MATERIALS & METHODS
The present study is involved in the analysis and assessment of conditions of patients suffering from chronic inflammatory and degenerative diseases like bone and joint inflammation, fibromyalgia, low backache, arthritis (osteo & rheumatoid), inflammatory diseases of skin like psoriasis and pemphigus (in acute and chronic phase). Clinical assessment, biochemical assessment of glycaemia, lipidemia and other metabolic status and assessment of hormonal status of the relevant endocrine glands were done.

Multiple organ dysfunction of acute phase like that occurring in multiple organ failure (MOF) syndrome occurring in acutely ill patients like stroke, myocardial infarction, diabetic acidosis and other metabolic storms were taken up for the study protocol. Chronic states of multiple organ dysfunctions occurring in bedridden cases and age related arthritis etc., were also included. It was noteworthy that the phenomenon of downhill course of disease processes involving the cascade of multiple organ dysfunction (ending up in multiple organ failure) happens in acutely ill cases of stroke, head injury and other severely traumatized cases of road traffic accidents. Under strange and unfortunate circumstances quiet often they appear to succumb to an inevitable end due to the rapid progression of the disease process, suddenly bringing in dysfunction and failure of various organs, which were at admission were apparently normal. The unfortunate outcome turns out as the patient dies even though the original disease factor (like the head injury or stroke) may have been clinically well controlled.
Patients for the study were selected from the various groups, consulting the author in his chamber at various cities, like Chennai, Hyderabad, Vijayawada, Nellore and Tirupati in India. At Chennai and Hyderabad, some of the patients were those who consulted him at Apollo hospitals in those places. Typically, cases of arthritis and low backache were the ones who had got no relief from the usual treatment modalities, like interferential anti inflammatory drugs, local short wave diathermy treatments and various physiotherapeutic manipulations. They were also willing to undergo the treatment protocol of the present study, probably, wanting to get some relief through some means! The patients were grouped according to their disease process like:

- Arthritis, rheumatoid and osteoarthritis.
- Chronic skin diseases. Psoriasis and pemphigus, morbid obesity with complications of respiratory insufficiency and intertrigenous nonhealing skin infections.
- Patients with complications like post-cerebral stroke, post myocardial infarction, patients undergoing critical care with the development of the critical multiple organ dysfunction ending in multiple organ failure.
- All the patients were grouped according to age and sex and were compared with the age and sex matched controls, taken from the hospital records, suffering from similar illnesses, but not treated according to the present study protocol.
All the cases were assessed clinically and studied by investigations of routine hematological, biochemical and radiological investigations. Scanning of various organs and assessment of dynamic organ functions were done by blood biochemical parameters, ultrasound and computerized tomography scans, magnetic resonance imaging and nuclear imaging techniques as warranted by the basic disease conditions. Hormonal assays were done as relevant to the disease condition and gender (depending on the endocrine involvement of the disease and also according to the treatment protocol with which treatment was given using the hormones like testosterone, oestrogen, progesterone and growth hormone). The assessments of the hormonal levels were done before and after treatment.

A total number of 57 patients selected from hospital records with the similar diseases selected for the present study but were given the conventional therapy formed the control group. This group included equal number of age, sex and disease matched subjects who were treated for arthritis, psoriasis, pemphigus, morbid obesity and multiple organ failure with conventional modes.
All the cases were assessed clinically, and studied by investigations of routine hematological, biochemical and radiological investigations. Scanning of various organs and assessment of dynamic organ functions were done by blood biochemical parameters, ultrasound and computerized tomography scans, magnetic resonance imaging and Nuclear imaging techniques, as warranted by the basic disease conditions. Hormonal assays were done as relevant to the disease condition and gender (depending on the endocrine involvement of the disease and also according to the treatment protocol with which treatment was given using the hormones like testosterone, estrogen, progesterone and growth hormone). The assessments of the hormonal levels were done before and after treatment.

Control group

A total number of 57 patients selected from hospital records with the similar diseases selected for the present study but were given the conventional therapy formed the control group. This group included equal number of age, sex and disease matched subjects who were treated for arthritis, psoriasis, pemphigus, morbid obesity and multiple organ failure with conventional modes.

