Can human fetal cortical brain tissue transplant (up to 20 weeks) sustain its metabolic and oxygen requirements in a heterotopic site outside the brain? A study of 12 volunteers with Parkinson's disease

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Summary

Background: Neural and stem cell transplantation is emerging as a potential treatment for neurodegenerative diseases from Parkinson's to Huntington's disease. Stereotactic placement of dopaminergic neurons in the caudate-putamen (striatum), is being attempted in centers of excellence and has proved to be beneficial. Basic research using cell transplantation indicates that structural development mechanisms seen in immature brains, i.e., fetal brains, can also function in the adult brain. The adult brain consumes 15% of the resting cardiac output for its metabolic needs. While most human tissues can sustain an anaerobic assault for a few minutes up to 30 minutes, a sudden total lack of oxygen supply to the brain cells in an adult will render the person unconscious within five to ten seconds.

Our team has been working on the problem of human fetal tissue response to antigenic assault for the last two decades. In the present series, 12 patients with prolonged histories of Parkinsonism, who were not responding to anti-Parkinsonian drugs, and could not afford costly stereotactic surgery or deep brain stimulation and other modalities of recent Parkinson's disease treatment, were enrolled in the study.

Materials and Method: After obtaining proper informed consents from the patients or their guardians and from the multidisciplinary ethical committee, the patients, varying in age from 45 to 75 years and suffering for many years with Parkinsonism, were enrolled in the heterotopic brain tissue transplant programme. We followed standard antiseptic, aseptic and premedication protocols, after selecting a proposed site of transplantation of the brain in the axillary fold of the skin, under local infiltration anaesthesia. In an adjacent OR, a fetus was collected from a consenting patient undergoing hysterotomy and ligation (before 20 weeks), under general anaesthesia. Within a minute of hysterotomy, the fetal brain tissue was dissected, and under the guidance of the operative microscope, 1 g of fetal cortical brain tissue was dissected and weighed in an electronic machine. The tissue was collected from around 1 cm of the frontal opercula of the developing human fetal brain and grafted in the already dissected and prepared subcutaneous site in the axilla and the skin was closed. Hematological parameters (Hgb; total count, Tc; differential count, Dc; erythocyte sedimentation rate, ESR) were estimated sequentially up to one month. A small portion of the transplanted tissue was retrieved after one to two months, and a serial histological study was done along with a clinical assessment of the disease condition as per the specifications of the Unified Parkinson's Disease Rating Scale. The results were matched with the pre-transplant ratings of the individual cases. Presenting dyskinesia was also rated (0-4), on the basis of objective criteria assessment like walking, putting on a coat, lifting a cup to drink, etc.

Results and analysis: Initially 30 patients suffering from advanced Parkinson's disease (PD) were approached after getting the necessary clearance from the institutional multidisciplinary ethical committee; however, we have been able to arrange transplantation in only 12 cases so far. These patients were evaluated at the pre- and one month post-transplant period by the Unified Parkinson's Disease Rating Scale (0-108) and the minimum score was 40 in the motor portion of the unified scale at the pre-transplant state. Evaluation of the patients after one month revealed mild improvement of the pre-transplant scoring (up to 33.3%) in 41.6% of the cases, and moderate improvement (up to 66.6%) in another 41.6% of the cases.

While 16.8% of the cases did not show any improvement from the basal score, i.e., the pre-transplant score, there was a definite sense of well being and rise in weight (2-4 pounds) noted in each case and there was also a reduction of the L-Dopa dosage in 75% of the cases. There was also a 58.3% improvement in the bradykinesia scoring from the pre-transplant level.

What is intriguing is the survival, growth and proliferation of the grafted fetal brain tissue in the HLA - and sex-randomized adult axilla without any immunosuppressive support.

