µg/ml). The cells were then placed in the humidified incubator supplied with 5% CO_2 at 37°C. After 72 hours medium was changed carefully and cells were fed with fresh medium thereafter. Non adherent cells were removed and replaced with fresh medium, observed culture under microscope to assess the degree of confluency.

Sub culturing of WJMSCS

Cells at 80-90% confluence were subjected to passage. Cells were dissociated using 1X Trypsin-EDTA for 4-5 minutes at 37°C and 5% CO_2 until the Cells began to detach. Detachment was confirmed by observing the flask under Phase contrast microscope, then neutralized with equal volume of complete culture medium. Cells were

plated in a 75 cm^2 culture flasks and placed in incubator. Cells at passage 3 were used for further experiments.

Characterization of WJMSCs

The cultured cells were plated in a 6 welled plate for characterization by immunocytochemistry for 24 hours. Media was removed and cells were washed with PBS (Invitrogen Inc., USA) three times for 5 minutes leading to fixation by incubating them in ice cold methanol for 20 minutes. Cells were incubated with 1% BSA for 30 minutes to block unspecific binding of the antibodies. Cells were Incubated with primary antibodies i.e. goat anti CD 90 and CD45 (Santa Cruz, USA) in a dilution of 1:100 at 4°C overnight. After washing, Flourochrome conjugated secondary antibody (FITC-rabbit anti goat) was added in each well. The cells were incubated with DAPI for 15 minutes rinsed with PBS, finally stained cells were observed under imaging station for expression analysis.

Population doublings

After every passage, cells were placed in a T25-flask, 80-90% confluent cells were trypsinized, counted and replated. This phenomena was repeated until passage 7. Population doubling was calculated using an online population doubling calculator. (<http://www.doubling-time.com/compute.php>).

Treatment with CRC plasma

The WJMSCs were plated in a 6 well tissue culture plates in equal number and divided in six groups. One of the groups was CRC patients' plasma. Cells treated with DMEM and 10% colorectal cancer patients' plasma. Several test was done on cells including cells viability assay, cell proliferation assay, LDH assay, ELISA, oxidative stress, cryopreservation of stem cells, and statistical analysis. LDH assay and ELISA, the protocol was the same mentioned above. The oxidative stress protocol was resemble to the protocol of GSH, Catalase, SOD and MDA which were mentioned above.

During the cell viability assay, Cells after treatment were provided with crystal violet for 20 minutes which were subsequently lyesd with DMSO after three washings with normal saline. Absorbance was taken at 540 nm.

Cell proliferation assay compare proliferative potential of all treatment cells. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay was performed. Cells monolayer was first washed with PBS. 500 µl 10% complete medium and 60 µl MTT solution was added to cells and incubated for 2 hours. Cells were lysed with 1.5ml DMSO and absorbance was taken at 570 nm.

Cryopreservation of stem cells

The cells in the growth phase were detached from the surface by 1X trypsin EDTA and centrifuged at 2000rpm for 10 minutes at 25˚C to obtain all the cells in pellet. Supernatant was discarded and pellet was suspended in 1ml plasma, 5µl heparin and DMSO (5%) 50µl. The cell suspension was saved in cryovials and stored at -20^0C for 2 hours then placed in -80^0C and then in liquid nitrogen for use in future.

Statistical analysis

All experiments were performed in triplicates. Statistical tests and graphical representations were performed using GraphPad Prism 5 software (GraphPad, San Diego, CA). Data is presented as mean± standard deviation. Unpaired student T tests were performed for comparison between two group's data Student's t-test was used for comparison of data between two groups while ANOVA was performed for comparison between more than two groups. The statistical significance was set at P<0.05.

Results

LDH assay

Cytotoxicity level was assessed in normal and different cancer patients' plasma by the LDH assay. It was observed that plasma from normal persons, released a significantly less LDH as compared to all cancer patients.

Sandwich ELISA

Sandwich ELISA was performed on normal and different cancer plasma. Pro-apoptotic genes BAX was significantly decreased in all cancers plasma in comparison to normal plasma (Fig. 2A), while significantly increased gene expression of pro-survival markers BCL2 was observed in all cancer group (Fig. 2B), whereas, p38 was significantly enhanced in all cancers (Fig. 2C), furthermore significant increased gene expression in all cancer group was observed in p53 tumor suppressor gene (Fig. 2D).