Arthritis patients were treated with non-steroidal anti-inflamatory drugs, physiotherapy measures with short wave diathermy, interferencial current etc. Their investigations of the disease state were comparable to those of the age and sex matched patient groups indicating the extent of disease involvement. Their basal values of the relevant hormones like oestrogen, LH, FSH, GH, TSH in female group and testosterone, LH, FSH, TSH and GH were assayed using
commercially available RIA and IRMA kits (DSL, Webster, Texas, USA; DPC, Los Angeles, Canada, USA). It may be inferred that the parameters judged clinically and assessed by markers of inflammation and organ function (Hb% indicating the status of haemopoietic milieu, enzymes SGOT, SGPT, γGTP, indicating the gross functional status of the liver, and comparing the integrity of the pituitary-gonad, pituitary adrenal axes), in the control and the study group were similar. However, there was significant difference in the outcome of treatment in the two groups. Though these parameters are more on the basis of clinical assessment and subjective information given by the patients and their relatives, the significance needs to be judged on a qualitative basis of patient’s feelings of pain-free status and ability to carry out the day-to-day activities without complaints or help and support of others.

Another factor of medical significance, which cannot be translated to statistics, is that the patients involved in this study were mostly non responders or poor responders with regard to the usual and conventional treatment, prior to being enrolled in the present treatment schedule. Hence, in a way each patient is control for the results of the present treatment as comparable to other mode of treatment. This was clearly evident in all the patients enrolled for the present study. This may be especially highlighted in the individual cases like the crippled and bedridden lady with arthritis who was up and ambulatory in about 10 days of the treatment schedule, and the bed-ridden quadriplegic man after cervical spine fracture and interventional corrective spinal surgery, who was practically helpless despite theoretical medical normalcy and residual defect, which was known to be
permanent in the available medical knowledge. The fact that he is moving about freely now after 2yrs of the mishap is more than rewarding and proof for the concerned (elaborated in page). The significance of the present study with limited number of subjects has to be in the background of difficulty in enrolling cases for newer methods of treatments in the light of current developments in field of molecular medicine.

The normalization of modestly elevated levels of TSH with the present treatment schedule, without giving replacement of thyroxine, is a clear fact that there is poly glandular dysfunction in chronic inflammatory, degenerative disorders, especially the condition of andropause in males and menopause in females, which can be corrected the treatment of the underlying inflammatory process, and does not warrant life long thyroxine supplementation. Ageing and degenerative changes of the body tissues have been linked to chronic inflammatory processes in recent scientific advancement of cell biology.

Multiple organ dysfunction of acute phase like that occurring in Multiple Organ Failure (MOF) syndrome occurring in acutely ill patients like stroke, myocardial infarction, diabetic acidosis and other metabolic storms were taken up for our study protocol. Chronic states of multiple organ dysfunctions occurring in bedridden cases and age related arthritis etc. were included for our study. It was noteworthy that the phenomenon of downhill course of disease processes involving the cascade of multiple organ dysfunction (ending up in organ failure) happens in acutely ill cases of stroke, head injury and other severely traumatized cases of road traffic accidents; and the succumb to the inevitable end even though
the primary illness on admission might have been abated and brought under control like the head injury or multiple fractures or the acutely diabetic state? Making the explanation of the untoward outcome of treatment rather difficult to understand for the layman related to the unfortunate patients. This is because of the shift of the central mechanism of disease courses to the relentless catabolic process of cell apoptosis leading to multi organ dysfunction and ultimate organ failures. This process understandably can be tackled by the use of recombinant growth hormone and the supportive gonadal steroids (testosterone, estrogen, progesterone): the latter helping in anti-inflammatory cytokine and prostaglandin mechanisms.
Figure 14A  Agarose gel electrophoresis of exon 10 of CHEK2 gene
Lanes 1-15 represents PCR products of CHEK2 gene. NC- negative control. M- molecular weight marker

Figure 14B  Representative reverse sequence of CHEK2 exon 10.
Provera 150 mg, manufactured by Ferring India Pvt. Ltd.) once in two weeks and transdermal estradiol applications daily at night (Oestrogel equivalent 0.625 mg, manufactured by Solvay Pharma India Ltd.). In patients having very low serum estradiol levels like less than 10 ng/dl they were given twice a day applications for first 10 days. Out of these, two male patients with rheumatoid arthritis, were given subcutaneous injections of recombinant growth hormone (Nordilets manufactured by Novo Nordisk Pharma) 3 units every night on a 5 day week schedule for 2 months. This was started after assessing the response to the treatment with only testosterone for two weeks. The additional benefits in these patients were also assessed clinically and by biochemical parameters. One female patient with rheumatoid arthritis who was severely affected and bed ridden was given injection of recombinant growth hormone after two weeks of giving hormone replacement therapy with progesterone and oestrogen (injections of Depo Provera 300 mg. and transdermal Oestrogel) she could afford only two weeks of injections of recombinant growth hormone and hence, the same was given along with hormone replacement therapy for two weeks and the latter continued for two months.

