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Clinical Use of Amniotic Fluid in Osteoarthritis: A Source of Cell Therapy

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38.1 Introduction

Nature is the finest physician: it takes care of everyone because its concerns are universal and genuine; however, it always follows certain guidelines and principles and scientists can learn from these. Giving birth is an act of nature through which the realm of creation is opened to the mother. Nature comes from the Latin word *Natus*, "to be born." During birth, if nothing is present, what will prevent infection from affecting the newly born baby? Nature's concern can be seen when the vagina is washed, prior to birth, by a fine baby-friendly liquid containing cell suspension and antibacterial elements, which gives it a disinfectant property; it also possesses lubricant and cell therapy properties that are crucial for the mother and the child at the critical time of birth. This fluid is known as the amniotic fluid. So far, no one appears to have used this fluid for any therapeutic purpose for more than 70 years in the practice of modern medicine. However, there has been some recent awareness about amniotic fluid as a source of mesenchymal stem cells, which can be converted into any cell type given the niche or the environment for its transdifferentiation property, the implication being that it can help in regeneration in a degenerating system.

Knee-joint problem is one of the commonest geriatric problems that makes a person aware that he is aging. Although the exact causes for painful knee joints may be difficult to ascertain in many cases and sometimes remain unknown, it is understood that degenerative damage, especially cartilage damage, plays a central role in the pathogenic mechanism leading to this disorder. Current treatment modalities include pharmacological support, physiotherapy, etc., to palliate the condition. There is growing interest in the development of novel technologies to repair or regenerate the degenerated knee joint.

In 1927, Dr Johnson, a famous American surgeon and investigator first reported on the use of human and bovine amniotic fluid as an agent that stimulates the defense mechanism if injected in a host at the site of the problem or injury. Initially, amniotic fluid collected from mothers undergoing cesarean section was used and later substituted with a bovine amniotic fluid concentrate.[1] Contemporary evidence of other workers suggested that the use of amniotic fluid after abdominal surgery prevents or at least minimizes postoperative adhesions[2-4]. Taking a hint from the available knowledge in the field,[5] Dr Mandell Shimberg, a noted orthopedic surgeon from Kansas, USA, used amniotic fluid in various pathological conditions affecting different joints in the body. He also used the amniotic fluid in a closed reduction attempt in difficult fractures involving or proximal to a joint. Dr Shimberg used the amniotic fluid in an intraarticular route in 46 patients with knee-joint affectations, namely, sympathetic joint effusion, subacute joint infection, atrophic arthritis, gonorrheal joint effusion, and also idiopathic joint effusion cases only to name a few, without facing a single mortality and a very low morbidity.[6]

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People at that time did not know that there is a group of cells known as stem cells with high telomere content, which possess a unique property of transdifferentiation, depending on the niche provided to them and can migrate to the site of injury, and thus can actively participate in tissue repair and regeneration.

Recent clinical use of stem cell biology includes autologous mesenchymal stem cells application in animal models, which can arrest intervertebral disc degeneration or even partially regenerate it and the effect is suggested to be dependent on the severity of degeneration.[7] It has become abundantly clear to scientific workers in recent times that a stem cell can renew itself through cell division and can be induced to develop into many different specialized cell types. Moreover, stem cells have shown the ability to migrate and engraft within various tissues, as well as exert stimulatory effects on other cell types through various mechanisms (e.g., paracrine effects, cell–cell interactions).[8]

Important investigators in the field, Kaviani and coworkers, have reported that "just 2 milliliters of amniotic fluid" can provide up to 20,000 cells, 80% of which are viable.[9] Actual amniotic fluid stem cells originate in the developing fetus. The cells are thought to be sloughed from the fetal amnion and skin, as well as the alimentary, respiratory, and urogenital tracts. Amniotic fluid also contains a mixture of different cell types and has the potential to differentiate into various cell types.[10] A number of different origins have been suggested for these cells.[11] Cells of both embryonic and fetal origins and cells from all three germ layers have been reported to exist in amniotic fluid.[12, 13]