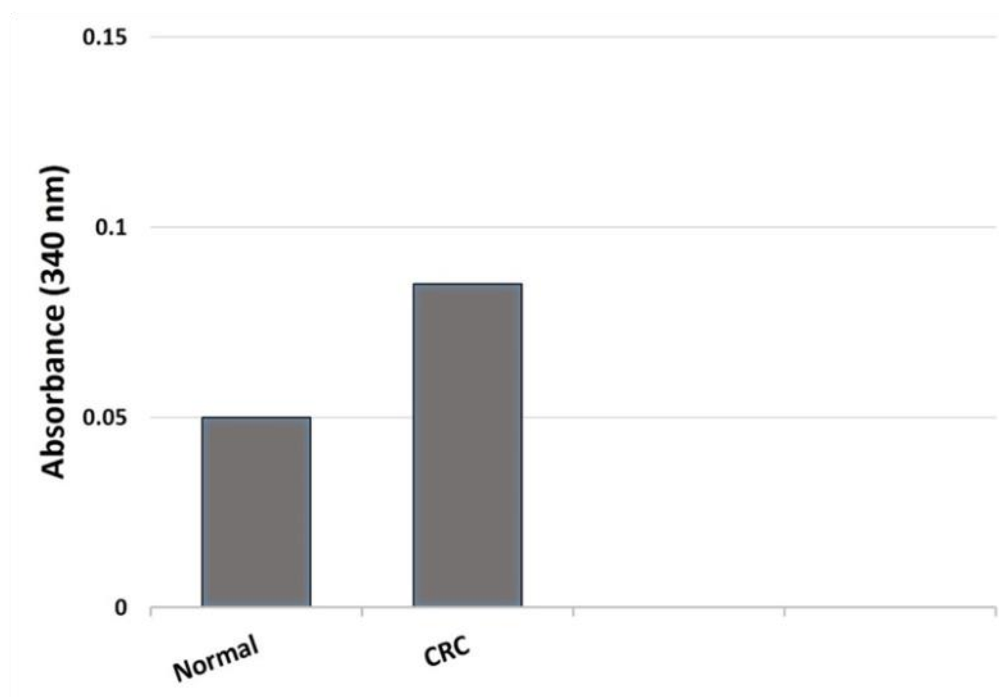

Fig. 1. Assessment of LDH release on normal and cancer patients' plasma. LDH release was significantly high in plasma from cancer patients. The data is expressed as mean ± SD and P≤0.05 was considered significant for all comparisons. *** denotes significant difference between normal and cancer groups.

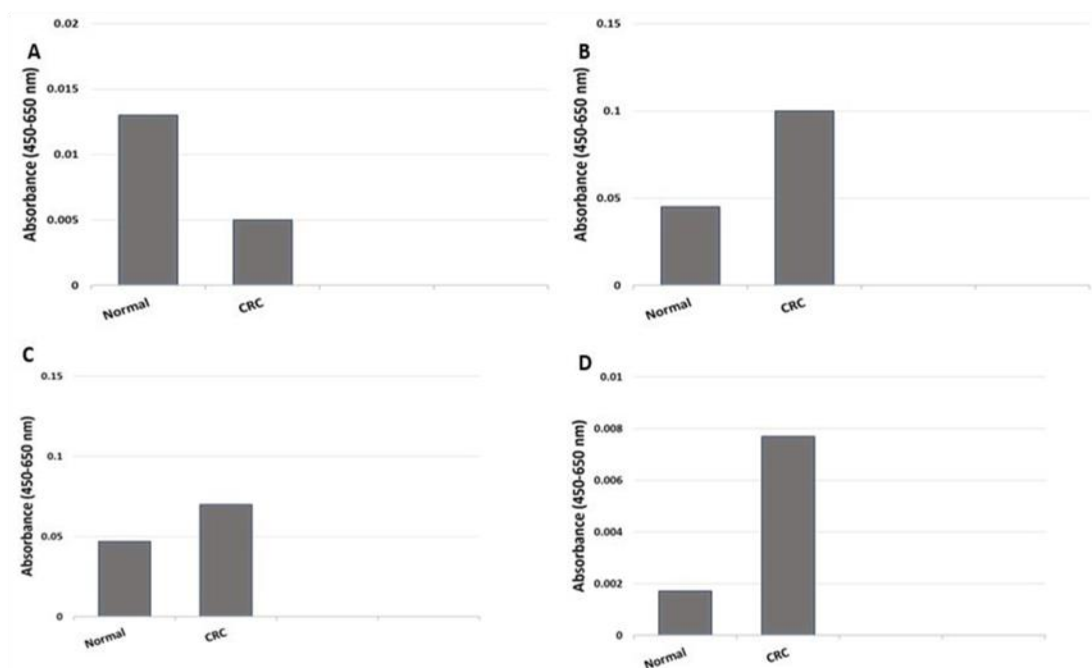

Fig. 2. Graphical representation of ELISA for A)BAX, B)BCL2, C)P38 and D)P53 on normal and cancer serum. The data is expressed as mean ± SD and P<0.05 was considered significant for all comparisons.

Oxidative stress

Oxidative stress was assessed by comparing plasma of normal and different cancer groups. It was observed that GSH release was increased in CRC (Fig. 3A), SOD levels were significantly decreased in all cancer except CRC and OC (Fig. 3B) whereas, in catalase assay no significant difference between was observed between normal and OC while significant high expressed was found in other cancer groups (Fig. 3C) and significant high ($P<0.05$) MDA level was observed in all cancer (Fig. 3D).