Three male patients and one female patient, age and sex matched with rheumatoid arthritis, whose data were taken from hospital records, were taken as controls to study the differences of results with other conventional modes of treatment they were getting, with the results of our treatment in the study group. The conventional treatment consisted of oral/parenteral non-steroidal
anti inflammatory drugs and physiotherapeutic measures like shortwave diathermy and interferential treatments.

The two male patients with osteoarthritis were given testosterone injections for two weeks and subsequently supplementation with recombinant growth hormone was added on for the next two months. The results of the treatment with the testosterone alone and with additional recombinant growth hormone were assessed clinically. Three female patients with osteoarthritis were given Oestrogel and Depo Provera for three months in the previously mentioned dosage schedule.

The same number of age and sex matched controls were studied from hospital records, for analysing the condition of them as regards the clinical and biochemical/radiological parameters before and after conventional treatment with non-steroidal anti inflammatory drugs and physiotherapy measures, and compared with the outcome in the study group.

Dermatological Cases

There were four females in the age group of 40-50 years (menopausal) with severe and extensive pemphigus. There were three female patients with severe and extensive psoriasis. They were treated with transdermal oestrogen (daily) and depot preparations of progesterone (Oestrogel and injection Depo Provera once in three weeks by intra muscular route) and recombinant growth hormone by subcutaneous route at night (3 units) daily for 5 days a week for one month and subsequently twice a week for two months. Since all of them had earlier irregular use of cortisone, they were weaned off from cortisol
The heights of female patients (132-140 cms) are, according to south Indian standards, average heights for females for that age group. Besides, these cases had their blood levels of growth hormone assessed prior to treatment to confirm that they did not have any element of hypo-somatotrophism.

The female cases of morbid obesity mentioned here, had irregularity of menstrual cycle and were estimated to have deficient levels of oestrogen and progesterone prior to treatment. Hence by giving them replacement therapy with estrogen and progesterone regularized their cycles, and gave beneficial effects on obesity, which is often considered as an extension of inflammatory process of adipocytes.
doses and their hypothalamo-pituitary-adrenal axis was readjusted by intramuscular ACTH injection (40 units of oil preparation of Acton Prolongatum manufactured by Ferring India Ltd.) intramuscular route once a week for five weeks.

There were five males in the age group of 40-50 years with extensive psoriasis and two in the same age group with extensive pemphigus. They were given depot preparations of testosterone (injection Sustanon 250mg intramuscularly once in 15 days) and three units of recombinant growth hormone (Nordilets) subcutaneously at night daily for five days a week for four weeks and twice a week for the subsequent eight weeks.

Patients with pemphigus and psoriasis of age and sex matched control subjects receiving conventional treatment for these conditions with local applications with steroid, salicylic, antibiotic creams and oral anti-inflammatory drugs and antibiotics were compared with study group for significant difference in treatment outcome.

Morbid obesity

There were 6 female subjects, all in the age group of 35-45 years with morbid obesity of weight more than 120 kilo grams and height in the range 130-145 centimeters. They were treated with recombinant growth hormone by subcutaneous doses of 3 units every night for 5 days a week for two months. In addition to this they were given high fiber normal protein, low fat, low carbohydrate reducing diet of 800-900 calories per day. They were also given hormone replacement therapy of progesterone and oestrogen by transdermal
estradiol (Oestrogel and injections of Depo Provera once a month). Two female subjects who were in the peri-menopausal phase with 4-6 months of amenorrhoea, were given 21-day cycle regulating low estriol type of preparations (Krimson 35 manufactured by Sun Pharma Ltd.).

Three male subjects with age group of 40-50 years and height of 160-170 centimeters and weight ranging from 120-140 kilo grams, all business executives by profession and sedentary type of office work. They were incapacitated in their official travel and day-to-day activities with their morbid trunkal obesity, due to respiratory insufficiency, difficulty of moving about and inter trigenal abrasive wounds of the axilla and groins. They were given testosterone injections of 250 mg intra muscular fortnightly and daily subcutaneous doses of recombinant growth hormone as 3 units at night daily on a 5 day week schedule they were also advised to be on a reducing diet of 900 calories consisting of low carbohydrate, low fat, normal protein diet with high fiber content. Before treatment they were unable to tolerate even 15 minutes of brisk activity, there by making them unable to exercise and burn the calories towards weight reduction. With the treatment their exercise tolerance improved in 7-10 days of starting treatment and they were subsequently encouraged to resort to brisk activity, as per their tolerance capacity.