The focus of the present chapter is on the use of amniotic fluid in osteoarthritis (OA). Osteoarthritis-related knee problem is the commonest cause of disability at older ages.[14] This chapter reports on a study undertaken over 7 years (1999–2006) and its follow-up, which was undertaken to examine the clinical efficacy of freshly collected amniotic fluid from consenting mothers undergoing hysterotomy and ligation, as an effective progenitor or stem cell source for cell therapy procedure to combat the varying stages and grades of degenerative osteoarthritis affectations of the knee. Further, a simple comparison was made with the time-tested palliative procedure of intraarticular, long-acting corticosteroid application aseptically in the O.T. The study got the necessary clearance of the institutional ethical committee.

38.2 Materials and Method

Fresh amniotic fluid was collected from women admitted for hysterotomy and ligation at Bijoygarh State Hospital (1999–2006) and was used for the present study for the treatment of patients with osteoarthritis of the knee joint.

As per the standing direction of the State Family Planning Department, hysterotomy and ligation may be allowed up to 20 weeks of pregnancy, provided the mother has two or more healthy children. For the present study, 10 cm^3 amniotic fluid was collected aseptically in the O.T. from each mother undergoing hysterotomy and ligation, from an intact sac after opening the uterus, when the amniotic membrane containing the amniotic fluid generally herniates outside the uterus. The sac was gently punctured and the amniotic fluid was sucked out aseptically with a wide-bore size 16 needle and syringe. The collection protocol started, after getting the donor's consent and the recipient's informed consent.

Initially, 62 patients volunteered for this project of amniotic fluid cell therapy on degenerative osteoarthritis of the knee joint. Ten cases were discarded due to the association of neurodegenerative diseases such as Parkinsonism, malignancy, dementia of varying etiology and other chronic disease burdens.

The 52 cases that were ultimately enrolled for this trial had earlier not responded to conventional pharmacological or nonpharmacological treatment. The pharmacological treatment had included use of NSAIDs, i.e., naproxen, ibuprofen, etc., as well as the cyclooxygenase-2 inhibitor group of drugs like celecoxib with supporting drugs such as glucosamine, chondroitin, and opiates, only to name a few. The nonpharmacological treatment had included anaerobic exercises, i.e., resistance training, suggestion of weight loss or use of crutch, use of brace for the patella, and correction of tilting or misalignment. Acupuncture for some temporary relief had also been suggested to them but there was either no response or noncompliance.

The patients suffering from osteoarthritis of the knee not responding to oral medication and physiotherapy were given the option of free cell therapy from freshly collected amniotic fluid source, or intraarticular instillation of long-acting steroid. These patients were randomized for age and sex, and eventually divided in two equal groups: Group A (26 patients; 14 male and 12 female, age varying from 39 to 78 years, mean 51.4±4.6 years

SD) and Group B (also contained 26 patients, female 14 and male 12, age varying from 41 to 82 years, mean 49 ± 6.4 years SD).

The patients were asked to mark presence or absence or overall impression of improvement or deterioration with treatment, of some simple clinical parameters like (1) knee pain at rest, (2) little walking is painful, (3) definite increase in walking distance, (4) decrease in flexibility of the joint, (5) swelling of the joint, (6) little power of the joint to move against gradual increasing resistance, (7) difficulty in the initiation of the movement, (8) stiffness of the joint and movement, (9) range of movement is severely restricted. If seven of the nine clinical functional parameters were positive and there was X-ray evidence confirming the osteoarthritis status (standard weight-bearing anteroposterior and lateral knee radiographs) osteoarthritis was confirmed. Each compartment (medial tibiofemoral, lateral tibiofemoral, patellofemoral) was graded 0–3 for overall severity of OA. Clinical assessment of joint effusion (positive bulge sign and patellar tap: present/absent) was documented by a specialist, and knee aspiration was performed via the medial approach, and the volume of aspirated synovial fluid (SF) recorded. Total and differential leukocyte counts were estimated in all SF samples, which were also examined for the presence of calcium pyrophosphate crystals by microscopy.

