# EFFECT OF VITAMINS 'A' AND 'E' ON SPONDYLOSIS



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# ABSTRACT

A biomedical study was undertaken to observe the effect of the anitioxidative roles of vitamins A and E on the degenerative process occuring in spondylosis in humans.

A single blind and a double blind study were done. Thirty two patients suffering from spondylosis were selected for the study on the basis of certain criteria. The patients were 40-60 years old and there were 24 males and 8 females. The patients were suffering from mild to severe neck pain and low back ache and the spondylotic degeneration in their cervical and lumber regions were confirmed by X-rays. The serum vitamin E level in most of the patients were lower (5.8±2.7 μmol) than normal (11-41 μmol/L. Vitamin E administration at a dose of 100 mg daily for three weeks resulted in a significant increase in serum vitamin E level. This was accompanied by complete relief of pain. This efficacy of vitamin E was observed in both the single and the double blind studies. The vitamin A level in serum of the patients was in most cases already within normal range (0.53-2 μmol/L) and vitamin A administration neither resulted in a signficant increase in serum vitamin A level nor was this vitamin found to be effective in relieving the pain and other symptoms of the disease.

The results therefore strongly indicate that vitamin E is effective in curing spondylosis and this effect is most probably due to its antioxidant activity.

# EFFECT OF VITAMIN E AND A ON SPONDYLOSIS

No. : Date:.....

Name of the patient .....

Age:..... Sex: ..... Religion: .....

Address: Present : .....

Parmanent.....

Occupation ..... Income.....

Educational status ..... Marital Status .....

**Symptoms:** **Duration:**

(1) Early morning stifiness

(2) Limitation of movement

(3) Pain

(i) Site (a) Posterior neck region (b) Lower lumber region

(ii) Radiation (a) Occiput (b) Chest

(c) Shoulder girdle (d) Arm

(e) Scapular angle

(4) Paresthesis

(5) Pain exacerbetaed by

(a) Prolonged standing (b) Hotion (c) Prolonged sitting (d) Lying

(6) Pain releived by

(a) Rest (b) Motion (c) Drugs (i) Non steroidal antiinflammatory (ii) Others

**Past history**

(1) Injury (2) Fall (3) Fracture (4) Debiliting disease

**On examination**

**General**

(1) Anaemia (2) Jaundice (3) Odema (4) Height (5) Weight

(1) Liver (2) Spleen (3) Heart (4) Lungs (5) Pulse (6) Blood pressure

(7) Temperature

**Physical**

**Inspection**

(1) Vertebrel prominence (2) Interspinous process (3) Scapular angle

**Palpation**

(1) Tenderness (2) Spinous process

**Percussion**

| Testing spinal movement | Cervical region | Thoracic & Lumber region |
|---|---|---|
| (1) Flexion | | |
| (2) Extension | | |
| (3) Lateral bending | | |
| (4) Rotation | | |
| (5) Prone | | |
| (6) Supine-straight leg test | | |

Motor system

Aupper extremity

| | | Biceps | Triceps | Interossei |
|---|---|---|---|---|
| (1) Inpection | Atropy | | | |
| | Tremor | | | |
| (2) Power | | | | |
| (3) Tone | | | | |

(4) Reflex

(5) Spinal movements Cervical region Thoracic & Lumber region

Flexion

Extension

Lateral bending

Rotation

Prone

Supine

(6) Motor system

Upper extremity

Biceps Triceps Interview

**Inspection**

Power

Tone

Reflex

Lower extremity

Thigh calf Interosses

**Inspection**

Power

Tone

Reflex

**II X-Ray findings**

B. Lower extremity

Thigh calf Interossei

**Inspection**

Power

Tone

Reflex

| **Laboratory** | | **Investigation** | **X-Ray** |
|---|---|---|---|
| Blood | | Urine | |
| Hb% | | Quantity | |
| TC | | Colour | |
| DC | Poly | Sp. Gr. | |
| | Lymph | Reaction | |
| | MOno | Albumin | |
| | Eosino | Sugar | |
| | base | Pus cell | |
| ESR | RBC | | |
| PCV | | C exalate crystals | |

**Vitamin A**

**Vitamin E**

**Treatment received**

Group A

Group B

Group C

Result:

I Elimination of symptoms

(1) Early morning stiffness

(2) Limitation of movement

(3) Pain

(4) Tenderness

| Testing spinal movement | Cervical region | Thoracic & Lumber region |
|---|---|---|
| (1) Flexion | | |
| (2) Extension | | |
| (3) Lateral bending | | |
| (4) Rotation | | |
| (5) Prone | | |
| (6) Supine-straight leg test | | |

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# I. INTRODUCTION

The term spondylosis has been given to a chronic, progressive, involutional process occurring due to degenerative changes in the intervertebral discs of the vertebral column. This is usually associated with secondary osteoarthritic changes in the adjacent vertebral joints [1].

Epidemiological studies[2] have demonstrated that in degenerative condition of the vertebral column, cervical disc degeneration has the highest prevalence. Both males and females between the ages of 65 and 74 years show radiological evidence of cervical disc degeneration. This condition is usually manifested by severe neck pain, yet some people tend to be symptom free throughout their life. Post mortem studies[2] have shown that 90% of people above 60 years of age were suffering from symptomless spondylosis.

In no other systems of the human body do the changes of aging culminate in more suffering than in musculoskeletal system. As age increases the degnerative changes of the vertebral joints along with other major synovial joints constitute a great burden. Even though these degenerative changes have been blamed on the modern ways of living, it was present in our primitive ancestors, discovered in the Nubian caves[3] dating back to 8,000 to 10,000 B. C.

Since 1950 [3] attention has been growing on the influence of aging on the fibrocartilaginous joints of the vertebral column. The importance of changes in these joints in which the intervertebral disc is situated has been emphasized by population studies. These studies[3] show that back pain is the most common cause of lost working time.

Back pain caused an estimated loss to industry of around 15,000,000 working days per annum in Britain[4]. It was also asserted[5] that more than 1 in 3 Swiss people became unable to work at leas once in their life time because of low backache. Impairments of the back and spine were recorded as the most freequent cause of limitation of activity in persons of less than 45 years of age and they ranked third after heart desease and rheumatism in those aged between 45 and 64 years[6]. Over 8,000,000 Americans suffer from chronic back pain and it affects 65% to 80% of Americans[7] sometime in their lives.

The intervertebral discs sandwiched between the vertebraes not only serves as the main weight bearing force but also help in the normal motion of daily life (lying, standing, walking, sitting). As such it suffers more degeneration than the other parts of the body. Causes for spondylosis has been searched for a long time. Finally it becane known that free radicals couldbe responsible for intervertebral disc degeneration which manifests itself in the clinical form of spondylosis[8].

Free radicals are groups of atoms which are produced during normal metablism 8. For example are superoxide and hydrogenperoxide. They interact with oxygen to give a continuous supply of free radicals. Many organic systems would have undergone degeneration due to these free radicals had they not been protected by antioxidants. Antioxidants are antidotes for free radicals.

Vitamin E has long been known to act as an antioxidant in the body[9]. Other substances believed to act as antioxidants are beta-carotene pro vitamin-A and selenium.

The degenerative changes of spondylosis seldom kill but cause endless suffering and countless days of limitation of physical activity. The exact etiology is yet not known and hence its control and prevention remains to be discovered.

Our present study is only an attempt towards fulfilment of this goal. We have tried to put into use the antioxidant role of vitamin E in controlling the degenerative process of spondylosis. Furthermore, we have also used vitamin A for its stability effect[10].

## II. LITERATURE REVIEW

Spondylosis seems to be a slow involutional process of wear ane tear due to constant weight bearing through out the years. The intervertebral discs sandwiched in between the vertebraes eachconsists of an outer ring of fibrcartilage (the anulus fibrosus) which encloses a central mass, the mucleus pulposus[11]. The nucleus pulposus consitsts of 70-80 percent water and its semifluid nature[12] is important in converting the vertical forces to which the annulus is subjected into radial forces, and allow deformation due to pressure during movement and weight bearing.

The structural molecule of the mucleus pulposus is made of proteoglycans synthesized by chrondocytes. These proteoglycans are complex protein molecules to which oligosaccharide chains are covalently attached to the polypeptide backbone[13]. The proteoglycan molecule exert an osmotic pressure of several atmospheres against it and is extremely important in maintaining the machanical function of the intervertebral disc.

Age and chondrocyte injury due to years of wear and tear slows down the capacity of the chorondocytes to produce proteoglycans[14]. Also degradative enzymes causes decrease in size of the proteoglycans. Furthermore the proteoglycans also lose their ability to form aggregates[15]. This may lead to dehydration of the nucleus pulposus. Degeneration of the nucleus pulposus is charactersed by the appearance of clefts due to fibres since it loses its water content and becomes moe fibro cartilaginous. These clefts spread into the annulus fibrosus and cause separation of the concentric lamellae[16]. This is likely to alter the mechanical characteristics of the intervertebral disc. The overall effect cause reduction in disc thickness. So there is weakness of the annulus fibrosus which bulges into the spinal cord. Herniation of the intervertebral disc is followed by osteoblastic reparative activity causing bony spurs from opposing bones coming in contact leading to stiff neck and pain of the affected part[1]. Compression of the spinal cord and impairment of its circulation by these bony overgrowth may cause numbness, weakness or muscular atropy.[17]

The degenerative reactions associated with ageing is already known to be due to free radical chain reaction[18]. The proteoglycans of the intervertebral discs are

also subject to these radiolytic damages. The hydroxy radicals causes oxidation of the proline residues [8] of the polypeptide core of the proteoglycans. This leads to their degradation.

As such many systems of the body would have undergone degeneration had these not been protected by antioxidants.

Such an antioxidant was discovered in 1936 as vitamin E or tocopherol. It acts as an antioxidant by virtue of its chain breaking action of the degeneratige process[19]. Probably it is the only chain breaking (i.e. peroxyl trapping) antioxidant in the human blood[20].

In a normal cell superoxide interacts with hydrogen to produce hydrogen peroxide during metabolism. Tocopherol[21] located in the cell membrane interacts with the peroxides to provide defense against these oxidative damages and removing the free radicals.

It was observed by Leonard et al[22], who studied 8 vitamin E deficient subjects. that vitamin E by virtue of its antioxidant role prevents haemolysis of RBC.

A study in Japan by Yoskikawa et al[23] done on female rats with induced arthritis were found to be significantly protected by a dietary supplement of vitamin E. This study accords with our study since arthritis is a disease pathologically and clinically similar to spondylosis.

During the late 1940's attention has been focused to retrolental fibroplasia of premature infants, where there is proliferation of reatinal velssels of the eye with fibroplasia and multiple haemorrhage in the vitreous body leading to separation of retina[24]. There is also bronchial fibroplasia of these infants. Since high oxygen tension is the main cause, low levels of the natural antioxidant tocopherol in the neonatal period might predispose to the disorder. If these children are supplied with exogenous vitamin E[25], then there is no such development due to the antioxidant role of vitamin E.

Exposure to high concentration of oxygen, ozone, nitrogen dioxide[26] and other gases results in oxidative damage both in the water soluble phase and membranous part of the alveolar cells of the lungs. These extensive pulmonary damages appear to be mediated by free radical formation but the lungs have biochemical defenses. Tocopherol[27] stationed in the membranes of the cell

there fore appears to be amongst the most important defensive systems against oxidative damage to the lungs. According to research at the University of California at Davis, USA these oxidative components are found in city smog[28]. Tocopherol may also protect the lungs from air pollutants.

Vitamin E deficient diet was seen to decrease hepatic DNA activity[29] found in the liver cells. The DNA damage was corrielated to other biochemical and physiological changes characteristic of cellular damage in aging and disease.

Aging is a very slow compolex process in which free radicals are formed in the various thssues and organs. These free radicals results in lipid peroxidation causing membrane damage. In fact the accumulation of the pigment lipofuscin[30] in the aging heart and liver proves the presence of free radical in these tissues. Theoritically[31] aging therefore might occur due to (1) continuous increased formation of free radicals and (2) diminished availability of antioxidants. So it has been suggested that since vitamin E as an antioxidant interacts with and neutralizes free redicals, it may perform[32] the same function in the body andslow the aging process.