Isolation and culturing of WJMSCS

The next step in the study was isolation and characterization of MSCs from WJ. The piece or explant after mincing released rounded cells in medium which attained mesenchymal appearance and flask got confluent after 15-20 days.

Characterization of WJMSCs

The WJMSCs were characterized for the expression of mesenchymal marker CD90 and hematopoietic marker CD45. WJMSCs expressed CD90 while they lack the expression of CD45.

Evaluation of population doubling time

The cells after every passage were collected to count the cell number. Estimation of population doublings rate of WJMSCs more accurately assesses the cells' growth at different passages.

Cells viability assay

Cell viability was assesses by staining the cells with dye known as crystal violet. The dye stained all the cells present in a particular well and on lyses absorbance of the color released was compared for the all treatment groups. It was observed that cells

cultured in normal plasma are more viable than those cultured in different cancer plasma.

Cell proliferation assay

WJMSCs were treated with normal and different cancer patients' plasma and were analyzed for cell proliferation by MTT assay. It was observed that cells cultured in cancer plasma showed a significantly low proliferation as compared to the control group. (Fig. 9A).

LDH ASSAY

LDH assay was performed on WJMSCs treated with normal and cancer plasma. LDH release was significantly higher in the medium of cells treated with cancer plasma. (Fig. 9B)

Sandwich ELISA

ELISA was performed on WJMSCs treated with normal and cancer plasma.

Oxidative stress

Oxidative stress was performed on WJMSCs treated with normal and different cancer plasma. It was observed that GSH release was significantly lower in medium of cells cultured with plasma of different cancer patients (Fig. 11A). Superoxide dismutase (SOD) release was significantly lower by the cells cultured in plasma of cancer patients as compared to untreated cells (Fig. 11B). Catalase release by cells of cancer plasma treated group was significantly lower as compared to normal untreated group as shown by less absorbance (Fig. 11C). MDA release by cells of cancer plasma treated group was significantly higher as compared to normal untreated group as shown by less absorbance (Fig. 11D).

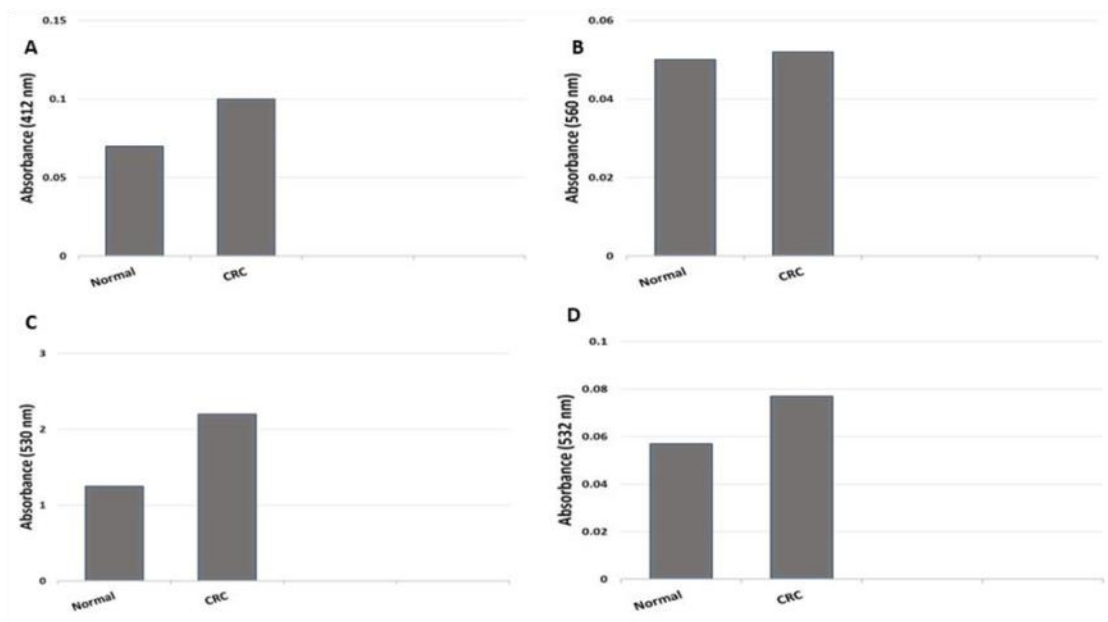

Fig. 3. Graph representing effect of normal and cancer patient's serum on A) glutathione B) SOD C) catalase and D) MDA release. The data is expressed as mean ± SD.