Following aspiration from the knee, if there was effusion or dryness, each knee would be randomly injected with triamcinolone acetonide (40 mg in 1 + 9 mL normal saline) for Group A or alternatively with only amniotic fluid 10 ml, as a source of cell therapy for Group B. The amniotic fluid was taken from consenting mothers carrying pregnancy (14 weeks to 20 weeks gestation, calculated from the first day of the last menstrual period [LMP]; mean gestation was 17.6 ± 2.1 weeks in the present study), who were undergoing hysterotomy and ligation as a family planning measure as already mentioned. If both the knees of the patient were affected, they were treated with identical dosages in each knee.

A thorough history of all the patients was taken, i.e., age, sex, height, weight, menstrual history, history of chronic disease like tuberculosis, hypothyroid, frank diabetes, or even altered glucose tolerance, history of diabetes in the family, lipid profile including uric acid level, apart from a history of specific involvement of cancer, systemic lupus erythematosus, ankylosing spondylitis, etc.

Specific rheumatological history with history of oral or intraarticular steroid intake, degree, and pattern of joint involvement with the duration of knee affec- tion were noted. The knee pain was noted on a 100 mm horizontal visual analogue pain scale (VAS). The other parameters that were assessed included the distance walked in 1 min (WD) and also a locally modified and local (Bengali) language-translated Modified Health Assessment Questionnaire[15] was filled up.

Individual features in each compartment (narrowing, sclerosis, osteophytes, cysts, and attrition) were graded 0–3 and presence/absence of chondrocalcinosis was also noted. At follow up visits (1st–6th, 9th, 12th, 18th, and 24th month), a specialist doctor made a subjective assessment of the clinical condition with objective correlation, as much as it was practicable, for all the patients blinded for the type of treatment offered to the patient, to clinically assess the overall status of the treated knee joint (worse, no change, improved). Pain score (VAS), WD, and HAQ were recorded.

Student's paired test (p value) was also conducted. Analysis of variance for repeated measures was used to compare differences that were assessed by simple regression analysis. The differences in patient opinion of overall change, and relationship between clinical evidences were calculated by contingency table analysis incorporating mean with standard deviation (SD). Differences that were significant at the 5% confidence interval are quoted in the follow-up chart record.

At the completion of the study, patients who received cell therapy were offered steroid therapy if they voluntarily requested for that procedure, and *vice versa*.

38.3 Result and Analysis

Patient demographic data were more or less similar in both patient groups A and B as noted in Table 38.1. Age varied from 39 to 82 years, and the study included 50% male and 50% female patients; weight varied from 49.8 to 112.6 kg, height varied from 4 ft 11 in. to 6 ft 1 in.; the period of illness varied from 3 to 14 years, with the majority showing involve- ment of both knees.

As mentioned earlier, out of the 52 patients, we randomize the overall results of the effusion group

Table 38.1 Showing the patients selected for this study (epidemiological profile)

No of patients enrolled for this study: $N = 52$
Age of group: 39–82 years
Sex: males 26 and females 26
Weight: 49.8–112.6 kg
Height: 4 ft 11 inches to 6 feet 1 inch
Duration: 3–14 years
Single knee effusion: 16
Both knee affection/effusion: 36
Treatment with analgesic including NSAID and Physiotherapy etc.: All of them

(32 cases) and the noneffusion group (20 cases). The clinical assessment is based arbitrarily on certain easy clinical parameters that the patient could understand irrespective of intelligence or education status.