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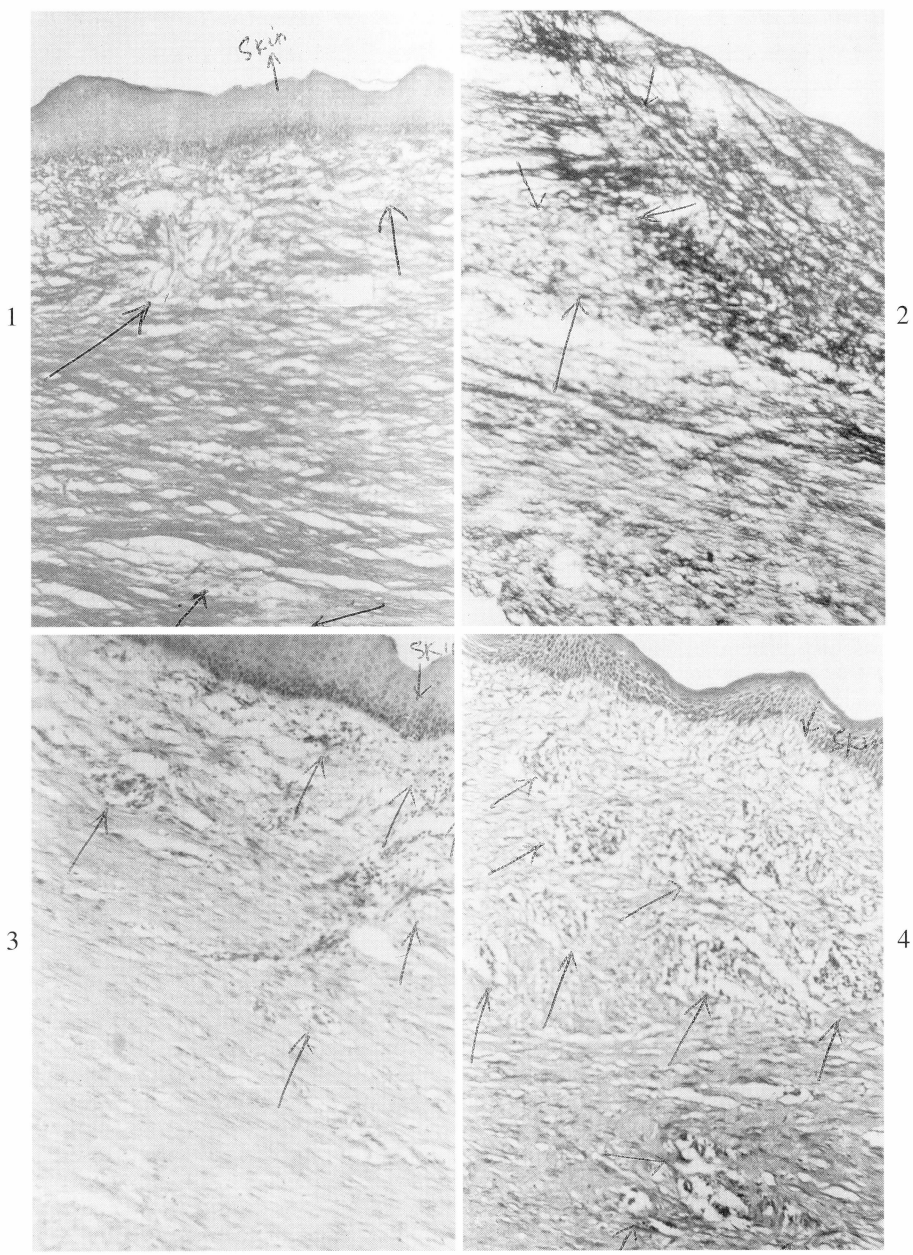
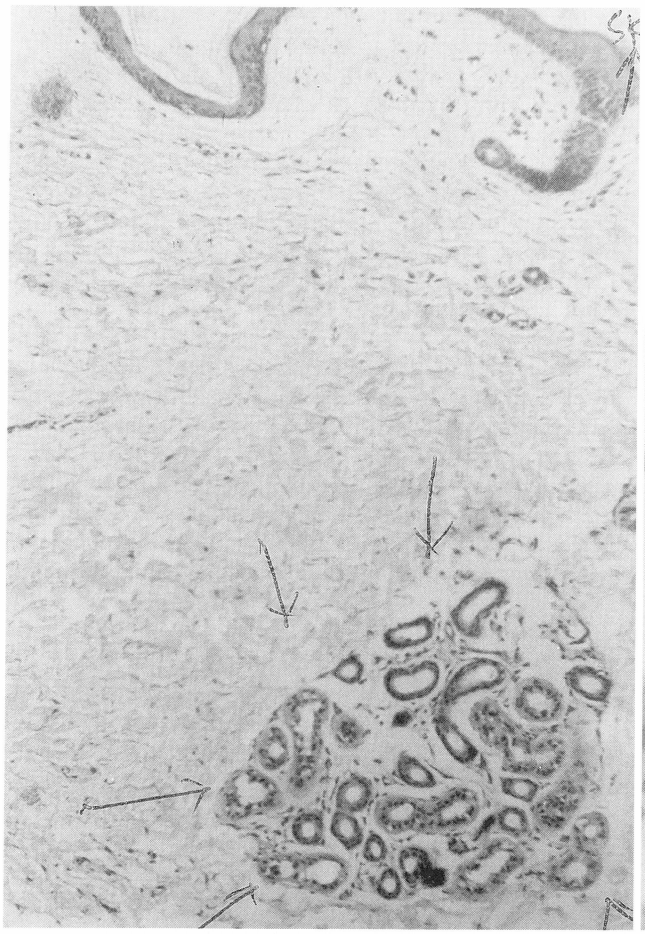
Not a single histological study of the fetal brain tissues after removal from the axilla showed any signs of graft vs. host or inflammatory reaction (Figures 1-9) but there were features of growth of the transplanted cortical brain tissue along with its different components like neurogenesis, gliogenesis, early neovascularisation and angiogenesis, etc. There was also no systemic leucocytosis or lymphocytosis.