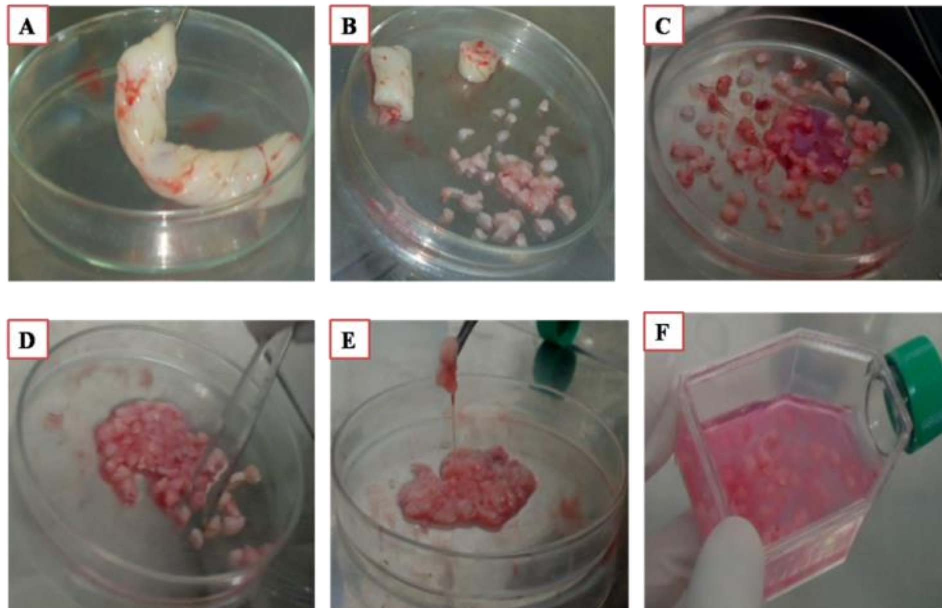

Fig. 4. Isolation and culturing of human WJMSCs (A) ~2 inches piece of umbilical cord was taken in a petri dish and washed with PBS. (B&C&D) The umbilical cord (UC) was minced into fine pieces. (E&F) WJ was obtained by removing the cord vessels and the amniotic epithelium, the thick jelly obtained was plated in culture flasks with DMEM and 10% Plasma. The cells were then placed in a humidified incubator.

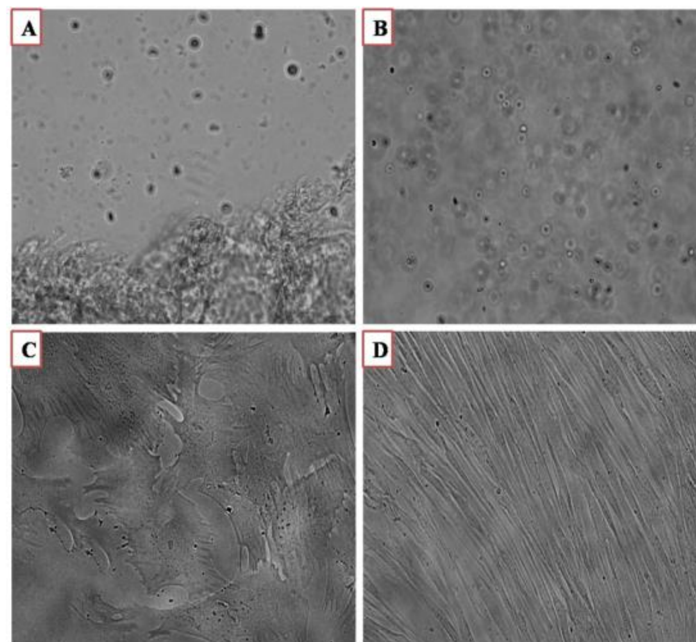

Fig. 5. Morphological appearance of WJMSCs. (A) Spherical Cells emerging from piece of cord was observed under microscope. (B) Digested material yielded small spherical cells at day 5. (C) Adhered cells formed mesenchymal extensions at day 10. (D) After 19 days of culture, the primary WJMSCs reached 80–90% confluency.