These parameters are nine in number, namely, subjective appreciable decrease in knee pain at rest, walking without pain for some time, definite increase in walking distance before pain reappeared, etc. If seven of the nine parameters were positive with objective verification, the result was termed as adequate clinical improvement with the therapy in either A or B schedule, whichever was followed by the individual patient. If the result satisfied less than seven clinical parameters out of nine, the result was considered as inadequate clinical improvement.

If the overall impact of treatment in Group A is assessed and compared with the results of Group B as noted in the Table 38.2 and Fig. 38.1, it can be seen that a mean 92.3% patients showed improvement in the steroid-treated group (A) compared to a mean 88.46% of the patients in the amniotic fluid group (B) at the completion of the first month from the procedure ($p < .01$).

At the completion of the second month from the initiation of treatment, mean improvement was reported in 57.69% in the steroid-treated Group A and in 84.61% of the amniotic fluid-treated Group B ($p < .01$). The benefit of treatment was sustained at the end of the third month in Group B particularly, with mean 80.76% in the amniotic fluid-treated Group B and 46.15% in the steroid-treated Group A ($p < .01$) showing continued improvement. Evaluation after completion of the fourth month of treatment showed that a mean 30.76% of Group A and 73.07% of Group B maintained the benefit of the treatment ($p < .02$). The value for the 5th, 6th, 9th, 12th, and 24th months for Group A were noted as decreasing uniformly: mean 26.92%, 23.07%, 19.23%, 15.38%, and 15.38%, respectively. The identical value for the 5th, 6th, 9th, 12th, and 24th months for Group B were mean 65.38%, 57.69%, 53.84%, 50%, and 46.15%, respectively.

Out of 32 patients (61.53% of the patients) who had clinical evidence of joint effusion, which was aspirated before instillation of steroid or the amniotic fluid in the joint space with adequate antiseptic and aseptic precautions, 21(40.38%) patients were treated with amniotic fluid after aspiration of the joint space; the rest, that is, 11 (21.15%) patients were treated identically with intraarticular steroid. The results are worth noting:

(a) In this study, 18 out of the 21 patients (85.71%) with clinical evidence of joint effusion showed benefit with amniotic fluid cell therapy (Group B) as seen after 1 month.
(b) This can be compared to Group A, where seven patients (63.63%) out of the 11 with effusion, treated with intraarticular steroid, showed improvement.
(c) Among patients in the noneffusion group, five patients (B) were treated with amniotic fluid, and four (80%) of them were satisfied with the outcome after completion of 1 month.
(d) Similarly, of the 15 patients of the noneffusion group who were treated with intraarticular steroid (A), eight cases were enjoying the benefits of therapy (53.33%) at the end of the first month period.

This variation of the results in the effusion group and the noneffusion group could be due to the state of the disease. In early stages of osteoarthritis there is irritation of the joint space with the erosion of the joint cartilage. This irritation of the synovial membrane that is responsible for the effusion eventually dries up with the progression of the disease and an initiation of fibrous ankylosis, or in a late stage, bony ankylosis, sets in.

The overall findings of treatment in the effusion and noneffusion group were further supported by the results of VAS, WD, and HAQ study as reported in Table 38.3.

The results are supported by analysis of the VAS (Visual Analogue Pain Scale), WD (walking distance in

Table 38.2 Showing the clinical results of treatment in Groups A and B (subjective and objective improvement of at least seven out of nine clinical parameters)