Discussion and conclusion: Histological evidence at the transplanted tissue site suggests that fetal cortical brain tissue can sustain life in sex-randomized, HLA-randomized adult hosts, without the support of immuno-suppressive drugs and the tacit support of the blood-CSF and blood-brain barrier and other specific requirements of adult brain cells in the skull. Whether the clinical improvement in PD is transient or long lasting is presently under investigation along with basic questions like, is it due to transplanted fetal dopaminergic or non-dopaminergic neurons or is it the growth factors and the cytokine mediated hitherto unknown reactions causing the clinical improvement.

Key words: Fetal brain; Heterotopic transplantation; Growth and oxygen demand; Parkinson's disease.



Figures 1-4. — Histological micro-photographs of the tissues of a 16-week fetal transplanted frontal lobe in the background of sex- and HLA-randomized adult axillary tissue, without the support of any immunosuppressives, taken out surgically one month after its placement. Figure 1 is a low power (80 x) histological section of Mrs. B. P. (45 years), also suffering from breast cancer. The stain is Van Gieson. Figures 2, 3, 4 are of the same patient. Figure 2 shows Gomori stain for reticular fibres in low power; Figure 3 shows Haematoxylin and Eosin stain in low power; Figure 4 shows staining with PAS stain.



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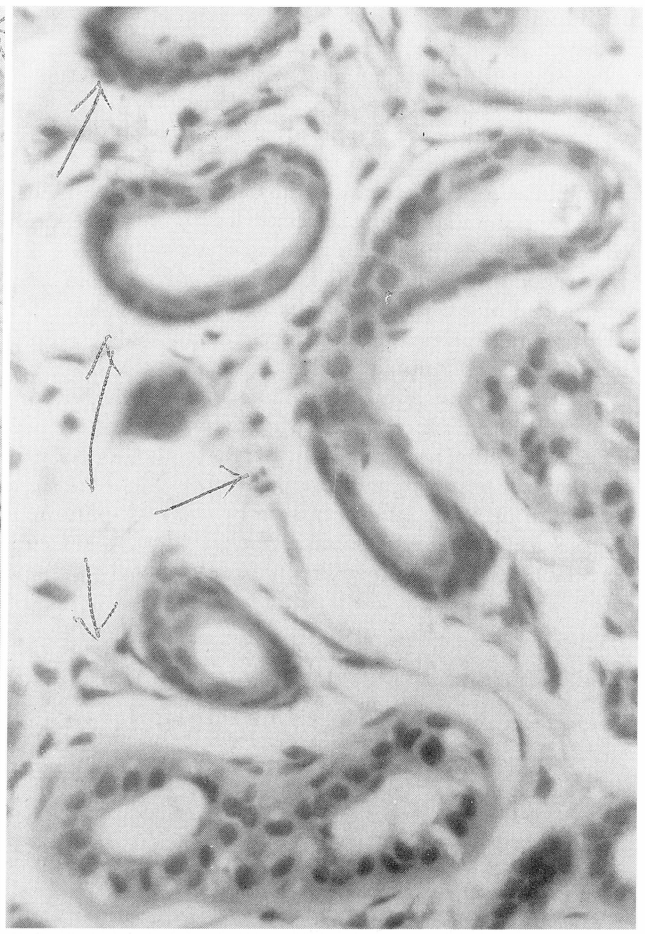