That vitamin A may play a role in ensuring the stability of cell membranes[33,34] was first indicated in the early 1920's. Also vitamin A is believed to be essential for the maintenance of normal cellular membrane structure and function. The structure and stability of erythrocyte[35] membrane was seen to be markedly altered in vitamin A deficient rats.

It was also observed that if Vitamin A was not available at the time of assembly of a cell or its membrane, assembly may not occur or a defective cellular structure may result[35].

## A. HISTOLOGY OF THE VERTEBRAL COLUMN:

Throughout the spine, weight bearing compresive forces are largely supported by one set of articulations-the intervertebral disc. The intervertebral discs are elastic organs composed of collagen fibres produced by chondrocytes, embedded in matrix or ground substance[36]. Each collagen macromolecule consists of three tropocollagen fibre or alpha chains each of approximately 1,000 amino acid sequences rapped together in an alpha helix structure. These helical molecules are held in rigid rod like form[37]. The tropocollagen molecules are cross linked into the extracellular matrix to from fibrils which in turn forms fibres[38]. These fibres are then arranged into a three dimentional lattice.

The chondrocytes, also produce the ground substance, the proteoglycans in which the nucleus pulposus of the intervertebral discs are very rich. 38

The proteoglycans are large complex molecules consisting of chains of sulphated glycosamino glycan polymers, namely, chondroitin sulphate and keratin sulphate, atached to a central protein core[13].

The function of the intervertebral disc depends on the properties of the somidehydrated proteoglycan molecules which exert an asmotic pressure of several atmospheres against the collagen network that retains it.

These proteoglycans are not only extremely important in maintaining the mechanical function of the disc but also in influencing the nutrient and metabolite trnsport [39].

## B. PATHOGENESIS IN SPONDYLOSIS:

Owing to man's upright position intervertebral discs are subjected to constant strain for which they were not originally intended so that degeneration in the discs is commoner than in any other organ with age. The commonest outcome associated with ageing and the intervertebral disc degeneration is the reduction in the ability to cope with in mechanical stress. The exact cause is yet to be known. It seems to be a slow involutional process due to the mechanical effects of trauma of wear and tear and weight bearing through out the years.

The nucleus pulposus of the intervertebral disc[14] has a high water content which decreases rapidly after the fortieth year and this loss is associated with degenerative change in the central portion of the disc. The progressive dessication and decrease of the protein mucopolysaccharide complex of the nucleus pulpsus encourages disc, degeneration as age inceases. Degeneration of the nucleus pulposus is characterised by a loss of resilience, apparent dessiction and fibrosis and the appearance of clefts which spreads at right angle to the surface[40]. These clefts may spread into the annulus fibrosus, producing what is referred to as cartilage fibrillation and cause separation of the concentic lamellae[16].

The dehydration of the nucleuas pulposus seems to indicate that changes in the proteoglycans play a major role in disc degeneration.

Side by side ageing and chondrocyte inury slows down the capacity of the chondrocyte to synthesize proteoglycans[14]. Since chondrocyte inury leads to the production of degradative enzymes like proteoglycanases and cathepsin. As such there is decreas in the over all size of the proteoglycans due to decrease in the chain length. It also appears likely that there is an increase in the interaction between proteoglycans and collagen with age[15]. This phenomenon diminishes the ability of the proteoglycans to form aggregates, which in tuern is likely to alter the hydration and mechanical characteristic of the disc.

The proportion of keratin sulphate increases while the water, hexosamine, chondroitin sulphate, ash and sialoprotein content decreases[41]. The collagen fibrils left unsupported becomes more vulnerable to the mechanical effects of the trauma of wear and tear[42]. The overall mechanism causes reduction in disc

thickness.The resulting narrowing of the disc space causes bulging and weakness of the annulus fibrosus, so that the disc tends to protude posteriorly into the spinal cord[43]. There is osteoblastic reparative activity following this herniation of the disc resulting in the joint[44]. They increase the available articular surface and may be compensatory in character.

These may form a bony bar or spondylotic ridge and enlarge backwards[42]. Large spurs may project from apposing bones, coming in contact with one another causing pain and limitation of motion[41]. The osteophytes may encroach upon the spinal canal, compress the spinal cord and adjacent nerve roots and the vertebral and segmental spinal arteries in the transverse and the intervertebral foramina [42].

There seems to be a vicious circle of changing mechanical conditions and attempts at structural adaptations [45]. Ultimately however permanent degeneration occurs causing the development of spondylosis.

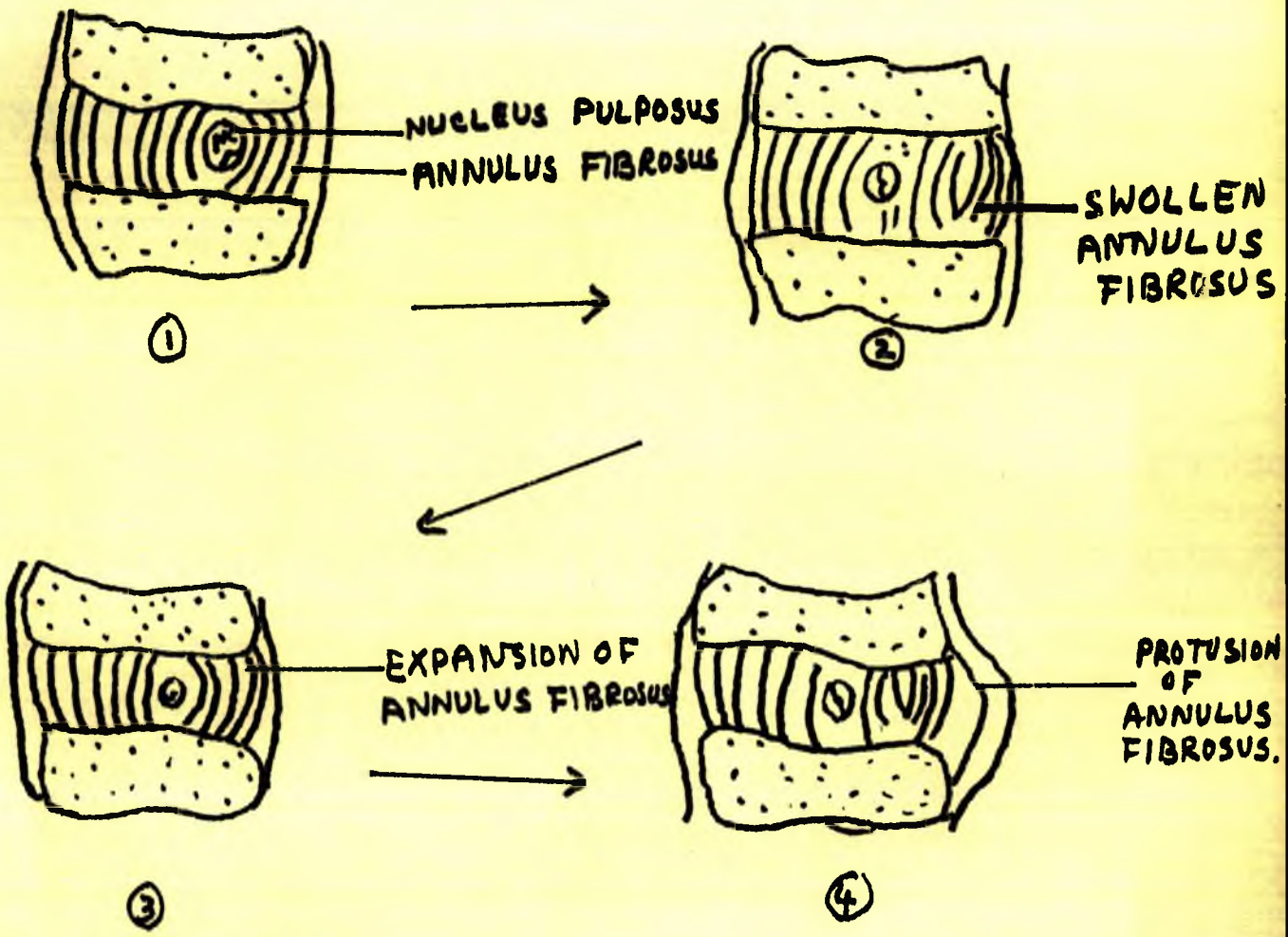



FIGURE 1 : PATHOLOGICL MECHANISM OF HERNIATION AND PROTUSION OF ANNULUS FIBROSUS OF INTERVERTEBRAL DISC.

## C. CLINICAL FEATURES

The cardinal clinical feature of spondylosis is pain of the affected part, which early in the disease occuers with motion and is relieved by rest. As the disease progresses the pain may occur with minimal motion and also at rest. It may often be troublesome at night, awaken the individual from sleep and is of aching quality and poorly localised. It may lead to protective reflex muscle spasm, which in turn causes further pain and may result in abnormal posture and limitation of movement. Stiffness for several minutes after inactivity and on awakening may occassionally occur.

The clinical features of spondylosis is much clearly understood if it is divided into the regions affected.

1. Cervical Region

A. Pain

Majority of the patients do not complain of neck pain or limitaion of neck movement. However some may experience dull, vague aching pain in the neck and crepitus when the neck is rotated.

Normally, the diameter of the spenal canal averages about 17 mm in the cervical area[41]. The protuding degenerated disc and the secondary osteophytes form a bar of calcified or fibrous tissue which narrows the diameter of the canal.

Patients with cervical spondylosis have been seen to have narrow spinal canals. When the diameter of the canal is decreased to 10-11 mm[46], then the spinal cord is likely to e compressed.

The cord compression may be further caused by certain other additional factors. The normally thin dentate ligaments which pass between the dura mater and the wall of the spinal canal may become anchored within the canal, rendering it susceptible to pressure during movement of the neck causing pain.

Narrowing of the disc spaces and shortening of the cervical spine produces malalignment of the vertebral bodies and inter peduncular joints., This further contributes to disc degneration, osteophyte formation and narrowing of the

spinal canal. Due to the osteoarthritic changes in the joints, pain arises in the neck and the patient complains of head ache. This leads to reduced range of movement accompanied by reflex muscle spasm[17].

Normally the nerve root occupies about one fourth of the intervertebral occupies about one fourth of the intervertebral foramina. Due to lateral encroachment of the digenerated discs and osteophytes, the forman becomes narrowed and the ane ve root becomes thickened by fibrosis. Thus the mobility of the nerve root is reduced increasing its susceptibility to compression. Such compression may easily produce wallerian degeneration in the posterior and lateral columns and loss of neurons in the grey matter of the anterior horns[47]. The pain which arises due to nerve root compression suddenly or insiduosly is often severe in nature.

Nerve root irritation may result from cervical disc protection or forminal encroachment by osteophytes. The area affected is in the distribution[48] of tne dermatomes supplied by the affected nerve root causing hyperaesthesia and hyperalgesia. When the sixth cervical root is irritated the pain ccurs in the neck and radiates down the anterior chest wall, shoulder, down the upper arm and forarm to the hand and index finger.

Further compression on th nerves causes a variable amount of wasting of the small muscles of the hand and forearm, [49] along with dyseastheise and paraesthsie. The deep tender reflexes (biceps, triceps) innervated by these nerve roots are absent or depressed.

A number of patients complain of sub occipital headaches. [17] Other symptoms such as vertigo and drop attack [50] may be precipitated along with rotation of the neck or its extension. These are symptoms of vertebral basilar arterial insufficiency. The vertebral and segmental ateries supplying the spinal cord passes though the transverse and intervertebral foramina. These may become compressed by the formation of secondary orteophytes specially when the head is rotated. Furthermore there may be kinking or obstruction of these arteries which may result in infarction of the spinal cord and even of the brain stem.

2. Dorsal Region

The dorsal spine is also a common site of spondylosis but its affection rarely produces symptoms. Occasionally there may be localised pain in the dorsal region due of osteoarthritic changes in the intervertebral discs. This may be accompanied with radiation of the pain to the appropriate intercostal space[51]. The pain may be exacerbated by percussion or palpation of the individually affected area. The pain may also be felt by rotating the upper part of the truck in either direction.