Group A treated with intra-articular steroid	Group B: treated with cell therapy	Satisfaction after	Special comment
N = 26 Assessment after 1 month showed improvement, i.e., mean subjective and objective assessment of definite relief in 92.3% ± 3.6% S.D. Lost follow-up (LFU) = Nil	*N* = 26 Assessment after 1 month showed improvement, i.e., mean subjective and objective assessment of definite relief in 88.46% ± 2.8% Lost-follow up (LFU) = Nil	1 month Group A = 24 Group B = 23	(p <.01).
Assessment after 2 months showed improvement, i.e., subjective and objective assessment of definite relief in mean 57.69% ± 4.8% (LFU) = Nil	Assessment after 2 months showed improvement, i.e., mean subjective and objective assessment of definite relief mean 84.61% ± 7.3% (LFU) = Nil	Satisfaction after 2 months Group A = 15 Group B = 22	(p <.01).
Assessment after 3rd month showed improvement, i.e., subjective and objective assessment of definite relief in Mean 46.15% ± 3.4% (LFU) = Nil	Assessment after 3rd month showed improvement, i.e., subjective and objective assessment of definite relief in Mean 80.76% ± 7.4% (LFU) = Nil	Satisfaction after 3 months Group A = 12 Group B = 21	(p <.01).
Assessment after 4th month showed improvement, i.e., subjective and objective assessment of definite relief in Mean 30.76% ± 2.9% (LFU) = Nil	Assessment after 4th month showed improvement, i.e., subjective and objective assessment of definite relief in Mean 73.07% ± 6.8% (LFU) = Nil	Satisfaction after 4 months Group A = 8 Group B = 19	(p <.02).
Assessment after 5th month showed improvement, i.e., subjective and objective assessment of definite relief in Mean26.92% ± 2.9% SD (LFU) = Nil	Assessment after 5th month showed improvement, i.e., subjective and objective assessment of definite relief in 65.38% ± 4.9% SD (LFU) = Nil	Satisfaction after 5 months Group A = 7 Group B = 17	(p <.01).
Assessment after 6th month showed improvement, i.e., subjective and objective assessment of definite relief in (Mean) 23.07% ± 2.2% SD (LFU) = Nil	Assessment after 6th month showed improvement, i.e., subjective and objective assessment of definite relief in 57.69% ± 4.9% SD (LFU) = Nil	Satisfaction after 6 months Group A = 6 Group B = 15	(p <.01).
Assessment after 9th month showed improvement, i.e., subjective and objective assessment of definite relief in 19.23% (Mean) ± 2.1% (LFU) = Nil	Assessment after 9th month showed improvement, i.e., subjective and objective assessment of definite relief in Mean 53.84% ± 4.4% percent (LFU) = Nil	Satisfaction after 9 months Group A = 5 Group B = 14	(p <.01).
Assessment after 12th month showed improvement, i.e., subjective and objective assessment of definite relief in 15.38% ± 2.2% (LFU) = Nil	Assessment after 12th month showed improvement, i.e., subjective and objective assessment of definite relief in 50% ± 4.3% (LFU) = Nil	Satisfaction after 1 year Group A = 4 Group B = 13	(p <.01).
Assessment after 24th month showed improvement, i.e., subjective and objective assessment of definite relief in 15.38% ± 2.2% (LFU) = Nil	Assessment after 24th month showed improvement, i.e., subjective and objective assessment of definite relief in 46.15% ± 5.4% (LFU) = Nil	Satisfaction after 2 years Group A = 4 Group B = 12	(p <.01).

meters), and HAQ (Health Assessment Questionnaire) assessments. Table 38.3 outlines changes in VAS, WD, and HAQ in steroid (Group A) and cell therapy (Group B) patients at the third- and sixth-month follow-up. The results demonstrated a significant improvement in VAS at third month, which was sustained at the sixth-month interval assessment in both groups, but more so in the cell therapy Group B (p <.001). Again, a better and more positive improvement trend was noted at the third- and sixth-month assessments in WD (walking distance in meters) in case of Group B (cell therapy group with amniotic fluid), when compared to the steroid-treated Group A. The health analysis questionnaire results also supported the VAS and WD results of Group A and B justifying the validity and superiority of cell therapy from steroid therapy in this preliminary report (p<.01).