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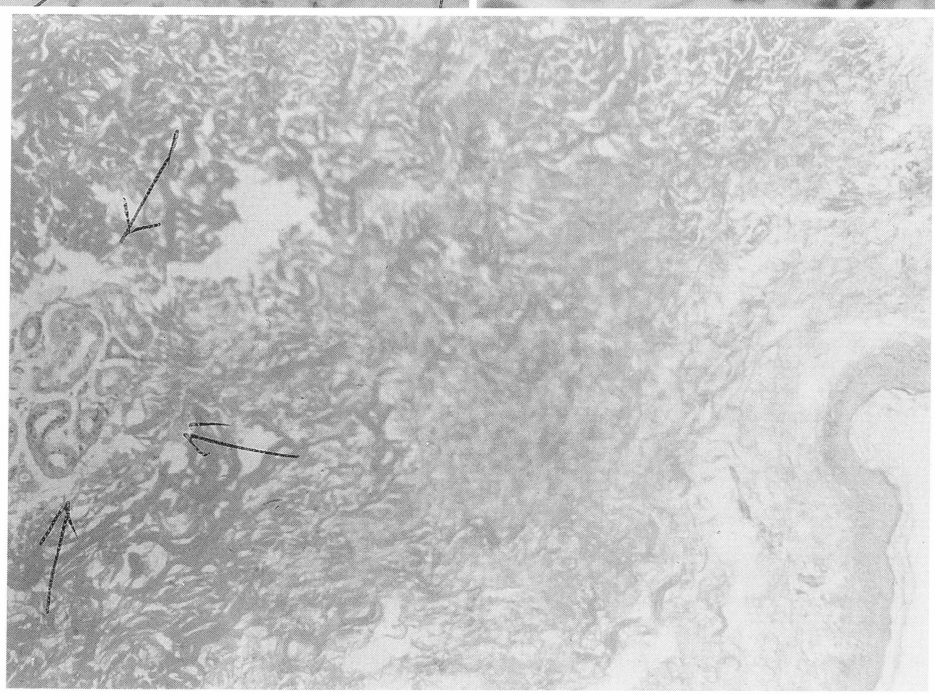

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Figures 5-7. – Histological micro-photographs of the tissues of a 16-week fetal transplanted frontal lobe in the background of sex- and HLA-randomized adult axillary tissue, without the support of any immunosuppressives, taken out surgically after two months of its placement. The patient, Mr. K. P., (65 years), was also suffering from stomach cancer. Figure 5 shows the tissue stained with Haematoxylin and Eosin in low power; Figure 6 depicts stain with Haematoxylin and Eosin in high power (275 times); Figure 7 shows Van Gieson staining in low power.

Introduction

The successful development of fetal cell/tissue transplantation in adults has resulted in the possibility of eventual therapeutic solutions in a variety of intractable diseases.

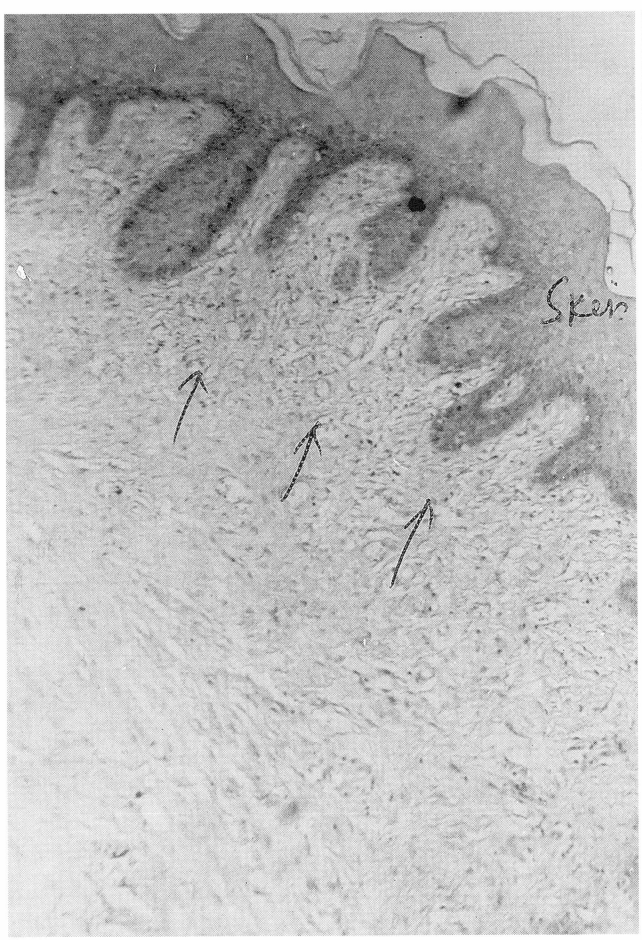
Umbilical cord whole blood transplantation in the adult system appears to be safe [1]. Our team has earlier reported on the successful transplantation of a fetal thymus in HLA-randomized adult axilla to combat leucopenia in the background of non-Hodgkin's lymphoma [2].

During intrauterine growth, the human fetus passes through the pre-immune phase (before 15 weeks) and subsequently through the hypo-immune phases of growth and maturation. The expression of hypo-antigenicity of the growing fetus in utero provides an excellent opportunity for fetal tissue/organ transplant [3].

Transplantation of human embryonic dopamine neurons in the brain, to be more precise, the CT-guided stereotactic placement of embryonic mesencephalic tissue in the putamen or putamen + caudate region, has shown marked improvement in Parkinson's disease [4, 5].