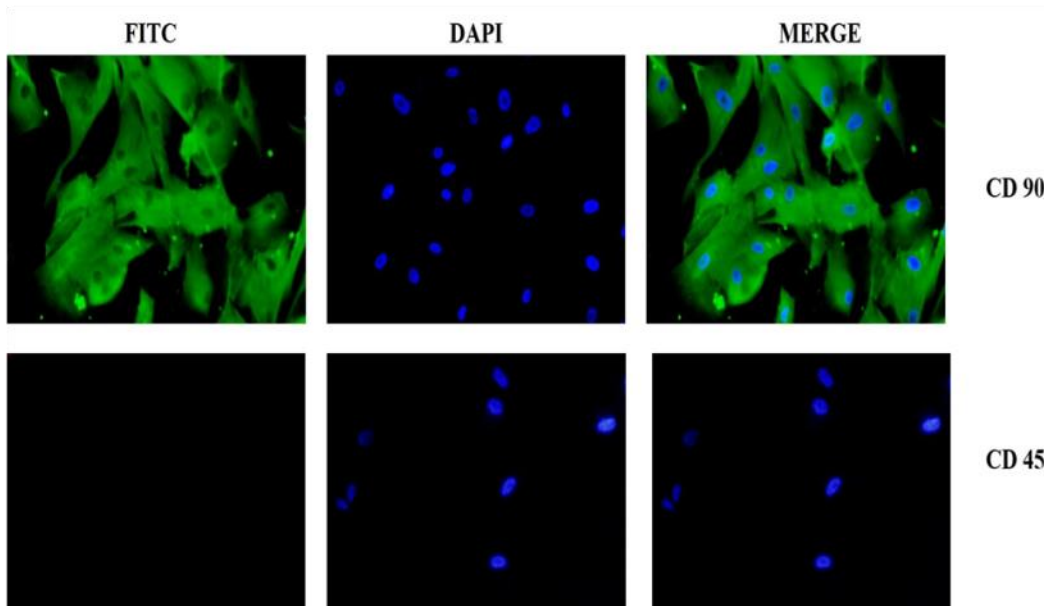

Fig. 6. Characterization of WJMSCS by Immunocytochemistry.

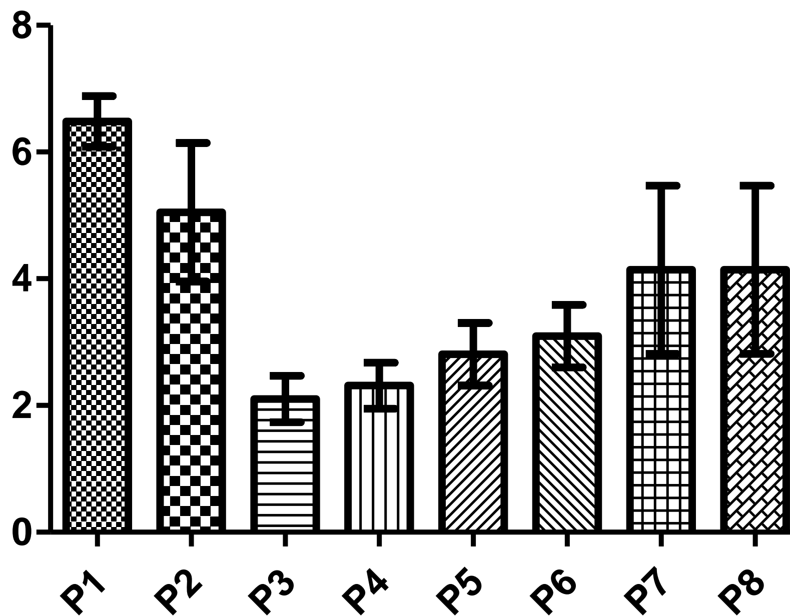

Fig. 7. Estimation of the population doubling level of WJMSCs. Population doublings shows an increase in growth rate of WJMSCs during 3-5 passages.

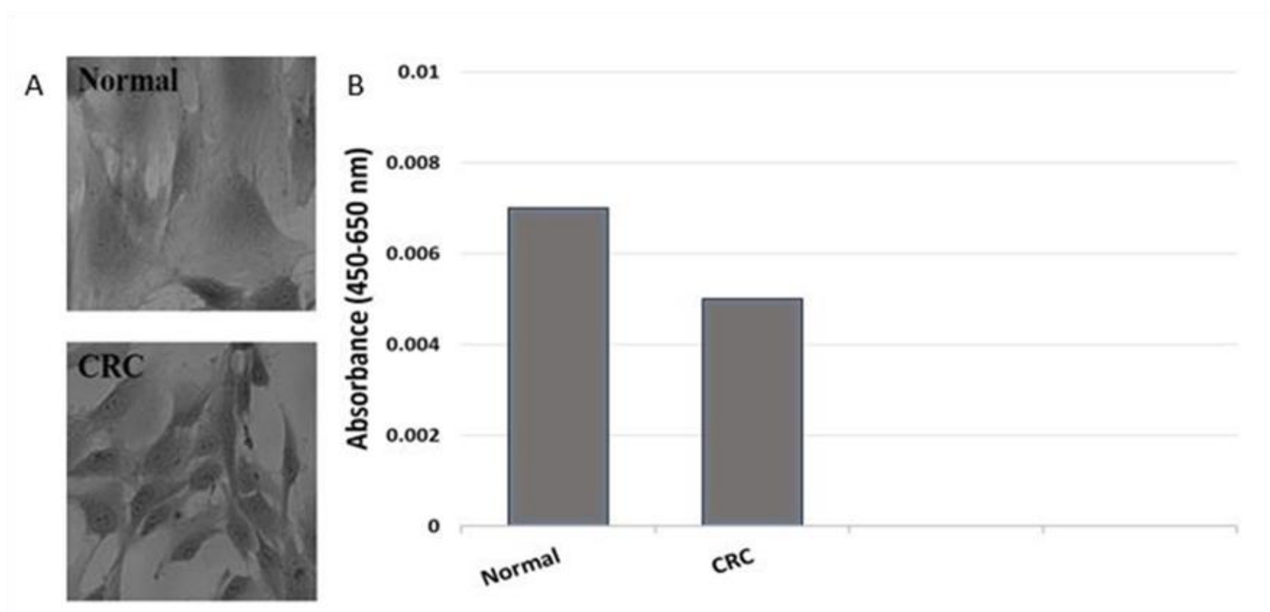

Fig. 8. WJMSCs treated with crystal violet to stain viable cells. (A) Comparison of cells viability on normal and cancer treated WJMSCs. (B) Graphical representation of cells viability of normal and cancer treated WJMSCs. The data is expressed as mean ± SD and P<0.05 was considered significant for all comparisons. *** denotes significant difference between normal and different cancer group.