3. The lumber region:

The lumber spine is also predisposed to spondylotic changes. The degenerative changes[52] that arise may be part of the generalized disorder associcated with advancing age or it may result from other disc lesions acquired earlier in life.

Severe backche is the usual presenting symptom[53]. There is flattrening of the normal lumbar curve, at the level of the prolapsed disc, so that the patient cannot stand errect. There is also reduction in the range of spinal movements in all directions (flexion, extention, lateral bending, rotation)[54].

Due to sponldylotic involvement of the lumber 4 nerve root, there may be radiation of pain to the anterior part of the thigh and the sciatic nerve resulting in sciatica. Compression of the lumber 4-5 and sacral nerve root by acutely prolapsed disc result in pain in thegroin and anterior thigh along with absent knee jerk[55].

There may be weakness and spastic paralisis of the lower limbs. Involvement of the spinothalmic tracts leads to disturbace of pain and thermal sensation in lower limbs[17]. There is also development of increased deep tendon reoflexes, ankle clonus and bilateral extensor plantar reaponses. The gait may be spastic and ataxic[48]. Walking or standing immobile for hours together or leaning backward tends to precipitate the symptoms.

## D. ROENTOGRAPHIC FEATURES:

The use of roentographs is so self evidently important that its limitations are often over looked. However it still remains the conventional screening investigation for spondylosis despite its limitation.

To make an adequate diagnosis and evalution of the extent of the spondylosis, it is better to take anteroposterior and lateral views of the affected part in the position of flexion and extension. It is even better to supplement it by two obligue views (right and left) of the vertebral column. These films permit adequate visualizsation of the disc spaces, measurments of the anteroposterior diameter of the spinal canal and evaluation of osterophyte formation on the vertebral bodies and around the interpeduncular joints.

Due to destruction of the articular cartilage the redioluscent interosseous joint spaces can usually be seen to be narrower than normal. Degeneration of the intervertebral discs result in narrowing of the spaces in between the vertebral bodies. A vaccum sign or marked trnaslucency in the intervestbrel disc may be seen. Evidence for disc degeneration is usually documented in [56] the anteroposterior and lateral roentgenogram.

The oblique views are of particular importances for visualization and assessment of the degree of encroachment in the Intervertebral foramina by the formation of the osteophytes. Oblique views are also valuable for defining bony sclerosis and joint spaces and narrowing of the spine[57].

The distance from the target site shoud not be more then 85cm, ray given in 60-65 KB and 25-30 MAS. The exposure time is 5 seconds for the cervical spine and 7-11 seconds for the spinal coulmn [58].

## E. CLINICAL TREATMENT

Uptil now prevention or life long relief from spondylosis has not been found.

Patients who have sudden onset or exacerbation of pain may benefit somewhat from medical therepy.

During the acute phase, hed rest and adequate use analgesies may help. Diazepam (5 mg thrice daily) may be given as a muscle relaxant. For treatment of pain and spasm indomethacin (25mg twice daily) is often effective.

When the pain is controlled traction[7] may be given with the patient in a prone position. The initial period should not exceed 15 minutes and the initial weight not more than 10 lbs.

If there is no obvious benefit from traction withing a few days, it should be discontinued and a neck collar may be substitued. Certain types of physical therepy like short wave diathermy, infra red radiation and massage are useful in reducing muscle spasm and alleviating pain. A course of active exercises designed to strengthen the muscles of the affected part may bebeneficial to some patients.

Partients who have persistent and disabling symptoms of nerve root compression or cord compression or arteraial insufficiencey may also require surgery [59]. Nerve root compression may be treated by tbe anterior approach, with removal of degnerated disc material and the osteophytes encroaching on the intervertebral foramina, spinal cord compression is best treated by a wide laminectomy and the dentate ligaments are sectioned to give the cord mobility.

The postoperative course is short since immobiliazation and bed rest are unnecessary and the patient may begin walking the day after the surgery.

Even so, many patients are not relieved after such treatment and tend to suffer throughout life.

## F. AUTOOXIDANTS AND ANTIOXIDANTS

It is now widely recognised that the basic mechanism in degenerative reactions associatied with ageing is free radical chain reactions[18].

Free radicals can be defined as atoms or groups of atoms having an odd (unpaired) number of electrons that may enter into chemical bond formation. These may be positively charged, neagatively charged or neutral. They are potentially extremely reactive and chemically unstable and usually occur in low concentration. They generally induce chain reaction that occur slowly under normal conditions but may be rapidly accelerated if chain initiators other free radicals are introduced.

(1) Initiation $RH \longrightarrow R'$

$R' + O_2 \longrightarrow ROO'$

(2) Propagation $ROO' + RH \longrightarrow R' + ROOH$

Normal cellular metabolism produces superoxide and bydrogen peroxide which reacts with each other to produce hydroxyl free radicles (OH). A continuous supply of free radicals are provided which interacts with oxygen during oxidation-reduction reactions[60].

Free radical propagation occurs principally through atom transfer or addition reaction.

In an unprotected cell these free radicals produced, attack the membrane lipids at the double bonds of the polyunsatrurated fatty acids to form free radical intermediates and semi stable lipid peroxides. This propagates a chain reaction with the formation of lipid hydroperoxides OH that result in membrane damage and the production of terminal aldehydes. Free radicals can also catalyze amino acid oxidation, protein-protein cross linking and protein strand scission.

The intervertebral disc of the vertebral column in susceptible to these sort of free redical or radiolytic damage. It is the polypeptide[8] core which appears tobe more susceptible than the glycosaminoglycan. causing damage to the proteoglycans. The OH radical is said to have a 100 fold greater reactivity with proteins than with polysaccharides. The hydroxy radicals mediate protein

damage in a nonrandom fashion, possibly due to oxidation of its proline resdues.

Protein rich in proline such as the collagen of the proteoglycans of the cartilage matrix gove rise to many fragments of defined length after exposure to OH.

Less potent oxidants than OH produce subtler modification to protein but increase the susceptibility of the proteins to hydrolysis.[18] Other than that well defined inflammatory changes are also sometimes seen in the early stages of the disease.[18]

Antioxidants may be defined as substance which reduce or altogether prevent auto-oxidation in the body. It falls in two classes.[13]

1. Primary or preventive antioxidant which reduce the rate of chain initiation that is formation of new free radicals. For example catalase and other peroxidasew.

2. Secondary or chain breaking antioxidants, which interfere with chain propagation by trapping down the highly reactive peroxyl free radicals.

In vivo the principal chain breaking antioxidants are superoxide dismutase and vitamin E.[61]

The tocopherols act as chain breaking antioxidants as a result of their ability to transfer a phenolic hydrogen to phenoxyradical[19].

## G. VITAMIN " E "

### 1. History

Vitamin E was discovered in 1922 when Evans and his colleague Bishop, [62] while experimenting on the effect of nutrition on rat reproduction, discovered that female rats even though ovulating and conceiving normally, suffered foetal death and resorption, when fed on a rancid lard diet. The situation could be prevented on feeding either lettuce or whole wheat. Subsequently it was found that wheat germ oil contained the factor. The unknown substance was designated vitamin E by B. Sure in 1924. During the 1922 to 1924 period, Mattilland and his collaborators Carman and Clayton were also engaged in work on this new substance, so the vitamin nature of his new compound was readily accepted long before the vitamin discovering era reached its zenith.

In 1936. Evans, Emerson, and Emerson[63] finally isolated pure vitamin E and named it tocopherol after the greek word `tocos' which means child birth and the verb pherein meaning to bring forth. The 'ol' was added to indicate the alcohol nature of the factor.

In 1938, Fernholz established the structure of tocopherol and shortly after that Karrer of Switzerland and Smith of USA first synthesized $\alpha$ tocopherol.

Olcott and Emerson [9] in 1936 nad discovered the antioxidant properties of tocopherol but the attracti veness of the action of vitamin E on the process of repro duction somewhat overwhelmed the significance of this study and for many years assays for vitamin E were largely related to repro ductive organs functions. Now the full significance of the function of tocopherol as an antioxidant, which can protect the integrity of compounds in the tissues has been moreclearly understood.

### 2. Chemistry

Vitamin E is a generic term for a group of lipid soluble compounds, the tocopherol and to cotrienols that possess varying degrees of vitamin activity.

The multiple nnture of this vitamin was first noted in 1936 [64] by Evans et al, when they first isolated a tocopherol and β. tocopherol from wheat germ oil.

Two distinct classes of compounds comprise the vitamin E group. All are isoprenoid subtituted 6 hydroxychromanes ring structure and a side chain, The first series-the tocopherols derive from tocol, which contains a 16 carbon saturated isoprenoid side chain The second series, the toco trienols have a similar structure with a triple unsaturated side chain with double bonds at 3. 7 and 11 position of the side chain.

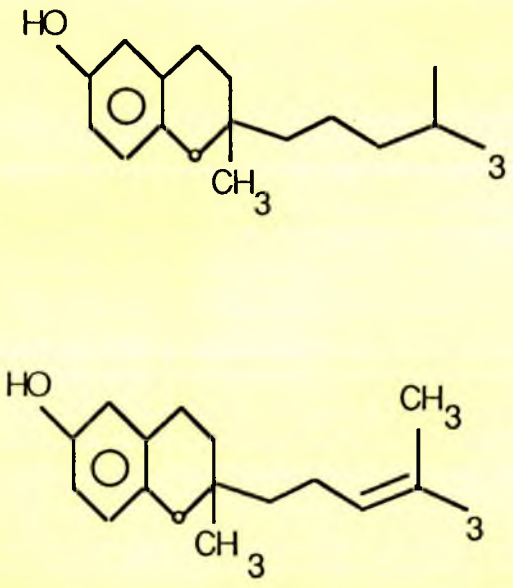



Fig. 2. Structures of tocopherols and tocotrienols

Within each series the campounds differ only in number and position of metryl groups in the ring struchure, The ring structure of the corresponding tocotrienol is similiar to the tocφpherols designated α. β. γ. δ.

TABLE 1.

| Tocols | Tocotrienols | $CH_3$ Substitution |
|---|---|---|
| $R_1$= ($CH_2$ - $CH_2$ - CH- $CH_2$) $_3$ H | | $R_2$=($CH_2$ - CH- C-$CH_2$) $_3$H |
| αT($R_1$- $R_2$=$CH_3$) | αT- $_3$($C_1$=$R_2$=$CH_3$) | 5,7,8 Trimethyl |
| βT ($R_1$=$CH_3R_2$=H) | βT- 3($R_1$=$CH_3$, $R_2$=H) | 5,8 Dimethyl |
| γT($R_1$=H, $R_2$=$CH_3$) | γT- 3 ($R_1$=$H_3$, $R_2$=$CH_3$) | 7,8 Dimethyl |
| δT ($R_1$=$R_2$=H ) | δ-T- 3 ($R_1$=$R_2$=H) | 8-Methyl |

Thus there there are four tocopherols and four tocotrienols occuring in nature out of which alpha tocopherol is the most widely distributed in nature and is the most active biologically of the compounds.

3. Food Sources

Even though tocopherol is widely distributed in nature, they are not very stable and there are often signifcant losses from food during storage and during cooking [65] For example there is 48% loss of tocopherol in potato chips after storage for two weeks at room temperature, Losses of natural tocopherol during storage of vegetable oils are usually minimal, but during cooking can be appreciable. The vitamin E content of vegetable oils and fats has been reported to vary according to source of the plant, time of harvest, stability after harvest and refining procedure[66]. Losses also occur during food processing, particularly if there is any significant exposure to heat and oxygen.

So to know the precise value for vitamin E intake, analytical assay of all foods just prior to ingestion has to be done.

Wheat germ and wheat germ oil offer the richest source of these factor, but it is also widely distributed in nature. All other vegetable oils, like sunflower seed oil, cotton seed oil, palm oil, rapeseed oil, coconut oil etc. are rich source of vitamin E.

TABLE 2.