Even fetal pig neuron cells have shown survival in patients with Parkinsonism when placed in the caudate + putamen region [6]. Hence, neuro-transplantation has been proposed as a potential treatment for neuro-degenerative disorders from Parkinsonism to Huntington's disease. Whether the placement of the developing brain tissue from a human fetus can have any role in relieving symptoms of advanced PD in a surgically prepared heterotopic transplant site, is the principal question behind the present study.

Parkinson's disease is a common neuro-degenerative disease and an important cause of disability. It may cause severe autonomic motor and cognitive deficiency resulting in significant disability. The cause of PD is not known, however pathologically there is pallor of the normally pigmented substantia nigra and loss of dopaminargic cells from the substantia nigra to the caudate and putamen region (striatum). The primary manifestations



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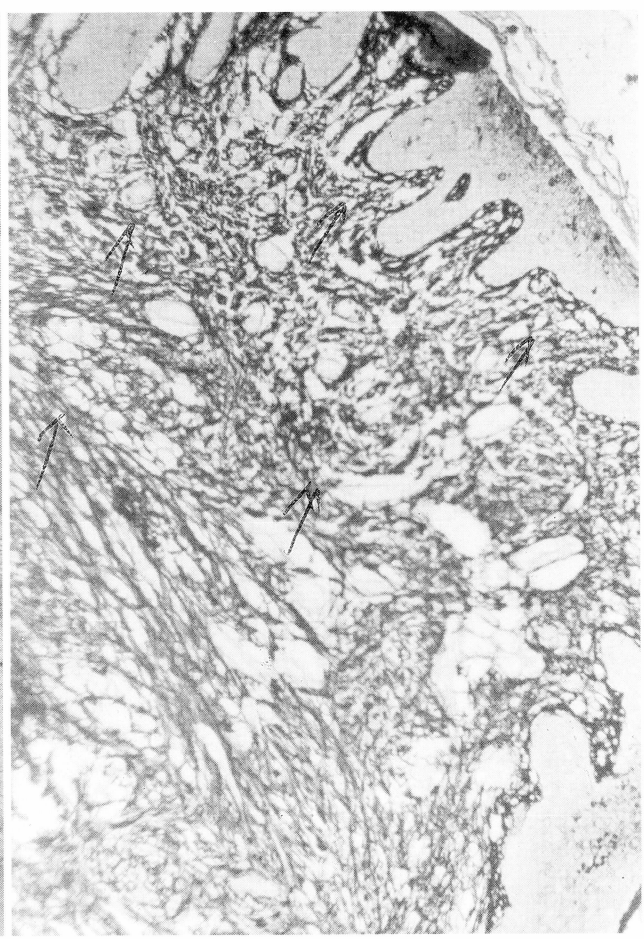

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Figure 8-9. – Figure 8 shows Gomori staining in low power and Figure 9 shows PAS staining in low power. Histological conclusion: At around 16 weeks of gestation there is extensive growth of capillaries inside the developing neuronal cells or neuroblasts. There is also, in this age group, development of sulcus in the fetal brain. From the ventricles (neural tubes), the neuroblasts arise, differentiate and migrate peripherally. Centrally, the fibres predominate and the neural cells become sparse and smaller in size. Here, the background shows the subcutaneous tissue and the skin without any inflammatory or immunological rejection by the adult host site, with conspicuous absence of the typical features of endarterites, mononuclear invasion, vascular disruption and thrombosis, even after two months in the case of Mr. K. P. (Figures 5-9), or one month in the case of Mrs. B.P. (Figures 1-4.).

are tremor, rigidity and slowness of movement. At least two of the manifestations along with definite improvement of Levo-Dopa help in the diagnosis of PD. The problem lies with the treatment of advanced PD, where higher doses of agonist/Levo-Dopa and other drugs are needed. There may be motor fluctuations with the wearing-off effects of Levo-Dopa before four hours which can cause a treatment crisis.

Materials and Method

Twelve patients were finally enrolled in the present programme. They were suffering from PD of long duration and presenting with at least two of the major criteria like (a) slowing of emotional or voluntary movement, (b) muscular rigidity, (c) tremor along with a good response with L-Dopa. These patients were included in the study of fetal brain transplant protocol after obtaining informed consent from the patient's guardian and approval from the multidisciplinary ethical committee headed by a standing District Court Judge. Taken into consideration were: (a) possible clinical benefits and costs, (b) privacy and safety of the donors and the recipients, (c) utmost care so as to eliminate the possibility of secondary gain for undergoing an abortion, and (d) the right of the woman to choose abortion and donate tissue for research [7]. Criteria for exclusion included major psychiatric illness, cognitive impairment and other substantial medical problems like advanced cancer, uncontrolled diabetes, ulcerative colitis, malignant hypertension, hyperthyroid, etc. [8].