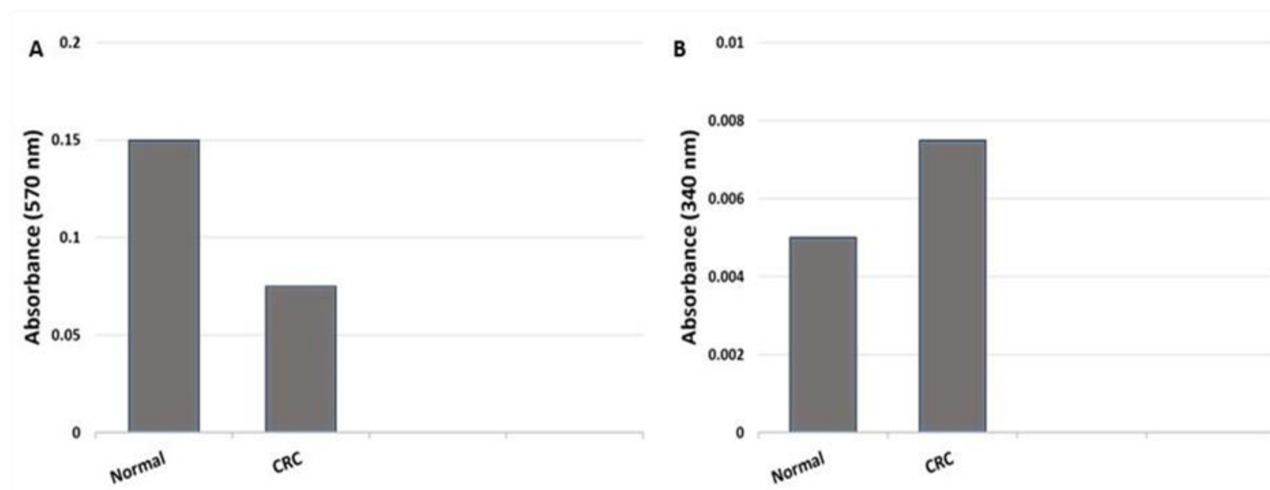

Fig. 9. A) Graph representing proliferative potential of WJMSCs treated with normal and cancer plasma. MTT cell proliferation assay revealed an increased rate of proliferation potential in normal cells as compared to cancer treated WJMSCs. B) Assessment of LDH assay on WJMSCs treated with normal and cancer plasma. The data is expressed as mean ± SD and P<0.05 was considered significant for comparison.