Tocopherol Content mg/100gm of Food [67, 68, 69]

| Vegetable Oils | Total Vit. E | αTocopherol | αTocotrienol |
|---|---|---|---|
| Wheat germ oil | 254.58 | 149.44 | |
| Sunflower seed oil | 63.62 | 59.50 | |
| Cotton seed oil | 65.24 | 35.26 | |
| Palm oil | 35.53 | 18.32 | 11.46 |
| Rape seed oil | 44.81 | 17.65 | |
| Coconut oil | 3.58 | 0.35 | 1.29 |
| Olive oil | 12.64 | 11.92 | |
| Soyabean oil | 93.74 | 10.99 | |
| Rye | 192.11 | 71.42 | |
| | | | |
| | | | |
| | | | |
| Bean | | 0.05 | |
| Lentils | 0.07 | | |
| Peas | 2.71 | | |
| | | | |
| | | | |
| | | | |

| | | | |
|---|---|---|---|
| Fruits | | | |
| Apple | 0.66 | 0.59 | |
| Banana | 0.32 | 0.27 | |
| Mango | | 1.12 | |
| Pineapple | 0.10 | 0.10 | |
| Orange | 0.24 | 0.24 | |
| Tomato | 0.49 | 0.34 | |
| | | | |
| Cereal grains | | | |
| | | | |
| Rice Brown | 2.04 | 0.68 | |
| White | 0.39 | 0.11 | 0.05 |
| Flour Dark | 6.68 | 1.41 | 2.89 |
| Light | 0.93 | 0.43 | 0.17 |
| Puffed rice | 2.15 | 0.36 | |
| Shredded rice | 4.05 | 1.06 | |
| | | | |
| Nuts | | | |
| Cocount | | 0.7 | |
| Cashew nut | 4.20 | 0.19 | |
| Peanut | 16.37 | 8.33 | |
| Sesame | 11.0 | 1.8 | |

| | | |
|---|---|---|
| Vegetables | | |
| Beet | | 0.03 |
| Cabbage | 1.67 | 1.67 |
| Carrot | 0.13 | 0.12 |
| Cauliflowers | 0.09 | 0.03 |
| Cucumber | 0.31 | 0.15 |
| Egg plant | | 0.03 |
| Garlic | | 0.01 |
| Lettuce | 0.75 | 0.40 |
| Mint | | 5.00 |
| Onion | 0.31 | 0.12 |
| Potatoe | 0.07 | 0.06 |
| Sweet Potatoe | 4.60 | 4.56 |
| Pumpkin | | 1.02 |
| Radish | 3.76 | 3.06 |
| Spinach | | 0.21 |
| Squash | | 0.02 |
| Turnip | | 0.03 |
| Animal Products | | |
| Beef | 0.43 | 0.41 |
| Mutton | 0.46 | 0.43 |
| Liver (Cow) | 0.67 | 0.67 |

| | | |
|---|---|---|
| Chicken | 0.34 | 0.29 |
| Liver (Chicken) | 1.44 | |
| Duck | 2.80 | |
| Pigeon | | |
| Egg (Chicken) | 1.06 | 0.70 |
| | | |
| Dairy Products | | |
| | | |
| Butter | 2.40 | 2.40 |
| Cheese | 0.63 | |
| Milk (Cow) | 0.90 | |
| | | |
| Fish | | |
| | | |
| Carp | 0.31 | 0.63 |
| Cod | | 0.23 |
| Herring | 2.00 | 2.00 |
| Mackerol (Liver) | 5.97 | |
| Mackerol | 1.2 | 1.52 |
| Lobster | | 1.47 |
| Prawn | | 2.85 |
| | | |

| Processed Food | | |
|---|---|---|
| Bread | 1.19 | 0.01 |
| Biscuit | 2.48 | |
| Cake | 7.27 | |
| Candy | 0.48 | |
| Coffee | 0.48 | |

Improved analytical techniques in the past decade not only have resulted in more accurate determination of the tocopherol content of foods but have also permitted a more complete description of the content of other tocopherols and tocotrienols.

Relatively high intakes of vitamin E are possible only when the dietary fat derived from vegetable oils is abundant. The tocopherol content of most beans and seed oils (soybean, corn, peanut etc.) is much higher than that of animal fats.

So it is very much probable that many diets throughout the world which are low in vegetable fats and oil contain less than 5 mg of tocopherol (17.5 IU). The most typical example is the vitamin E content of typical rural diet in Bangladesh[70], where 400-500 gm of polished rice is the daily staple food. This indicates at most 3.5-4 mg of tocopherol. In many of these low income population, fat intake is very low, 10-15% 0f total calorie. Vitamin E there fore mostly comes from the staple grain.

This means that vitamin E intake is not only determined by geographical consideration but also by economic factors , since vegetable oils are usually an expensive dietary component for low income group, especially in developing countries.

4. Metabolism

a. Absorption

Like other fat soluble vitamins the relative absorption of tocopherol and its esters is only 20-40% depending on the animals ability to digest and absorb fat. Tocopheryl esters are hydrolyzed in the gut lumen prior to absorption. The intestinal absorption of vitamin E follows the pattern for fat and other fat soluble nutrition of vitamin E follows the pattern for fat and other fat soluble nutrients, reaching the blood as chylomicrons through the lymph[71] The percentage of dietary vitamin E absorped seems to be relatively constant over a narrow range of normal dietary intakes and it dcreases with inceasing dose from high intakes of tocopherol.

The predominant route of absorption is the lymph, in which 45% appears almost enirely as unchanged tocopherol. Only 10% is absorped via the poortal vein.

b. Transport

Most tocopherol enters the blood stream via lymph where it is associated with chylomicrons and very low density lipoproteins[72]. There is no evidence of a specific transport protein. Upon entering the blood, the tocopherol in chylomicrons rapidly equilibrates with the other plasma lipoproteins. Most of it is transported in the lipoprotein fraction of blood.

There is positive corelation between total plasma lipid and plasma tocopherol in both children and adults. Normal plasma tocopherol values in adults range from 0.5 mg to 1.6 mg per kl. Some investigatorws prefer measurment of vitamin E adequacy in the ratio of plasma tocopherol (mg) to total lipids (g); a ratio of 0.8 or greater is considered normal[25]. New born infants tend to have low plasma level of vitamin E ($\frac{1}{3}$ rd of of adult level) due not noly to lower concentration and also to limited placental trnsfer. It begins to rise after brith, more in breast fed infants and reaches normal concentration by about one month ot age[73].

c. Storage

Circulating vitamin E is readily taken up from plasma lipoprotein by crythrocytes and other body tissues, maximum concentration reaching 4-8 hours after intake. The uptake of tocopherol into tissues varies directly with the logarithm of the tocopherol intake over a period of timel Adipose tissue differs however in that it continually accumulates tocopherol. So excessive adiposity can result in reduced concentratiolns of tocopherol in some tissues even though blood levelare high. The adrenal and pituitary glands, testis and platelets have the highest concetration of the vitamin. The adipose tissue, liver and muscle represent the major storage depots of the vitamin[74].

TABLE 3.

Tocopherol contents of human tissues[74]

| | ug/g | mg/g lipid |
|---|---|---|
| 1. Plasma | 9.5 | 1.4 |
| 2. Erythrocytes | 2.3 | 0.5 |
| 3. Platelets | 30.0 | 1.3 |
| 4. Adipose tissue | 150.0 | 0.2 |
| 5. Kidney | 7.0 | 0.3 |
| 6. Liver | 13.0 | 0.3 |
| 7. Muscle | 19.0 | 0.4 |
| 8. Ovary | 11.0 | 0.6 |
| 9. Uterus | 9.0 | 0.7 |
| 10. Heart | 20.0 | 0.7 |
| 11. Adrenal | 132.0 | 0.7 |
| 12. Test's | 40.0 | 1.0 |
| 13. Pituitary | 40.0 | 2.0 |

The mitochondria comprise the richest subcellular fraction. It appears to be concentrated in the phospholipid of the cellular and subcellular protion.

TABLE 4.

Subcellular distribution of Tocopherol ug/mg protein

| Cell fraction | Liver | Heart |
|---|---|---|
| Soluble | 0.002 | 0.02 |
| Mitochondria | 0.17 | 0.27 |
| Microsome | 0.08 | 0.37 |
| Nucleus | 0.03 | 0.25 |

The rate of depletion of tocopherol upon withdrwal from the diet varies considerably from tissue to tissue, being relatively rapid in plasma and liver, slower from skeletal asnd heart muscle, and very slow from adipose tissue.

d. Excretion

The major route of escretion is through bile into faeces as unaltered vitamin E. Its oxidative products are tocoquinone and tocohydroquinone[75]. Some oxidative products are also excreted in the urine.

5. Deficiency

Vitamin E deficiency state may be produce in human with severely impaired intestinal fat absorption. The signs of vitamin E dificiency in humans are muscular weakness, creatinuria and fragile crythrocytes[76]. All disappear after administration of tocopherol. So vitamin E dificiency not only causes pathology in the vascular and nervous system but also results in pathology in the adipose tissue, liver and testes.

TABLE 5.

EFFECTS OF VITAMIN E DEFICIENCY

| TISSUE | OBSERVATIONS | SPECIES |
|---|---|---|
| 1 Skeletal muscle | Myopathy | Monkey, pig, rat, rabbit, chicken, duck, horse, calf |
| 2 Heart muscle | Myopathy | Pig, rat, cow, sheep, goat |
| 3 Uterine muscle | Liporfuscin Accumulation | Rat |
| 4 Blood vessels | Foetal death and resorption | Pig, rat, mouse, cow, chicken |
| 5 Erythrocytes | Anamia, haemolyse, shortened life span. | Men, monkey, rat |
| 6 Platelets | Increased number | Man, rat |
| 7 Brain | Encepheloimalacin | Chicken |
| 8 Nerves | Axonal dystrophy | Rat |
| 9 Adipose tissue | Liposfuscin accumulation | Pig, rat, mouse |
| 10 Liver | Neurosis | Pig, rat, mouse |
| 11 Testis | Atrophy | Monkey, rat, pig, rabbit, chicken. |

There are some special conditions in which adequate vitamin E status may develop. This has been clearly demostrated in premature infants, who have low plasma vitamin E level because of its poor transfer across the placental barrier

and immaturity of the intestine reduces the absorption of dietary vitamin E. As a result vitamin E deficiency syndrome characterized by haemolytic anaemia, thrombocytopenia, oedema and skin lesions may develop[77].

Vitamin E supplementation in premature infants has been reported to decrease retrolental fibroplasia and chronic bronchopulmonary dysplasia[78].

Adequate vitamin E may be important in protecting the luntgs against oxidative damage in patients receiving pure oxygen and against damage by air pollutants, such as O zone ($O_3$) and nitrous oxide (NO)[79].

Patients with cystic fibrosis and other conditions secondary to intestinal malabsorption, showing a lowered plasma vitamin E and decreased RBC survival time, has been seen to correct all abnormalities with tocopherol acetate or vitamin E supplementation promptly reverses the condition.

6. Normal blood tocopherol level

TABLE 6.

NORMAL BLOOD TOCOPHEROL LEVEL[80]

| | | mg/dl | μ mol/L |
|---|---|---|---|
| 1 | Adults | 0.95 | or 22.00 |
| 2 | Adolescent | 0.86 | or 20.00 |
| 3 | Children 2-12 years | 0.76 | or 17.65 |
| 4 | Ihfants | 0.40 | or 09.29 |
| 5 | Premature infants | 0.26 | or 06.04 |
| 6 | Children with PEM | 0.45 | or 10.45 |
| 7 | Patients with cystic firosis | 0.23 | or 05.34 |

7. Human Requirement

Most studies of requirments of vitamin E for mammals have been conducted on small animals with short life span. When vitamin E has been removed from the diets of such animals, the various, severe pathological reactions obtained have presented such a confusing picture that it has been difficult to relate these multiple disorders to human beings. It has been seen that vitamin E is required for the maintenance of the structural and functional integrity of endocrine tissue, skeletal muscle, cardiac muscle, smooth muscle and the peripheral vascular system of growing animals. Furthermore there are accumulations of lipofuscin products in man's muscle and brain cells, which tend to increase with age.