Following standard antiseptic, aseptic and premedication protocols, the brain transplant recipient's sites were prepared using one percent lignocaine infiltration anaesthesia at the proposed site in the axillary fold of the skin. A 2-cm long and 2-cm deep tissue space with good vascularity was dissected and prepared in each patient selected to receive the brain tissue from a developing fetus. In an adjacent OR, a fetus was collected from a mother undergoing hysterotomy and ligation (up to 20 weeks) under general anaesthesia for each case. The fetus and the intact sac were taken out and brought to the transplant recipient's table. The second group of surgeons took out around 1 g of the fetal cortical tissue from the developing frontal lobe, i.e., within 1 cm of the frontal opercula of the fetus under an operative microscope and immediately weighed it in an electronic weighing machine before placing it in the recipient's prepared site at the axilla. The skin was then closed by 00 vicryl atraumatic interrupted suture. Haematological parameters (Hgb, Tc, Dc, ESR, platelets, etc.) [2] were studied in the preoperative phase and continued postoperatively up to six weeks from the date of transplantation. After one month of the primary operation, some brain tissue was taken out with a small elliptical incision under local anaesthesia for serial histological evaluation by Haematoxylin and Eosin stain [9], PAS stain [10], Van Gieson stain [11], Gomori stain [12], Giemsa stain [13], etc. In one case, the transplanted tissue was taken out after two months and in all cases skin suturing of the transplantation site was completed by 00 atraumatic vicryl.

Results and analysis

Initially 30 patients were approached and cleared by the Institutional Ethical Committee for enrollment in this fetal brain tissue transplantation study; however, we could offer this transplantation service to only 12 patients who completed our rigorous clinical protocol for diagnosis and treatment [14]. In the present series the diagnosis of PD remains clinical and was conducted by an experienced consultant in order to exclude early Parkinson's disease with mild to moderate disability and without any motor fluctuations. The problem in treating this disease in developing countries is partially due to a mindset which refuses to accept prolonged treatment which has diminishing returns, *vis a vis* the cost of treatment. Our referral service provides the option of stereotactic surgery/ablative or deep brain stimulation procedures in various brain nuclei/dopaminargic cell implantations, which can be conducted in private hospitals in our country or abroad at a prohibitive price. All 30 patients refused these options. They also refused our suggestion to purchase a peripheral apomorphine pump. We are following-up the clinical profile of the patients who underwent our protocol for transplantation surgery along with a serial study of brain-specific enzymes at the heterotopic transplant site and some cytokines from the retrieved tissue, which will be reported at a later date.

In all the enrolled cases, methodical history-taking revealed that the symptoms were insidious at onset and the common features included tremor, stiffness involving at least one limb, difficulty in walking, fatigue, depression, softness of voice, dysarthria and poor emotional and motor responsiveness. However, the classical features of akinesia with flexed posture and difficulty in the initiation of walking, followed by rapid small steps and freezing of movement during change of direction [15], were present in only eight out of the 12 cases. Details regarding the patients enrolled for the fetal cortical transplant are given in Table 1.

Parkinson's disease generally starts in middle or late life and progresses as a chronic progressive disorder. Signs of PD are very common in the elderly with 15% involvement in the age group from 65 to 74 years and more than 50% of individuals after the age of 85 will have some degree of extra-pyramidal disorder [16]. In the present series, with a fetal brain tissue transplant, moderate clinical improvement was noted in 41.6% of the cases and some clinical improvement (mild) was noted in 41.6% of the cases, as per the unified Parkinson's disease rating scale. In 75% of the cases there was some reduction in the L-Dopa dosage and in addition there was an improvement in 58.3% of the cases of bradykinesia one month after the pre-transplant rating. Another interesting thing was the sense of well-being and weight gain after the transplant in all the cases. Whether fetal tissue with its many unique properties plays a growth-promoting role is also currently being investigated. Serial assessment of p21 and p27 levels of the recipients of the transplanted brain tissue is done before surgically removing a part of the fetal brain tissue from the axilla for histological studies.

We know that in a developing fetal brain, there are phases of proliferation, neurogenesis, gliogenesis, angiogenesis, neovascularization; but all these events vary spatio-temporarily with location and cell types. Our study of transplanted brain tissue showed features of growth, proliferation and neurogenesis in small groups as well as in a cluster or cell nest-like formation (Figures 1-9).

Table 1.