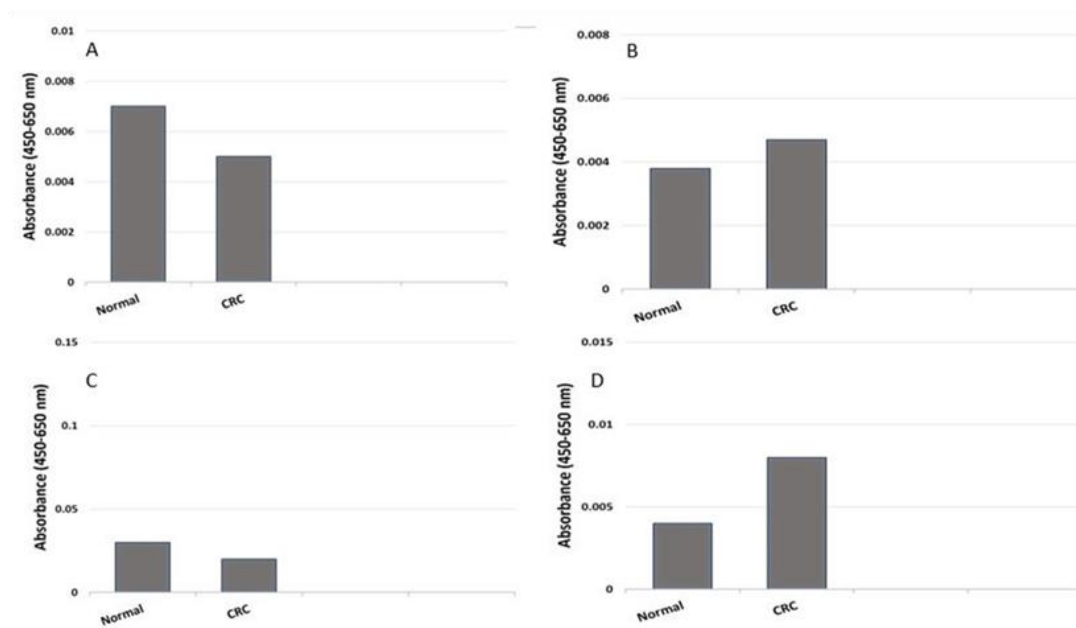

Fig. 10. Graph showing effect of BAX, BCL2, P38 and P53 on WJMSCs treated with normal and cancer plasma. A. Pro-apoptotic genes BAX expression was significantly decreased in WJMSCs treated with cancers plasma in comparison to WJMSCs treated with normal plasma. B. Increased gene expression of pro-survival markers BCL2 was observed in cancer group. C. p38 kinase expression was decreased in CRC. D. Significant increased gene expression in cancer group was observed in p53 tumor suppressor gene. The data is expressed as mean ± SD and $P<0.05$ was considered significant for all comparisons.

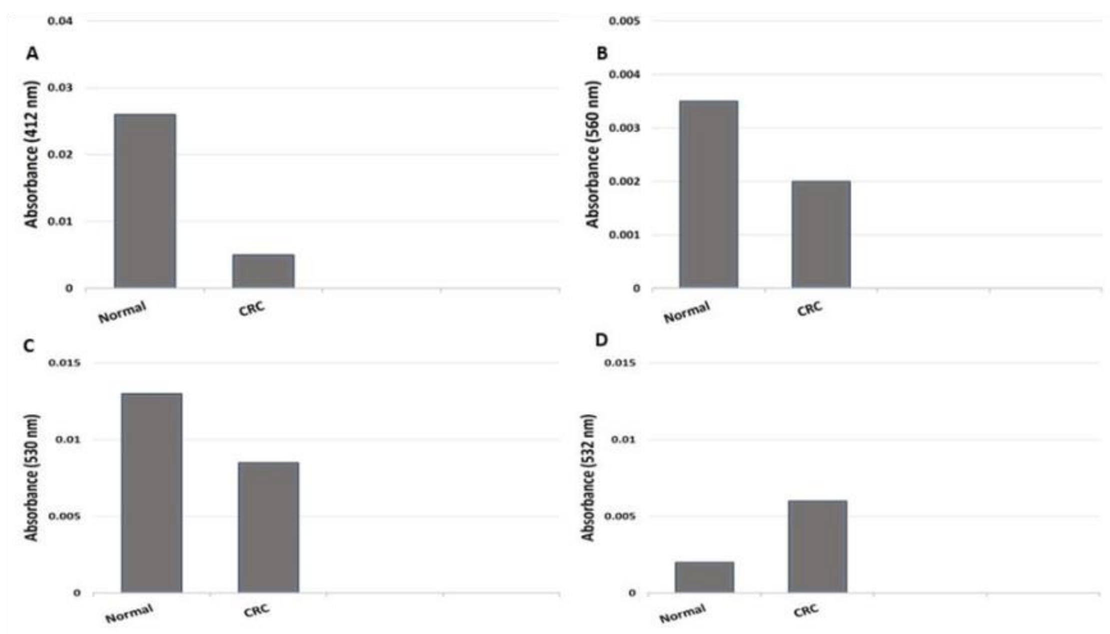

Fig. 11. Graph showing comparison of effect of WJMSCs treated with normal and cancer plasma's on A) Glutathione B) SOD C) Catalase D) MDA release. The data is expressed as mean ± SD and $P<0.05$ was considered significant for all comparisons. ** denotes significant difference between normal and cancer group

Discussion

The present study describes the potential behavior of WJMSCs in different cancers which was assessed by culturing these cells in plasma received from different cancer patients. One of the cancers was CRC. Before utilizing the plasma for the study, it was tested for 18 different hematology parameters, oxidative stress and paracrine factors like BAX, BCL2, p38, and p53. WJMSCs were effectively isolated from normal human umbilical cords and characterized for mesenchymal behavior preceding their culturing in plasma from cancer patients at passage 3. It was observed that cells cultured in plasma of cancer patients show decrease in cells' survival rate and proliferation while high LDH leakage indicated an increased cells death and a marked oxidative stress was also observed.Higher levels of LDH indicates acute or chronic cell damage in the blood. In present study, a significant increase in LDH release in CRC patient's plasma was observed. It has been documented that higher LDH levels was demonstrated colorectal cancer (Fig. 1) (22). Pro-apoptotic protein Bax regulate apoptosis (23).

Loss of function of Bax has been linked to tumorigenesis (24). Bcl2 and Bax are involved in regulation of apoptosis, in cancer cells (25). In present study a significantly underexpression of Pro -apoptotic Bax protein was observed in CRC plasma by ELISA (Fig. 2A).