Since the complete removal of vitamin E from the diet has produced severe and often irreversable pathology in all animals that has been investigated, it would not be ethically proper to study such study on the human being.

One long term study was sponsored at Elgin, Illinois,. USA by the Food and Nutrition Board of the N. R. C. where adult human subjects were fet a partially dificient diet, providing 5 IU of vitamin E, for more than five years[80].

From the data on rates of depletion and repletion recorded during the Elgin studies, an esrtimate of human requirements ofr vitamin E was obtained.

a. Recommended daily allowance

i. Adults

It is generally accepted that a dietary intake of vitamin E that maintained a blood concentration of above 0.5 mg/dl will ensure adequate concentration in all tissues.

However the adequacy of these intakes will vary if the polyunsaturated fatty acid content of the diet deviates significantly from the customary. Therefore the ratio of tocopherals to PUFA in tissues should be such that the normal physiological function is permitted.

Even though the daily intaskes may very considerably, the average over several days should fall between 7 mg and 13 mg of a tocopherols equivalents (10-20 IU) from diets supplying 1800 K cal to 3000 K cal. The recommended allowance

for boys and men is 10 mg TE (15 IU) beginning at 15 years and 8 mg TE (12 IU) for girls and women beginning at 11 years.

ii. Children

Vitamin E allowances for children increase

with increasing body weight being about 7-12 IU.

iii. Infants

The blood tocopherol level of breast fed infants rises from 0.37 mg/dl to 0.72 mg/dl, which is the adult level within two to three weeks. Therefore the vitamin E content of human milk, 2-5 IU/L is enough to provide an adqate intake for nursing infants up to the first year. The RDA for infants is therefore 4-5 IU days.

The adult female allowance for vitamin E is increased to about 10 mg TE (15 IU) during pregnancy and to 11 mg TE during lactation.

TABLE 7.

RECOMENDED DIETARY ALLOWANCES RDA

| | AGE YEARS | WEIGHT Kg | VITAMIN E IU | TE mg |
|---|---|---|---|---|
| 1 Infants | 0-0.5 | 6 | 4 | 2.5 |
| 2 Children | 1-3 | 9 | 5 | 3.5 |
| | 4-6 | 13 | 7 | 4.5 |
| | 7-10 | 30 | 10 | 6-5 |
| | 11-14 | 44 | 12 | 8 |
| 3 Adolescent | 15 | 70 | 15 | 10 |
| and adult | | | | |
| male | | | | |
| 4 Adolescent | 15 | 58 | 12 | 8 |
| and adult | | | | |
| female | | | | |
| Pregnant and | | | | |
| lactating | | | | |
| female | | | 15 | 10 |

b. Factors Affecting Vitamin E Requirment

i. Polyunsaturated fatty acids (PUFA)

It has been seen in animals that the requirment for vitamin E is increased by increased intake of PUFA[81]. However in human diets, the significance of the relationship between dietary tocopherol and PUFA is not clear. Since the toicopherol rich food like soybean, cotton seed and corn oils are also the usual sources of PUFA, increased PUFA intake are usually accompanied by an increased intake of vitamin E. However during the process of processing, storing, and cooking the oils, the vitamin E content is reduced. Further if an individual who has been consuming excessive quantities of vegetable oil such as more than 20g/day for a long period of time should abrupotly terminate this intake, a state of relative deficiency could develop, since tocopherol is lost from the tissues faster than polyunsaturated fatty acids[82].

ii. Selenium:

Selenium spares vitamin E or reduces vitamin E requirement in at least three ways:

a. Selenium is required for normal pancreatic function and their for the digestion and fat soluble lipids including vitamin E.

b. As a component of glutathione peroxidase[83], selenium helps destroy peroxides and thereby reduces the peroxidation of polyunsaturated fatty acid layer of the membranes. The diminished peroxidation greatly reduces the vitamin E requirment for the maintenance of membrane integrity.

c. In some unknown way, selenium aids in the retention of vitamin E in the blood plasma lipoproteins.

However most of the classical symptoms of vitamin E dificiency, such as foetal resorption and testis degneration in rats, muscular dystrophy in rabbits and encephalomalacia in chickens are not responsive to dietary selenium. In cases of exudative diathesis it has been seen that selenium can either spare the requirment for vitamin E or completely replace the vitamin.

Conversely vitamin E appears to reduce the selenium requirement, at least in experimental animals, by preventing loss of selenium from the body or maintaining it in an active form.

ili. Iron

When iron is given, vitamin E is required for its absorption, therefore the availability of vitamin E is decreased with iron supplementation.

iv. Drugs

Oral contraceptives lower plasma vitamin E levels, suggesting that women taking the 'pill[84] should increase their vitamin E intake. Animal experiments have shown that subjects receiving slcohol, acetaminophen, aspirin and adriamycin are required to increase their intake of vitamin E.

In conclusion, therefore the choiice of a satisfactory level of vitamin E intake to be recommended to the public will however remain controversial for some time. That is so, because in man, rapid development of tocopherol deficiency does not apparently occur owing to the resistance of tissue stores to depletion as well as the wide distribution of the vitamin in foodstuffs. Therefore it seens improbable that all of the animal deficiency syndromes will be found in men. Also the free radical promoted peroxidative processes that are inhihited by the tocopherols produce slow tissue changes that connot be evaluated in less than thirty years. Obviously small animals do not live long enough to facilitate such studies and humans cannot be subjected to such study of such tissue changes.

## H. VITAMIN "A"

(1) Chemistry:

Vitamin A is a generic term referring to all compounds other than carotenoids, that exhibit the biological activity of retinol. Its correct structure was first proposed by Karrer et al[87] in 1931. It is a polyisoprenoid compound containing a cyclohexeryl ring.

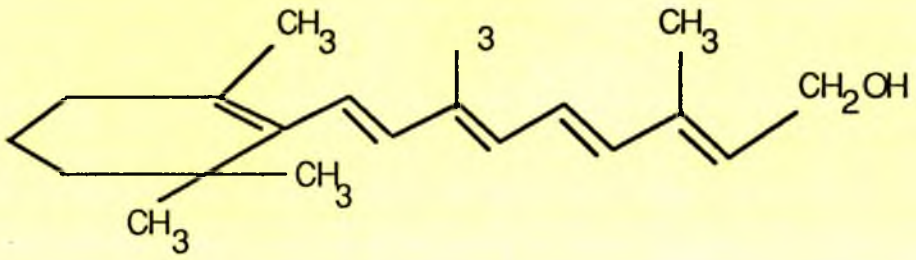



Fig-3 : Structure of Retinol

### Vitamin "A" or Retinol

Vagetable foods also has vitamin A activity which was found to be related to their contents of carotenoids-carotene, cryptoxanthin and other yellow pigments frequently found in association with chlorophyll and largely responsible for the colour of red and yellow vegetables. In 1932 it was found by Moore[86] that the carotene were precursers of vitamin A. Beta carotene is the most active form, one molecule yielding 2 molecules of vitamin A but the human body is only 50% efficient at this conversion. Alpha and gamma carotenes yield only one molecule of vitamin A, the other half being inactive. Animals cannot synthesise carotene but can convert it to vitamin A.

Vitamin A has been isolated[87] in pure form as pale yellow, almost colourless crystals, soluble in fat and fat solvents but not in water.

Both retinol and carotene are stable to ordinary cooking methods, although some loss may occur at above 100$^{o}$Ctemperature. Fruits and other food stuffs lose much of their vitamin A potency when dried in sunlight. But this vitamin is rapidly destroyed by rancidity or oxidation of fats.

(2) Food Sources[88]

The dietary sources of preformed vitamin A are all animals products. Human and animal orgenisms tend to concentrate most of the Vitamin A in the liver where it is stored. Other significant pools of the vitamin are found in the kidney, milk and plasma.

Therefore the best sources of preformed vitamin A is the animal liver and milk. Milk products and eggs are also rich sources of vitamin A. The milk of cows on green pasture is usually higher in vitamin A than the milk of stall fed animals. Butter, cheese and egg yolk is generally quite rich in Vitamin A. The yellow colour usually present in diary products, cheese, butter and ghee is due to carotenoids present.

The richest natural sources of vitamin A are fish liver oils, which are usually classed as food supplements, They vary according to the species and the season when caught.

Although more than 80 naturally carotenoids are known, only 10 have provitamin activity. These are found chiefly in dark green leafy vegetables. Other sources are yellow and red fruits and vegetables. The deeper the green or the yellow of the vegetable, the more carotene (provitamin) it contains.

Among the vegetable oils, the ricghest cource of provitamin A is red palm oil.

Vitamin A (IU/100 gm) [88]

| | |
|---|---|
| Milk (cow) | 150 |
| (human) | 170 |
| Cheese | 1000 |
| Cream | 500 |
| Butter | 3500 |
| Liver(beef) | 5000 |
| (sheep) | 4500 |
| Egg (whole) | 1000 |

| | |
|---|---|
| (white) | 0 |
| (yolk) | 3000 |
| Fish-codliver oil | 7500 |
| Carotene (IU/100 gm) | |
| Carrots | 11,000 |
| Sweet potatoes | 8000 |
| Green spinach | 8000 |
| Cabbage | 130 |
| Cauliflower | 50 |
| Lettuce | 970 |
| Peas | 500 |
| Pumpkin | 1600 |
| Fruits | |
| Apples | 50 |
| Bananas | 190 |
| Melons | 251 |
| Orange | 84 |
| Pineapple | 100 |
| Plums | 300 |
| Nuts | 0 |
| Bread(corn) | 300 |
| Cereals | Neglegible |

Animal foods containing preformed vitamin A seems to be sufficient as a source of this factor. However the ample availability of plant food containing carotene may well contribute a large share of vitmin A requirement.

(3) Absorption[89]

In animal products, vitamin A exists as long chain fatty acid esters of retinol. These retinyl esters are hydrolysed within the intestinal lumen and absorped in this form accross the mucosal cell membrane into the intestinal cell.

In vegetables, dietary vitamin A esters are hydrolysed within the intestinal lumen and absorped in this form across the mucosal cell membrane into the intestinasl cell.

In vegetables,[90] dietary vitamin A[89] exists as a provitamin in the form of β carotenes (yellow pigments) The ingested β carotenes are oxidatively cleared by β carotene dioxygenase requiring molecular oxygen and the bile salt lectithin. Two molecules of retineldehyde (retinal) is obtained which is further reduced by a specific reductate, utilising NADPH, to retinol.

(4) HUMAN REQUIREMENT

Requirements of vitamin A are usually expressed in terms of international units.[91] A unit is equivalent to the activity of 0.344 ug vitamin A acetate, 0.3 ug vitamin A alcohol or 0.6ug β carotene.[90]

The general assumption has been made that about two-thirds of vitamin A of the agerage diet consists of carotene precursers and one-third of preformed vitamin A. But in most developing countries a much higher proportion comes from carotenoids.

The recommended daily allowances [92] for the normal male and female adult are 1000 and 800 retinol equivalents per day respectively (1 R.E. = 1ug retinol or 6ug B carotene) and 400 to 700 μg R.E. for children up to ten years.

(5) PHYSIOLOGICAL FUNCTION

Vitamin A has a number of important functions in the body. It plays an essential role in the function of the retin[93]. It is apparently essential for growth and differentiation of epithelial tissue[94]. The vitamin is required for growth of

bone[95], rrproduction and embryonic development. It also has a stabilizing effect on various membranes and acts to regulate membrane permeability[95].

Many physiological functions are affected by vitamin A. It is believed to be essential for mainenance of normal cellular membrane structure and function. The structure and stability of cell membranes are markedly altered in vitamin A deficient rats.

(6) NORMAL VITAMIN "A" SERUM LEVEL

Studies[97] undertaken in the United States, Great Britain, South Africa and Norway indicates a serum vitamin A level of approximately 40ug/100ml. Serum levels do not reflect liver reserves of vitamin A. Only atfter complete exhaustion of liver reserves, serum vitamin A level falls drastically.