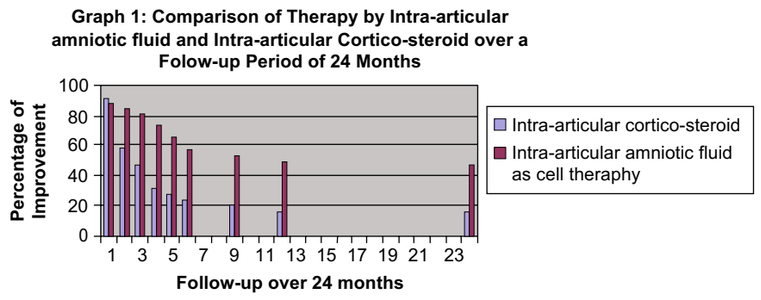

Fig. 38.1 Showing the overall 24-month follow-up of treatment of Group A (steroid-treated) and Group B (amniotic fluid cell therapy group)

Table 38.3 Shows the value of the VAS, WD, and HAQ in steroid (Group A) and cell therapy (Group B)

(Pretreatment mean ± SD) VAS (mm)	(3rd month mean ± SD) VAS (mm)	(6th month mean ± SD) VAS (mm)	*p* value
Mean Group A values with SD: 56 ± 11.30	21 ± 6.47	32 ± 4.8	(p <.02).
Mean Group B values with SD: 57 ± 10.2	17 ± 3.4	12 ± 4.8	(p <.002).
Walking distance in meters (WD)			
Mean Group A values with SD 38.6 ± 4.8 m	51 ± 4.8 m	42.2 ± 4.8 m	(p <.01)
Mean Group B values with SD 39.8 ± 3.8 m	58.6 ± 6.9 m	61.4 ± 7.2 m	(p <.01)
Local language Modified Health Analysis Questionnaire (1–11)			
Mean Group A values with SD 2.2 ±.2	2.3 ± 0.2	2.2 ± 0.4	(p <. 002)
Mean Group B values with SD 2.4 ± 0.3	2.1 ± 0.12	1.8 ± 0.31	(p <.01)

The *t*-test, one-way analysis of variance (ANOVA) and a form of regression analysis

38.4 Discussion

Randomized clinical trials (RCTs) are regarded as the most reliable method of evaluating the effects ofinterventions in health care. RCTs are also considered the "golden standard" for providing research evidence for interventions in evidence-based health care.[16]

The validity and reliability of trial results are, however, largely dependent on the study design and the methodology in its conduct. Jadad A.R.[17] has defined the quality of a trial, with emphasis on the methodological quality, as "the confidence that the trial design, conduct, and analysis have minimized or avoided biases in its treatment comparisons." In this paper, the attempt was to follow the basic guideline to minimize investigator or other biases as far as practicable. Our subjective assessment of that scoring in this study is possibly 3 on the Jadad scale.

The present study is the first global report on a clinical comparison of the effect of amniotic fluid cell therapy and the impact of standard intraarticular palliative treatment in case of varying degrees of osteoarthritis-induced degenerated knee joints.

Cell therapy describes the process of introducing new cells into a tissue in order to treat a disease. The material used for cell therapy in this study is freshly collected amniotic fluid from women admitted by the family planning department in a government hospital for hysterotomy and ligation. Under normal circumstances, the fetus and the amniotic fluid contained sac are immediately disposed for eventual clearance through the incinerator of the hospital.

To recapitulate, amniotic fluid is to be found in the amniotic cavity that protects the fetus as a buffer and also helps growth and movement, and prevents adherence to the placenta or the surrounding structures. This clear watery fluid is contributed principally from the maternal blood via the amniotic fluid epithelium but freely intermixes with secretions from the fetal lung, kidney, gastrointestinal tract, and the skin; hence, the properties of this specialized fluid compartment is quite complex with contributions from both the maternal and

the fetal side. Toward the outside, the amniotic cavity is delimited by the amniotic epithelium, the chorion laeve, and the decidua capsularis. The main constituents are water and electrolytes (99%) together with glucose, lipids from the fetal lungs, proteins with bactericide properties, and fetal epithelium cells.