Apoptosis is known to plays a key role in normal or cancerous cells. Bcl2 is the most main regulators of apoptosis (23). Bcl-2 is the major anti-apoptotic protein, but over expression of Bcl2 leads to cancer cells over proliferation (25). Increase in Bcl2 levels appear to be more important for cell survival than the down-regulation of Bax (26). In present study, a significantly increased gene expression of pro-survival markers bcl-2 was observed by ELISA in CRC group (Fig. 2B).

The p38 MAP kinases, has been involved in complex biologic process, including proliferation, migration and death (27). Increased levels of activated p38 indicate poor prognosis (28).

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In present study a significantly high expression of p38 protein was observed in CRC plasma by ELISA (Fig. 2C).

Mutations are commonly observed genetic abnormalities in the tumor suppressor gene p53 (29). Cancer patients have higher risk of being p53 antibody positive in comparison to healthy normal (30). In present study a significantly high increased gene expression in CRC was observed in p53 tumor suppressor gene (Fig. 2D).

Cancer cells generate Reactive oxygen species (ROS) (31). ROS promote damage to the DNA producing mutations and formation of tumors (32). In tumor cells, the expression of glutathione (GSH) in tumor cells has been assessed as a prognosticator for the prognosis. GSH, an antioxidant enzyme that prevents damage to important cellular components induced by ROS. It has been reported that (GSH) levels in comparison to normal had significantly reduced in CRC (33). In our study, it was observed that GSH release was raised in CRC (Fig. 3A).

Recent studies show that SOD levels was significantly decreased in CRC (34). However, in our study we didn't observe this decreasing (Fig. 3B).

Catalase is an enzyme which is common in living organisms. It is responsible in conversion of hydrogen peroxide to oxygen and water (35). It has been reported that the levels of catalase significantly reduced in colorectal cancer. In present study, catalase levels was significantly high in CRC (Fig.3C).

Malonedialdehyde (MDA) is a type of ROS produced as a result of lipid peroxidation. It has been reported in previous studies that MDA concentrations were higher in CRC which is according to our findings (Fig. 3D).

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Next step was to isolate, culture and characterization of the stem cells from Wharton's jelly of human umbilical cord (WJMSCs) leading to their culturing in plasma of colorectal cancer patients and other cancers.

Stem cells were successfully isolated from human umbilical cord tissue following explant method. Cells when freed from pieces of cord afterwards adhered to the plastic surface forming mesenchymal extensions (Fig. 4 & 5). After that the isolated cells were characterized for mesenchymal stem cells behavior. Cells were found to be positive for MSC marker CD90 and negative for hematopoietic marker CD45 (Fig. 6). These results were according to the previous studies(28, 36). Faster doubling time is a common feature for MSCs derived from fetal blood, cord blood, and Wharton's jelly (37). The isolated cells presented nearly constant rate of population doubling till passage eight and a regular increase in the population size (Fig. 7).

These cells at passage 3 were provided with plasma of colorectal cancer and healthy people plasma in same percentage i.e.10% for 3 days. Rate of cell survival, proliferation and cell death were estimated by crystal violet staining, MTT assay and LDH release respectively while different paracrine factors like BAX, BCL2, p38, and p53 were estimated by ELISA. Oxidative stress and its enzymes were also analyzed in the study.

Staining dye known as Crystal violet was used to assess cell viability. It was observed that cells cultured in normal plasma were more viable than those cultured in different cancer plasma (Fig. 8).

MTT cell proliferation assay revealed a significantly reduced cell proliferation rate in all cancer treated cells compared to healthy plasma treated cells (Fig. 9A).

LDH assay was performed on WJMSCs treated with normal and different cancer plasma. LDH release was significantly high in the medium of cells treated with cancer plasma (Fig. 9B).

ELISA was performed on WJMSCs treated with normal and different cancer plasma. A significant decreased expression of BAX was expressed in WJMSCS treated with cancer plasma (Fig. 10a). An increased expression of pro-survival markers BCL-2 was expressed cancer treated WJMSCs (Fig. 10b). Whereas, p38 kinase expression was significantly decreased in colorectal cancer (Fig. 10c). Significant increased gene expression in CRC was observed in p53 tumor suppressor gene (Fig.10d).

Oxidative stress was performed on WJMSCs treated with normal and different cancer plasma.

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It was observed that GSH release was significantly lower in medium of cells cultured with plasma from cancer patients (Fig. 11A). Superoxide dismutase (SOD) release was significantly lower by the cells cultured in plasma of cancer patients as compared to untreated cells (Fig. 11B). Catalase release by cells of cancer plasma treated group was significantly higher as compared to normal untreated group as shown by less absorbance (Fig. 11C). MDA release by cells of cancer plasma treated group was significantly higher as compared to normal untreated group (Fig. 11D).

Conclusion

The present study demonstrates that WJMSCs can be isolated and cultured effectively in a large number. When cultured in plasma from colorectal cancer patients these cells show reduced viability, proliferation and increased apoptosis. For the future, certain measures should be taken to improve viability of WJMSCs in cancer conditions.