Levels below 20ug/100ml serum are certainly on the low side and levels below 10ug/100ml are very low indeed.

The range of normal plasma vitamin A level is between 15-60ug/100ml[48]. or 0.53-2.1 mmol/L.

(7) DEFICIENCY STATE:

In man, vitamin A deficiency rarely occurs in isolation. It is a frequent accompaniment of marasmus, kwashiorkor[98] and other malnoiurished states. Ther is also evidence that a deficiency of circulating plasma protein, to which vitamin A is bound, may be a contributory factor in tissue depletion of the vitamin. Carotene is poorly abosrbed when the diet is low infant. In the case of low plasma levels of vitamin E, vitamin A may be oxidised.

1. The Eye:

Xerophthalmia, the most ancient of human diseases is a descriptive term applied to any acular manifestation of vitamin A deficiency. Clinical classification of Xerophthalmia[99].

Night blindness (XN)[100]

Conjuctival xerosis (XIA)

Bitot's spot (XIB)

Corneal xerosis (X2)

Corneal ulceration (X3A) $\frac{1}{3}$rd

Corneal ulceration (X3B) $\frac{1}{3}$rd

Corneal Scar (XS)

Xerophthalmia fundus (XF)

Night blindness (XN)[100]

It results from inmpaired visual pigment metabolism vitamin A is required for regeneration of the visual pigment rgodopsin.

Conjuctival Xerosis (XIA)

Vitamin A deficiency induces a squamous metaplasia throughout the conjunctiva, making the surface irregular.

Bitot's Spot (XIB) [100]

It is a small plaque of a silvery gray hue, with a foamy surface situated on the bulbar conjunctiva.

Corneal Xerosis (X2)

There is corneal haziness and loss of luster and the corneal surface becomes dry.

Corneal Ulceration (X3A)

Small corneal ulcer, sharply demarcated limited to less than $\frac{1}{3}$rd corneal surface appear.

Corneal Ulceration (X3B)

There may be localised stromal necrosis. Complete unilaternal or bilateral corneal necrosis may occur.

Corneal scar (XS)

There is corneal scarring

Xeropthalmia fundi

Description of the globe and complete blindness .

THE SKIN[48]

There may be a widespread dryness, wrinkling, state gray discolouration and hyperkeratosis of the skin. There may also be loss of luster of the hair a dry irregular, straight appearance of the eye lashes, Longitudinal furrowing with hormy apearance of the finger and nail has been seen.

## III. PURPOSE OF PRESENT STUDY

Spondylosis is one of the leading causes of chronic limitation of activity in person in all countries.

It contributes to an enormous financial expense as well as lost time and energy. The following table represents the morbidity and disablement from selected conditions (expressed as rates per 1000 persons of both sexes in the United States and Great Britain.[2, 7]

| CONDITION | MORBIDITY | DISABLEMENT |
|---|---|---|
| U.S.A. | | |
| ARTHRITIS | 79 | 21 |
| SPONDYLOSIS | 52 | 18 |
| CARDIAC PROBLEM | 29 | 20 |
| HYPERTENSION | 47 | 9 |
| GREAT BRITAIN | | |
| ARTHRITIS | 30 | 22 |
| SPINAL PROBLEM | 34 | 20 |

Even though no specific study on spondylosis has been done is Bangladesh, it appears that a large number of people attend a physician with complaints of bnackache and neck pain at some time or other in their lives. X-ray studies of these patients clearly indicate signs and sypmtoms similar to those found in spondylosis. Therefore it can be assumed that in our country too, the prevalence of the disease is not negligible.

We have therefore undertaken a project to make a biomedical study on patients suffering from spondylosis. The purpose of the present study is to investigate the known antioxidant effects of vitamin E and vitamin A on the degenerative process seen in these patients as confirmed by X-ray studies.

## IV. PATIENTS AND METHODS :

The patients were first diagnosed by prominent orthopedic surgeons of Dhaka city and referred to the Institute of Nutrition and Food Science. By using predetermined criterion we considered thirty two patients for inclusion in our study.

The criteria are:-

(1) subjects were between forty to seventy years.

(2) subjects were of both sexes

(3) subjects had the following salient features.

(a) Morning stiffness of the affected joints on getting up after night rest.

(b) Limitation of movement and posture

(c) Pain of dull aching quality at (i) Postorior neck region and/or Lower Lumbar region

(d) Radiation of pain to (i) Occiput (ii) Chest (iii) Shoulder girdle (iv) Arm (v) Scapularngle (vi) Other sites (e.g. groin, back of thigh).

(e) Conditions exacerbating pain (i) Prolonged standing (ii) Motion (iii) Prolonged sitting (iv) Lying at rest.

(f) Conditions relieving pain (i) rest (ii) Motion (iii) Pain relieving drugs.

(4) Subjects had no other complications

(5) Subjects had radiological evidences of spondylosis.

(6) Conventional treatment had no effect on their symptoms.

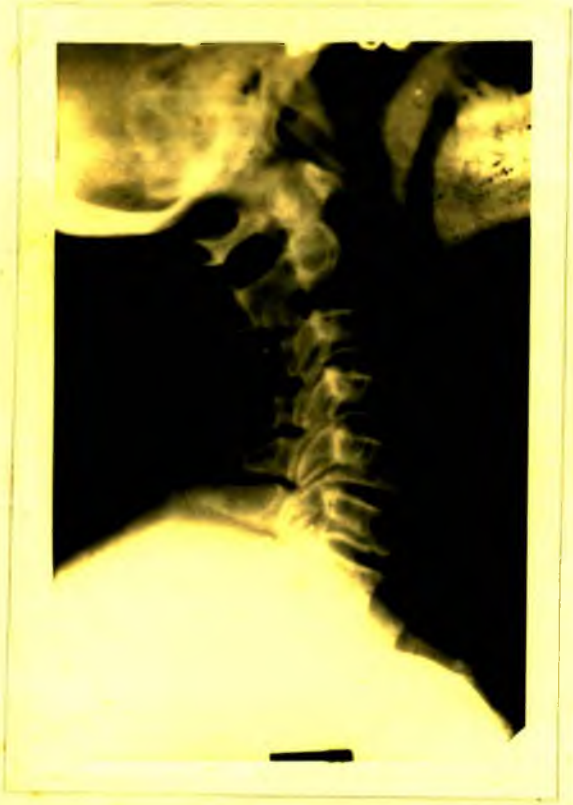
A questionnaire developed by us which also ncluded a general history (name, sex, religion, address, occupation, income, educational status) and past history (injury, fall, fracure or any other debilitating disease.

A general examination of height, weight measurement, pulse rate and blood pressure checking was also done.

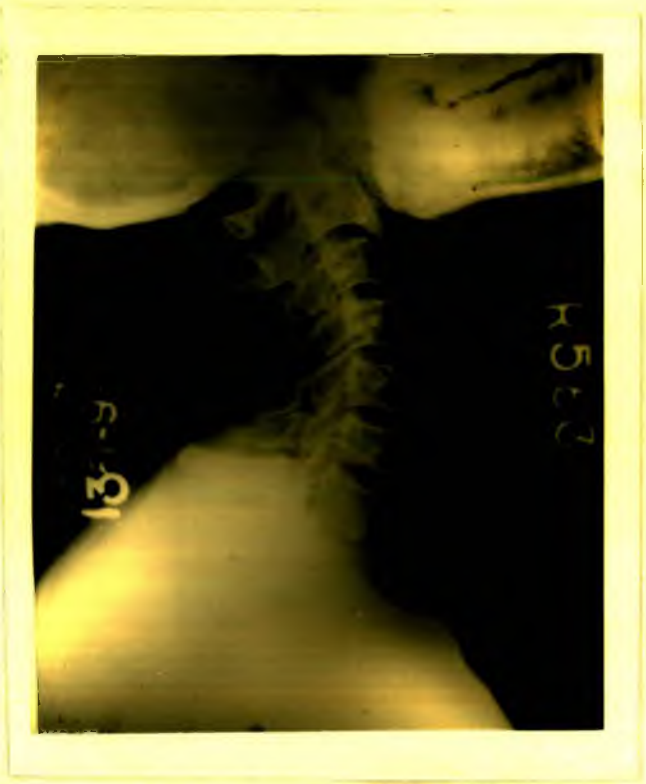
A physical examination based on inspection, palpation, percussion and testing of spinal movement was done. The testing included flexion, extension, lateral bending and rotation. The motor system of both the upper extremity (thigh, calf and interossei) was examined for muscular atropy, tremor, power, muscle tone and reflex.

X-Ray of the affected region (cervical or lumbar) on anteroposterior and lateral view was taken to confirm the diagnosis.

P.T.O



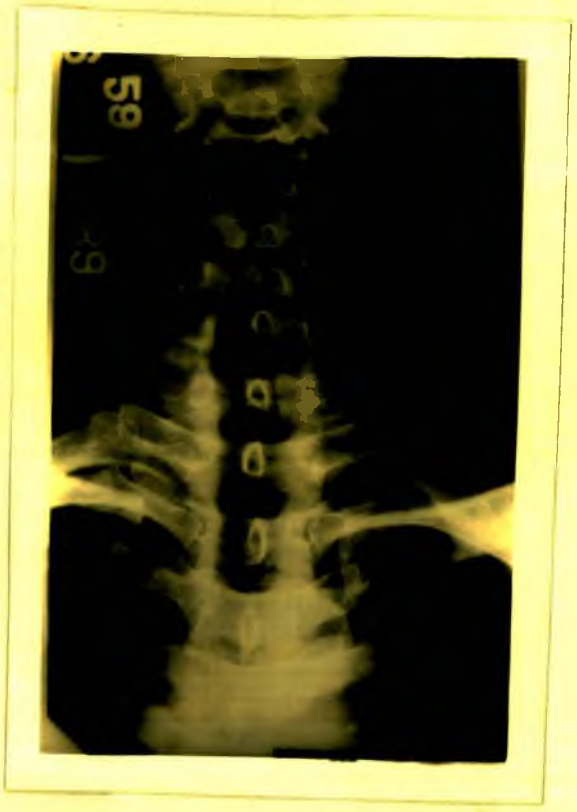
PHOTOGRAPH 1



PHOTOGRAPH 2

PHOTOGRAPH 1: X-RAY OF CERVICAL REGION (LATERAL VEIW) SHOWING DEGENERATIVE CHANGE BETWEEN $C_5$-$C_6$ AND OSTEOPHYTE FORMATION BEFORE TRIAL

PHOTOGRAPH 2 : X-RAY OF CERVICAL REGION (LATERAL VEIW) SHOWING NO FURTHER DEGENERATIVE CHANGE IN SAME PATIENT AFTER TRIAL.



PHOTOGRAPH 3


PHOTOGRAPH 4

PHOTOGRAPH 3 : X-RAY OF CERVICAL REGION (ANTERO POSTERIOR VEIW) SHOWING DEGENERATIVE CHANGE BETWEEN $C_5$-$C_6$.

PHOTOGRAPH 4 : X-RAY OF CERVICAL REGION (ANTERO-POSTERIOR VEIW) SHOWING NO FURTHER DEGENERATVIE CHANGE IN SAME PATIENT AFTER TRIAL

An informed consent explaining the purpose of the study was taken from the study subjects.

Five millilitres of blood were aseptically collected with a disposable plastic syringe from the antecubital veins of each subject for vitamin A and E assay. The plasma was separated by centrifugation with due care to prevent haemolysis and transferred to a 5 ml labelled, screw capped glass test tube, wrapped in aluminium foil and stored under-20°C until analysis.

To test the efficacy of the experimental drugs (Vitamin A and E), twelve of the thirty two were given a single blind trial. The patients were unaware of the fact that the experimental drugs were vitamins A and E. One tablet of vitamin A contained 50,000 IU retinol and one tablet of vitasmin E contained 100mg tocopherol. The subjects were divided into three groups. One group was supplied with both vitamins A and E. One group was given only vitamin A and the third group received only vitamin E. The subjects were asked to take one tablet daily. The drugs were administered for three weeks and the patients were examined again after the third week.