These subjects were counselled to meditate and follow the principles of kinesthesiology (by intense visualization and feeling of, after effects intense exercise like high heart beat feeling of warmth and high tone of muscles), even though they could not tolerate the physical part of the same.
The cases of arthritis, morbid obesity, cerebral stroke and dermatological cases were given sex steroids, to begin with. Either on finding partial response, or with a view to see the additional response that recombinant growth hormone can give to such patients, these patients were started on doses of recombinant growth hormone—the dosages were daily or by weekly depending on the severity of disease process-i.e. dermatological cases needed less frequent doses of growth hormone than the stroke patients in ICU. In further studies, we intend to design dosage schedule specific for each site of inflammatory/ degenerative disease.
Age and sex matched controls of comparable height and weight who were on conventional management of similar obesity treated with 800-900 calorie reducing diet (low carbohydrate, low fat, normal protein, high fibre) and tolerable exercise with equal amount of counselling as in the study group. Their clinical assessment and check of investigational profile were studied as comparable with the study group.

Cases of complications of cerebral stroke, paralysis & myocardial infarction

There were three cases of male and two cases of female in the age group of 45-65 years, with complications of diabetes mellitus in skin, joints, retina and peripheral nerves. They also had cardiac and renal functions deranged to a mild degree. All of them were being treated traditionally for post-cerebral stroke and post-hemi-paralytic episodes. There were 2 male patients and three female patients who had hemiplegia and were being rehabilitated at home with physiotherapy and supportive measures. As per our study protocol the two male patients were put on injections of testosterone 250 mg intramuscular fortnightly and subcutaneously recombinant growth hormone, 3 units subcutaneously at night on a five day week schedule for two months. The female patients were put on injections of depot progesterone fortnightly and transdermal estradiol daily. They were also given subcutaneous recombinant growth hormone of 3 units per day (daily on a 5 day week schedule) for two months. Course and progress of disease processes with similar ailments undergoing treatment schedules of standard, conventional nature in age and sex matched control subjects were studied and
compared with the data in the study group. Their pretreatment and posttreatment investigational assessments were comparable with those of the study group.

One male patient of age 63 years had complications of cervical spine fracture due to road traffic accident and he was having quadriplegia after the surgical manipulation and interventional plate and screw fixation, he was discharged from the hospital and was advised physiotherapy. After two weeks of physiotherapy there was no satisfactory improvement in muscle power and mobility. The family members were facing the problem of his getting irritated with physiotherapy and refusing to continue the same. It was at this juncture that he was taken up for the study protocol treatment. The promise of a new possible treatment to his miserable and disappointing fate made him enthusiastic and willing to accept this method of treatment and he had rewarding results.

Regarding this patient, his own progress of medical condition prior to starting the study protocol of therapy was as good as a control study.

There were three male patients and two female patients in the age group of 40-55 years, who were practically bed ridden after myocardial infarction. For no justifiable medical reasons, these patients were still unable to move about after 4 weeks of discharge from hospital after a typical myocardial infarction. Apart from the vitiated ejection fraction of the cardiac function in the range of 40-60% (assessed by their echocardiography), all other parameters were normal medically. They were given treatment as per
the study protocol, with gonadal steroids (testosterone in male patients and oestrogel and depot progesterone in female patients, in the dosage schedules mentioned earlier) and recombinant growth hormone (in the dosage of 3 units at night, five days a week for two months).

Here again the patient's condition before initiation of the present study protocol of treatment with hormones and after the treatment could be appreciated significantly.

**Multiple organ failure**

We had seven cases of critically ill patients undergoing acute care in intensive care units. Four of them were males and three of them were females in the age group of 30-50 years. All of them were developing or had developed multiple organ dysfunctions, as evidenced by the impairment of function of at least 3 organs, which were apparently normal clinically and biochemical at the time of admission to the intensive care. These cases were admitted for traumatic head injury (road traffic accident and other cases of severe injuries). Two male patients were in metabolic crisis due to diabetic acidosis and electrolyte imbalance, even though they were not known diabetics prior to admission. During the course of the critical care, these patients had developed multi organ dysfunction episodes of a pneumonic patch in the lungs, cardiac dysrrhythmias, rise of serum creatinine and urea. They also had high titers of hepatic enzymes (SGOT, SGPT, GGTP) and three of them had haematorrheological (abnormality of haematopoietic cellular count and abnormality of platelet number and coagulatory function)
complication ending with Disseminated Intravascular Coagulation (DIC). They were taken up for the treatment schedule with subcutaneous recombinant growth hormone (3 units subcutaneous Nordilets at night per day on 5 day week schedule) and the replacement of gonadal steroid, testosterone in male and oestrogen/progesterone in female.

Analysis of data regarding treatment outcome in age and sex matched subjects undergoing treatment schedules of conventional critical care were done to compare with the outcome of the present treatment schedule in the study group.