Name	Age & Sex	Date of transplant	Duration of PD	Treatment received earlier	Age of the fetal brain	Clinical improvement before graft removal	Date of graft removal	Sequential Tc, Dc, ESR study
G.R.	65 M	5.2.2001	5 years	A-H	14 weeks	Mild	5.3.2001	No gross variation
A.M.	55 M	8.2.2001	5 years	-do-	20 weeks	Moderate	8.3.2001	"
P.M.	71 M	19.2.2001	6 years	-do-	20 weeks	Moderate	19.3.2001	"
C.P.	66 M	19.2.2001	11 years	-do-	20 weeks	Mild	22.3.2001	"
M.S.	48 M	26.2.2001	7 years	-do-	10 weeks	Mild	27.3.2001	"
B.P.	45 F	27.3.2001	4 years	-do-	16 weeks	No variation	27.4.2001	"
K.P.	65 M	27.3.2001	11 years	-do-	16 weeks	Moderate	27.5.2001	"
T.P.	75 F	22.5.2001	9 years	-do-	10 weeks	Moderate	22.6.2001	"
B.M.	56 M	4.6.2001	6 years	-do-	10 weeks	Mild	4.7.2001	"
S.B.	45 M	27.6.2001	13 years	-do-	18 weeks	Mild	27.7.2001	"
T.C.	73 M	27.6.2001	4 years	-do-	18 weeks	No variation	27.7.2001	"
S.T.	55 M	30.6.2001	5 years	-do-	10 weeks	Moderate	30.7.2001	"

- All the patients noted in the table were treated with: A) Levo-Dopa; B) Dopamine agonist group of drugs; C) Catechol-O-methyl transferase (COMT) inhibitor group of drugs; D) Anti-cholinergics; E) Mono-amino oxydase-B inhibitors; F) Physiotherapy; G) Behavioral therapy to combat depression, dementia, psychosis, etc.; H) Symptomatic treatment for autonomic dysfunction.
- A minimum score of 40 points is required for enrollment in the motor portion of the Parkinson's disease unified rating scale [16] when the patient had been without medication. Scores in this scale varied from 0 to 108. Higher values indicate greater severity of symptoms and motor complications that can not be controlled by pharmacological therapy alone.
- In the present study an objective improvement of 33.3% from the pre-transplant level to the date of assessment, i.e., one month after the installation of the fetal cortical graft, is noted as mild improvement. Similarly, 33.4% to 66.6% improvement from the base level score in the unfied Parkinson's disease scoring system is characterized as moderate improvement. Any further improvement is noted as good response.

Table 2. — *Clinical impact of fetal cortical tissue transplant after one month on Dyskinesia Rating Scale and L-Dopa dosage.*

Name	L-Dopa dosage before transplant	L-Dopa dosage one month after transplant	Dyskinesia rating (0-4) before transplant	Dyskinesia rating (0-4) one month after transplant	Weight gain (in lbs)	Sense of well-being after the transplant
G.R.	4 tabs/day	2 tabs/day	3	1	3	Present
A.M.	5 tabs/day	2 tabs/day	2	1	2	-do-
P.M.	6 tabs/day	3 tabs/day	4	3	3	-do-
C.P.	3 tabs/day	3 tabs/day	2	2	4	-do-
M.S.	4 tabs/day	3 tabs/day	2	2	2	-do-
B.P.	5 tabs/day	4 tabs/day	3	2	4	-do-
K.P.	4 tabs/day	1 tab/day	3	1	3	-do-
T.P.	3 tabs/day	1 tab/day	2	2	2	-do-
B.M.	6 tabs/day	4 tabs/day	4	2	3	-do-
S.B.	2 tabs/day	2 tabs/day	3	3	4	-do-
T.C.	3 tabs/day	2 tabs/day	3	2	2	-do-
S.T.	2 tabs/day	2 tabs/day	2	2	2	-do-

0 = no dyskinesia; 4 = severe dyskinesia on the basis of objective criteria assessment like walking, putting on a coat, lifting a cup to drink, etc. [17]. Each tablet of L-Dopa contained 250 mg.

What is intriguing is the lack of any leucocytosis or lymphocytosis in the hosts' system even after the graft survived for one month or more in the host's axilla. We have had similar experiences in cases of thymus [2] and other fetal tissue transplants like the fetal heart, liver, lung and pancreas [3]. When we studied the histological appearance of the transplanted tissue and the interaction with the host tissue, we observed that the brain tissue

was growing as a cell nest under the axillary subcutaneous tissue of the host without any inflammatory or immunological reaction. It is well known that in pregnancy the uterus is considered as a privileged site due to its decidua and hormone-cytokine backup support along with HLA-G. The axilla has never been considered as an immunologically privileged site. There are, however, certain immunological privileges of brain tissue, but that may be due to the blood-brain barrier and other site specificity. We are presently estimating the tissue dopamine of the transplanted tissue along with brain specific enzymes like glutamate dehydrogenase and gamma glutamyl transferase, along with cytokines like tumor necrotic factors, which will be reported in a subsequent paper.