As mentioned earlier, pleuripotent progenitor cells isolated from the amniotic fluid and the placenta possibly present an exciting contribution to the field of stem cell biology and regenerative medicine.

Compared with embryonic stem cells, progenitor cells isolated from the amniotic fluid have many similarities: they can differentiate into all three germ layers, they express common markers, and they preserve their telomere length. However, progenitor cells isolated from the amniotic fluid and placenta have considerable advantages. They easily differentiate into specific cell lineages and further, they avoid the current controversies associated with the use of human embryonic stem cells.

Pregnancy results in the acquisition of specialized and unique cells that may have clinical applications and therapeutic potential. Whether the pregnancy-associated progenitor cells (PAPCs) are hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), or are a new population of stem cells is an unresolved issue. It is also unknown whether PAPCs respond to all types of maternal injury or only those injuries that recruit stem cells.

It is possible that these cells, since they are fetal in origin, have a higher proliferative capacity or more plasticity than their equivalent adult (maternal) cells. In the current debate over the use of embryonic stem cells for treatment of disease, the discovery of a population of fetal stem cells that apparently differentiate from the ones in adult women, and can be acquired without harming the fetus, may be significant.[18, 19]

The growing fetus in the womb is an eternal source of stem cells. The initial interest in the field started with the use of placental blood-derived hematopoietic stem cells in Fanconi's anemia in 1988 by the legend- ary Prof Elaine Gluckman. Meanwhile, scientists have been able to isolate and differentiate only 30% of mesenchymal stem cells (MSCs) on an average, extracted from a newborn's umbilical cord jelly-like material, shortly after birth. The success rate for amniotic fluid-derived stem cells, on the other hand, is close to 100%. Analysis of surface markers shows that progenitor cells from amniotic fluid express human embryonic stage-specific marker SSEA4, and the stem cell marker Oct4, and do not express SSEA1, SSEA3, CD4, CD8, CD34, CD133, C-MET, ABCG2, NCAM, BMP4, TRA1-60, and TRA1-81 [51, 52].

38.5 Differentiation of Amniotic Fluid- and Placenta-Derived Progenitor Cells

The progenitor cells derived from amniotic fluid and the placenta are pleuripotent and have been shown to differentiate into osteogenic, adipogenic, myogenic, neurogenic, endothelial, hepatic, and renal phenotypes in vitro. Each differentiation has been performed through proof of phenotypic and biochemical changes consistent with the differentiated tissue type of interest. In 2007, Perin et al. showed that AFSC (amniotic fluid stem cells) could be induced to differentiate into renal cells when placed into an in vitro embryonic kidney environment.[20]

In this preliminary clinical study, freshly collected amniotic fluid has been utilized as a source of cell therapy with the hypothetical assumptions that the mesenchymal cells of the AF (amniotic fluid) will participate in the knee joint repair process, the viscosity of the amniotic fluid will assist lubrication, and the bactericidal property of the amniotic fluid will guard against inadvertent infection. The idea was to match/compare this new therapeutic protocol (cell therapy for regeneration) with the globally accepted standard protocol of intraarticular injection of long-acting steroid triamcinolone.

The problem of knee pain is very common after the age of 50 years. Varying stages and grades of osteoarthritis due to degeneration of the knee joint plays the most important role behind such painful knee problems. The main pharmacological treatments remain analgesics and nonsteroidal antiinflammatory drugs (NSAIDs) although the role of these two treatments in the management of OA has been questioned.[21–24] So far, the most important antiinflammatory drug available in rheumatology, which can give some real relief in osteoarthritis is corticosteroid. In this context, it is first necessary to explain the treatment with corticosteroids to understand the implications of the present study, since intraarticular injection of steroid is a common treatment for osteoarthritis of the knee practiced globally by rheumatologists.