A preliminary analysis indicated that vitamin E was found to play a role in relieving the symptoms of spondylosis.

Next we designed a double blind study. The experimental drugs were prepared in the same size, shape and colour by pharmacists. The samples were divided into three groups (1,2 and 3) and their codes were kept strictly confidential with the pharmacists. Each group of sample contained twenty one tablets. The remaining twenty patients participated in the double blind study and neither they nor the investigators were aware of the type of drug being given. Using a random number table, the investigators gave sample No. 1 to six subjects, sample No. 2 to six and sample No. 3 to the remaining eight subjects. The drug therapy continued for three weeks and the patients were requested to come back after the third weed for assesment of prognosis of their condition and assay of vitamin A and E levels in the blood.

Two sample t tests[101] were used to compare blood levels of vitamin A and vitamin E before and after intake of experimental drugs among the subjects.

Chemical and Reagents Used:

Trans-retionol, retinyl acetate, d a-tocopherol and tocopheryl acetate were purchased from Sigma Chemical Co. St. Louis, U.S.A., HPLC grade methanol, n-hexane, ethanol and nitrogen gas were supplied by the Institute of Nutrition and Food Science, Dhaka University.

Chromatography:

A Pye Unicom high performance liquid chromatograph (HPLC) of Model 2040 was used for the study. It consisted of a M-45 pump, a UK injector, a 5 cm guard column, 15mX4.5nm analytic column and a 440 UV absorbance detector. The latter was fitted with a 292 nm filter for vitamin E assay and a 326 nm filter for vitamin A assay. Both the guard and analytic columns were maintained at 31°C. The mobile phase was composed of 96% methanol in deionized water. Prior to use the mobile phase was filtered through a 0.5 u filter and degassed. The flow rate was maintained at 1.5 ml/min.

All samples and analytic materials were protected from light and their analysis was performed with minimum exposure to any light.

Methods of Estimation:

Vitamin A and E levels in plasma were estimated by the method of Bieri and Tolliver[102] with adaptation from Horwitt et al[103] and Huang et al[104].

ANALYSIS OF PLASMA VITAMIN A:

Reagents required:

(a) HPLC grade absolute alcohol (ethanol)

(b) Spectograde n hexane

(c) Nitrogen gas

(d) Retinyl acetate:

(1) 10 mg retinyl acetate was dissolved in 100 ml ethanol. The concentration of retinyl acetate in this solution was 100 ug/ ml. One ml of this solution was

diluted to 100 ml with ethanol to give a concentration of 1 ug/ml retinyl acetate. This was used as the internal standard for the serum.

(ii) 10mg retinyl acetate was dissolved in 100ml ethanol. The concentration of retinul acetate in this solution was 100ug/ml. Five ml of this solution was diluted to 100ml with ethanol. Now the concentration of retinyl acetate was 5ug/ml. 20ml of the latter solution was diluted to 50 ml with ethanol so that the concentration of retinyl acetate become 2ug/ml. This was used as the internal standard of the standard solution for the standard curve, when the above solution was mixed with 50 ml of the retinol solution, the concentration of retinyl acetate beocmes 1 ug/ml.

(e) Retinol:

10 mg retinol was dissolved in 100 ml ethanol. The concentration of retinol in this solution was 100 ug/ml Five ml of this solution was diluted to 100 ml with ethanol. Now the concentration of retinol was 5 ug/ml. One ml of this stock solution was diluted to 50 ml with ethanol so that the concentration of retinol beocme 0.1 ug/ml. When this was mixed with the 50ml solution of retinyl acetate internal standard solution (ii) the concentration of retinol became 0.05 ug/ml.

Similarly 2,4,6,12,16,20 ml of the stock solution was diluted in the same manner to get a retinol concentration of 0.1 ug/ml, 0.2ug/ml, 0.4 ug/ml, 0.6 ug/ml, 0.8ug/ml and 1 ug/ml. The standard curve for retinol was thus prepared.

Procedure:

The working standard solution of known calibration with the internal standard was injected daily before the samples to ensure quality and to determine line arity. Calibration curves obtained from the standard solutions of retinol (0.05-1 ug/ml that is 0.17-3.50 umol per litre) gave the linear regression equations $Y=4.46+0.52X$ and coefficients of correlation $r=0.91$. The within-day and the inter day coefficients of variations were 4.6% ($X=1.71$ umol/L$\pm$0.08) ($n=10$) and 4.6% ($X=1.07$ umol/L$\pm$0.05) ($n=10$) respectively. The mean recoveries ($n=3$) at each concentrations were 103% (0.05-1ug/ml retinol).

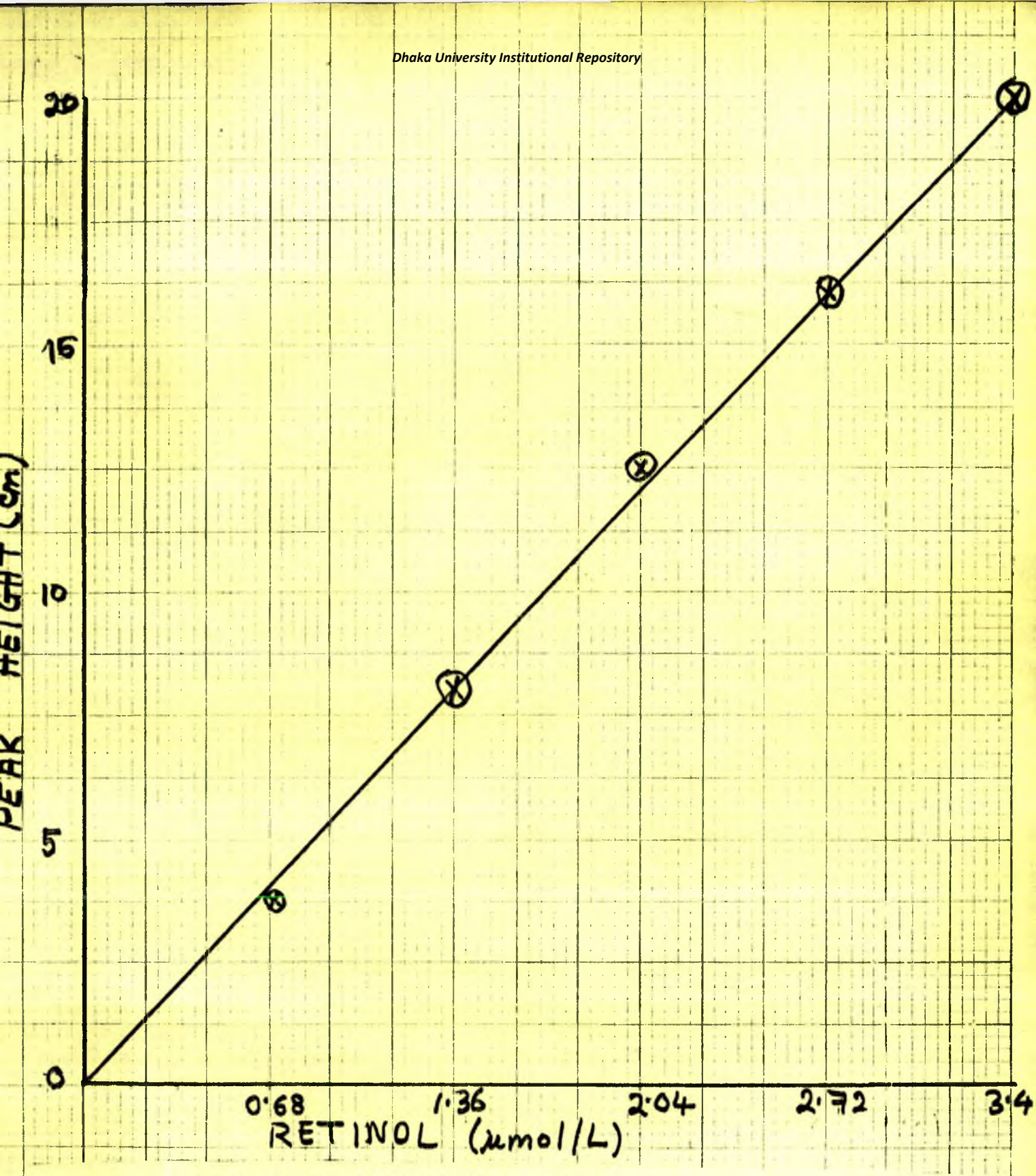



FIGURE 4 : STANDARD CURVE FOR RETINOL

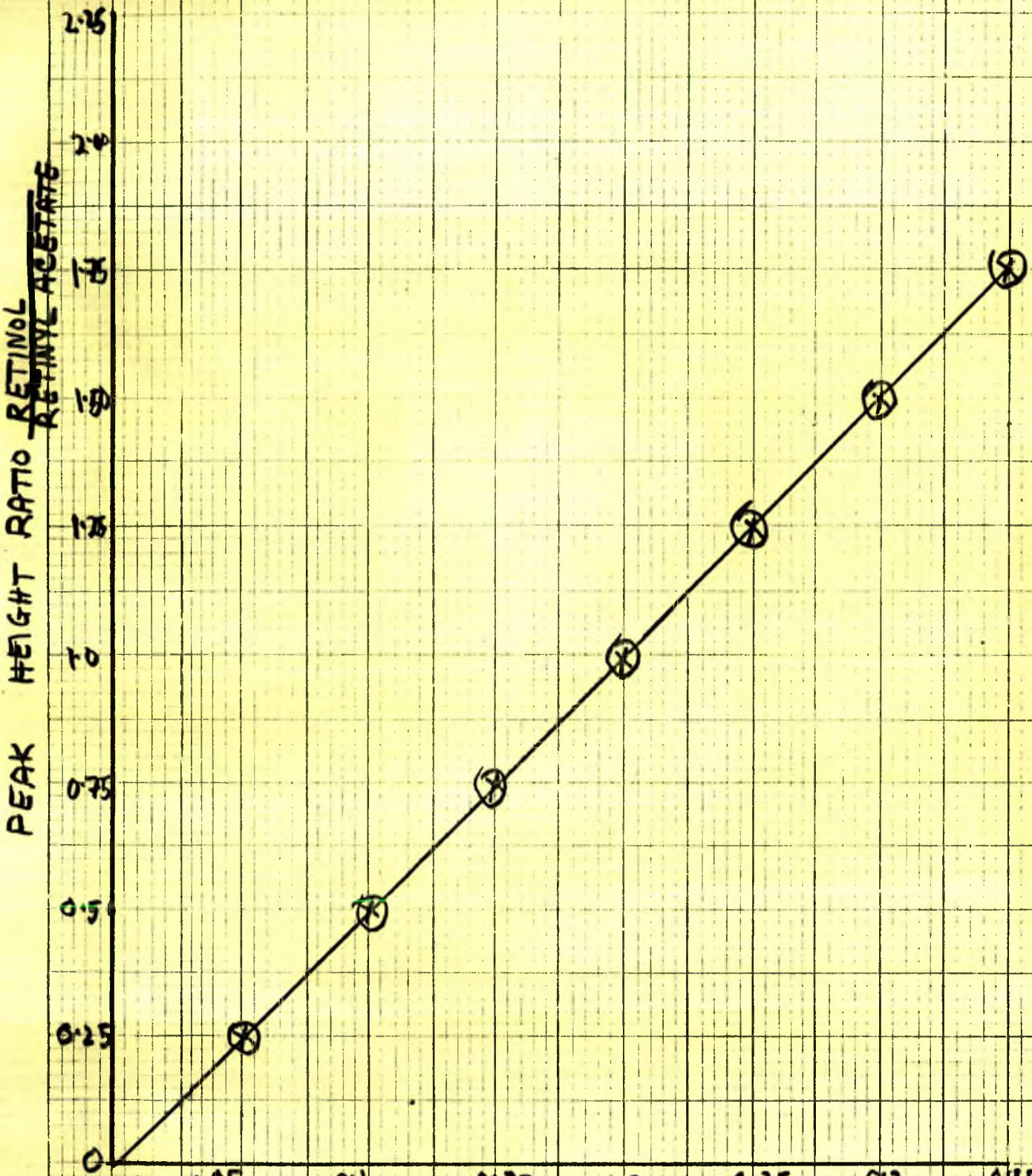



FIGURE 5

STANDARD CURVE FOR PEAK HEIGHT RATIO VERSUS WEIGHT RATIO FOR RETINOL : RETINYL ACETATE

Sample Preparation:

Plasma 100ul was taken into a 6X50mm glass test tube. To it was added 50 ul of the internal standard (retinylacetate (i) 1ug/ml in ethanol and 50ul ethanol. The contents were mixed and the lipids were extracted with 200 ul spectrograde n hexane with vigorous shaking, intermittently for 45 seconds, on a vortex misture. The tubes were centrifuged at 1200g for 10 minutes to separate the phases. As much of the solvent as possible was carefully drawn off from the upper phase with a 75ul Lang Levy pipette and trnasferred to a 3 ml conical centrifuge tube. The solvent was evaporated under a stream of nitrogen with the tube in a $60^0$C water bath. For injection into the chromatograph, the lipid in the centrifuge tube was dissolved in 50 ul absolute ethanol with gentle mixing by finger tapping. About 20 ul of the solution was gently injected followed by a flush of ethanol.