Whether transplantation of the developing fetal cortical tissue or the growth factors and other cytokines of the fetal tissue in causing some remission of PD, is under intense study is our laboratory. We are also meticulously following up the clinical remission and its duration and will also report on this later. There was no adverse event due to the HLA-randomized transplantation without immunosuppressive support. There was also no discharge, inflammation at the site of transplant nor leucocytosis justifying early removal of the graft.

The scar was healthy and the stitches were removed from the site on the seventh postoperative day.

Discussion and conclusion

The adult brain consumes 15% of the resting cardiac output for its metabolic needs and auto-regulation. While most of the tissues can sustain an anaerobic assault from a few minutes to 30 minutes in the human system, a sudden total lack of oxygen supply to the brain cells in an adult will make the person unconscious within five to ten seconds [18]. One of the serious complications of abnormal fluid dynamics is the development of brain cell edema, with the possibility of eventual destruction of brain cells as a result of decreased blood flow due to extra edema fluid compression as the brain is encased in a solid vault [18].

Blood-cerebrospinal fluid and the blood-brain barrier operate simultaneously by the fusion of adjacent endothelial cells to one another, thus decreasing the permeability. However, in the case of other tissues in the body, there are extensive slit pores [18].

In the last paragraph we have discussed in brief certain physiological peculiarities of adult brain function. The question now may be asked whether the developing fetal brain, up to 20 weeks of gestation (limit of hysterotomy and ligation), has acquired these specialized requirements needed for its survival as has been noted for the adult brain system. From the fetal brain tissue transplants that we have conducted, we have learnt that fetal brain cells can live outside their normal position, that is, the encasement of the skull, without the presence of cerebrospinal fluid or meningeal support as seen in adult brain cells. On the basis of our analysis of the histological data (Figures 1-9), we have noted that there is no rejection of the transplant in the form of thrombosis, endarteritis, mononuclear invasion, etc. The problem is why this is so. It is known that the cornea and cartilage are readily accepted as grafts. Moreover, the decidua of the uterus provides a certain degree of environmental privilege for the growth of an allogenic fetus. The human adult axilla, however, has never been cited in medical literature as a privileged site.

It is generally believed by biological scientists that human fetal growth is dependent on a unique symbiotic environment where the mother provides all the necessary factors and environment for growth, proliferation and differentiation. The fetal micro-environment is distinctly different from the adult neuro-endocrine and metabolic micro-environments [19].

Hence, it is possible that transplanted fetal neuronal tissue adjusts its own micro-environment to an altered metabolic situation, which is distinctly different from that of the mother which had supported the fetal growth so far in the uterus, using the advantage of its hypo-immune/pre-immune status for its survival strategy. Why and how the transplanted fetal brain tissue in a sex- and HLA-randomized non-primed (steroid or other immuno-suppressants) adult host escapes the immunological or inflammatory recognition system and becomes a human homologous *chimera*, can be explained by the lack of proper antigenicity in the growing fetal tissue.

Although the brain has more immunological or positional advantages due to its encasement and blood/brain-like physiological or functional barriers than the other tissues of the developing human fetus, we have had similar experiences with other tissues as well (see previous section).

Thus, we can conclude that all specialized fetal tissues [1, 2, 3] including fetal brain tissue up to 20 weeks of gestation, contrary to its known biological needs, can survive without the assistance of immuno-suppressive drugs in HLA-randomized, sex-randomized tissue and in the altered metabolic support of a non-mother host, due to the extreme versatility of its living potential.

The idea for this preliminary report was to communicate to the scientific fraternity that fetal brain cells/tissue can survive in a heterotopic site, e.g., adult non-pregnant females and also in males (HLA-randomized) and sustain its metabolic needs and oxygen demands, without concomitant support with immunosuppressives. We found this to be truly intriguing and exciting, and to the best of our knowledge it has not been specifically reported earlier. In conclusion, if the entire medial community combines its wisdom and examines this very complex issue of fetal tissue-adult tissue interactions in health and disease, there may be a major breakthrough in this hitherto vastly unknown and fascinating field, which has many important implications for understanding regeneration biology and engineering with strategies for tissue restoration to combat human miseries in the future [20].

It is of paramount importance to our group of researchers to determine whether the survival of brain tissue

and its clinical improvement are directly related to each other, or if the growth factors and different cytokines of the human developing fetal brain tissue are responsible for the apparent improvement. Another interesting aspect is to examine whether neuro-degeneration in idiopathic Parkinsonism [21] is site specific or not. If so, neuro-degeneration could be avoided in a heterotopic transplant site. These are very interesting issues to ponder and are currently under scrutiny.

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