The aim of treatment in patients with osteoarthri- tis (OA) is to reduce symptoms, minimize disability,

and limit the structural changes in the osteoarthritis-affected joint. But it has been observed that in OA a combination of factors, both mechanical and biochemical as well as immunological (and its effects) or cytokine effects, not only cause hyaline cartilage damage with time but also affects the synovial membrane. The subchondral bone, ligaments, and periarticular muscles also show varying degree of involvement and derangement. Synovial membrane inflammation in OA patients is probably related to the destruction of hyaline cartilage and the subse- quent release of cartilage breakdown products into the synovial fluid. Clinical evidence suggests that the benefits of even a strong antiinflammatory drug like steroid is short-lived, lasting for usually 1–4 weeks[25] In the present study we have followed the guideline of the use of 40 mg triamcinolone as recommended by the American College of Rheumatologists.[26]

Whether steroid injection flares the pain and deteriorates the joint is a valid question as has been isolated in two cases in this study that were later proved to have tubercular infection. But excepting in cases of undiagnosed tuberculosis of the knee, steroid injection does not appear to have any important adverse effect on the whole. Studies of cartilage damage, however, tend to suggest that changes are more likely due to the underlying disease than the steroid injection.[27,28,29]

38.6 New Horizon for Offering a Cure (Repair) for Osteoarthritis with Simple Cell Therapy

In the developing world, surgical abortion as a method of family planning is practiced widely. Hysterotomy and ligation is a standard surgical method of termination in government hospitals in India. Aseptic collection of the amniotic fluid is not a difficult job for experienced gynecologists and obstetricians who perform this simple surgery with skill and dedication. The aseptically collected amniotic fluid can be easily preserved in special containers in the vapor phase of liquid nitrogen chambers or jars.

This may work as an amniotic fluid bank that can supply amniotic fluid on demand. Amniotic fluid is a unique fluid made by nature; it is a cocktail of mesenchymal stem cells with antibacterial property, which is used in the present study as the cell therapy source for the repair of damaged cartilage, synovial membrane, supporting muscles, and supporting ligaments, as per the niche provided to these specialized stem cells for regeneration purposes, in advanced and degenerative osteoarthritis with satisfying results.

The amniotic fluid, because of its increased viscosity due to protein and other cellular suspension, differs from the steroid-treated fluid (normal saline), and may act as a lubricant that diminishes the irritation at the initial phase; and the mesenchymal cells, which do not express HLA antigens, may possibly help in the repair process of the adjacent structures in the joint space as a whole. Though the epidemiological background (Table 38.1) of Groups A and B are grossly randomized, the result of the therapy (shown in Fig. 38.1 and Table 38.2), strongly supports the potential of this new form of cell therapy in case of advanced osteoarthritis. The present treatment proved to be much superior to, and lasted longer than, the conventional widely practiced therapy with corticosteroid instillation at the joint.

Lastly, it may be noted with interest that in this simple method of cell therapy, Group B maintained superior patient's satisfaction in 12 cases only out of 26 enrolled patients, after completion of the 24-month follow-up period. The corresponding number for the standardized universally practiced protocol of intraarticular long-acting steroid (Group A) therapy for advanced osteoarthritis is a pathetic figure of four cases only (Fig. 38.1). The results are further supported by the VAS, WD, and HAQ assessments as mentioned in Table 38.3, which reiterated a significant improvement in VAS at third month and was sustained at the sixth-month interval assessment in both groups, but more so in the cell therapy Group B ($p < .001$).

38.7 Conclusion

Intraarticular amniotic fluid instillation is a new method of treatment in advanced osteoarthritis when the patient is not getting any relief with conventional analgesic and physiotherapeutic support.

The long-term follow-up result of this type of cell therapy justifies its procedural superiority over conventionally and universally practiced intraarticular long-acting corticosteroid triamcinolone (($p < .001$).

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