ANALYSIS OF PLASMA VITAMIN "E" :

Reagents required:

(a) HPLC grade absolute alcohol (ethanol)

(b) spectrograde n hexane

(c) nitrogen gas

(d) tocopheryl acetate:

(i) 10mg tocopheryl acetate was dissolved in 100ml ethanol. The concentration of tocopheryl acetate in this solution was 100 ug/ml. One ml of this solution was diluted by mixing with 1 ml ethanol. Now the concentration of tocpheryl acetate in this solution was 50 ug/ml. This was the internal standard for the serum (ii) 10 mg tocopheryl acetate was dissolved in 100 ml ethanol. The concentration of tocopheryl acetate in this solution was 100 ug/ml. 50 ul of this internal standard when mixed with 50ml of the tocopheryl solution gave a tocopheryl acetate concentration of 50 ug/ml.

(e) Tocopherol: 10 mg of tocopherol was dissolved in 100ml ethanol. The concentration of tocopherol was 100 ug/ml. One ml of this stock solution was diluted to 50 ml with ethanol so that the concentration of tocopherol was 2

ug/ml. When the latter solution was mixed with 50 ml of the internal standard solution (ii) the concentration of tocopherol was 1 ug/ml.

Similarly 5,10,15,20,25ml of the stock solution was diluted in the same manner to get a tocopherol concentration of 5ug/ml, 10 ug/ml, 15ug/ml, 20 ug/ml, 25 ug/ml. The standard curve for tocopherol was thuse prepared.

Procedure:

To quantitate tocopherol in the samples the working standard of know quantity was prepared daily. It was injected before analysis daily to determine linearity. Calibration curves obtained from the standard solutions of increasing concentrations of tocopherol (1-25 ug/ml that is 2.32-58 umol/L) gave the linear regression equations: Y=0.25+1.005X and coefficients of correlation r=0.63. The within-day and inter-day co-efficients fo variations were 2.6% (X=13.47 umol/ L±0.35) and 2.2% (X=10.34 umol/L±0.23). The mean recoveries (n=3) at each concentration were 90% (1-25 ug/ml tocopherol).

Sample Preparation:

Plasma 100 ul was taken into a 6X5mm glass test tube and to it was added 50ul of the internal standard (tocopheryl acetate(i) 50 ug/ml in ethanol) and 50 ul ehtanol. The contents were mixed well. For extraction of the lipid 200 ul spectrograde hexane were added and the contents mixed vigorously intermittently for 45 seconds on a vortex mixture. The tubes were centrifuged at 1200g for 10 minutes. to separate the phases. As much of the solvent as possible was carefully drawn off from the upper phase with a 75ul Lang Levy pipette and trnasferred to a 3 ml conical centrifuge tube. The solvent was evaproated under a stream of nitrogen with the tube in a $60^0$C water bath. For injection into the chromatograph, the lipid in the centrifuge tube was dissolved in 50 ul absolute ethanol with gentle mixing by finger tapping. About 20 ul of the solution was gently injected followed by a flush of ethanol.

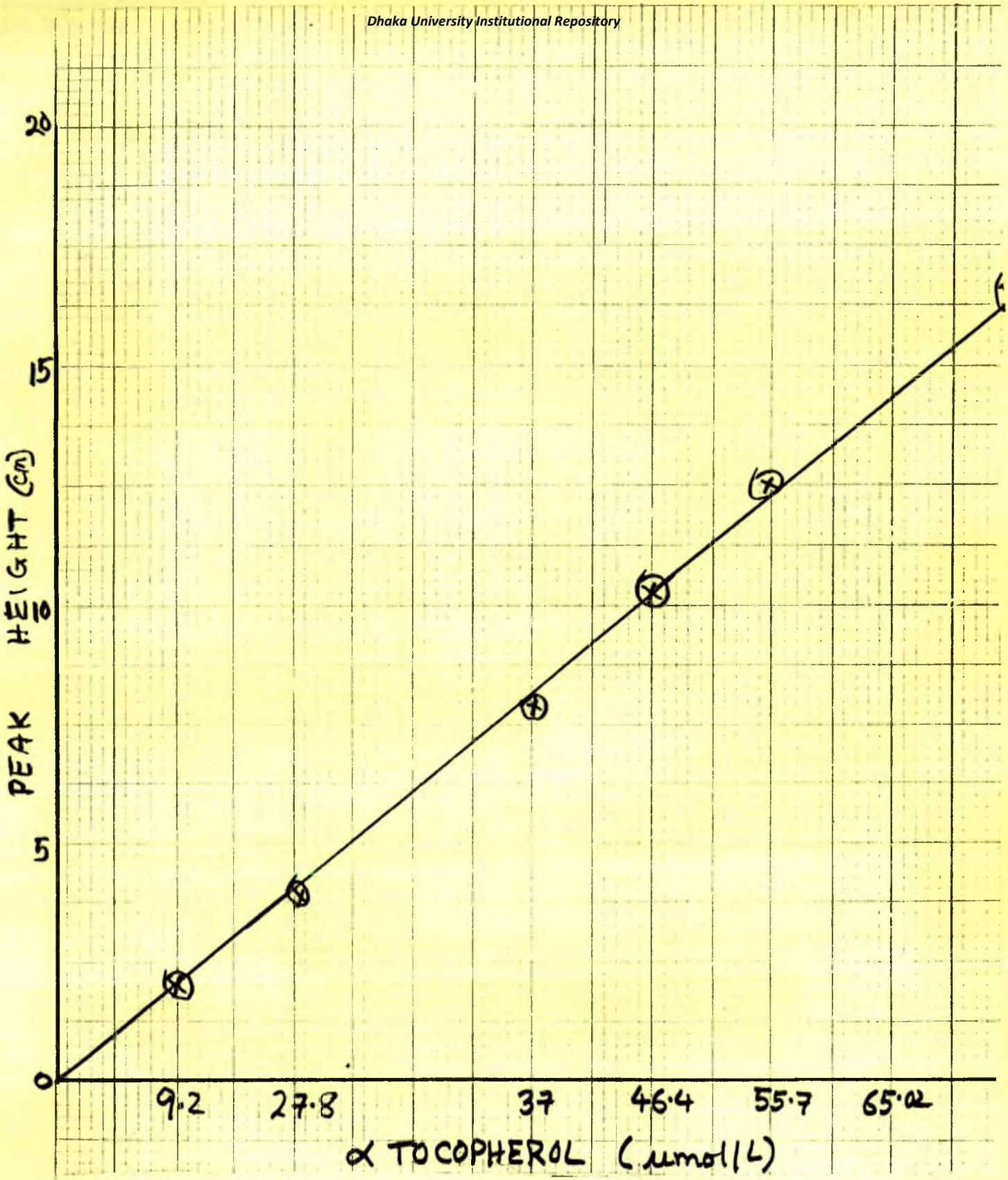



FIGURE 6: STANDARD CURVE FOR α TOCOPHEROL

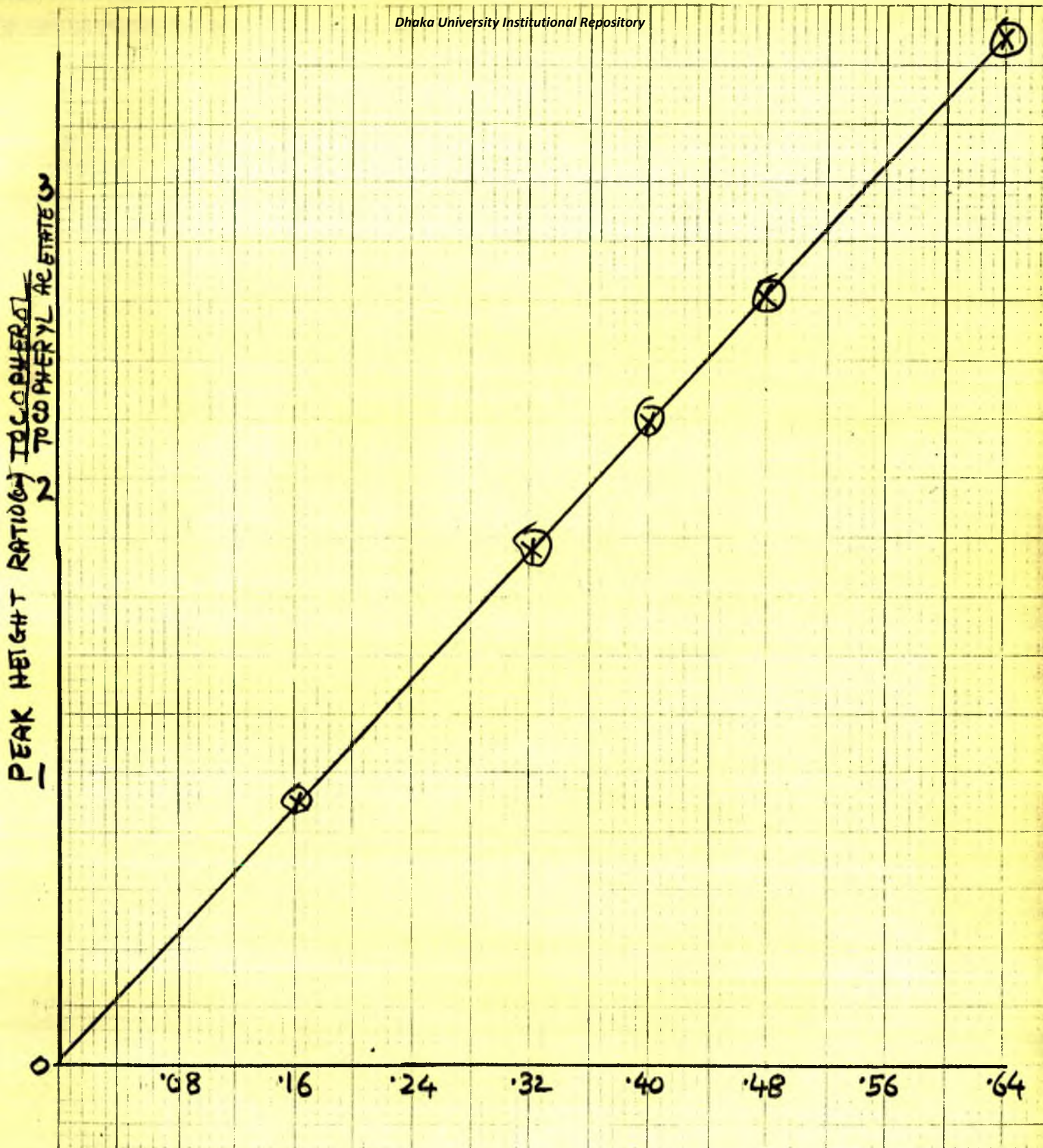



FIGURE 7
STANDARD CURVE FOR PEAK HEIGHT RATIO VERSUS WEIGHT RATIO FOR α TOCOPHEROL : TOCOPHERYL ACETATE

## RESULTS:

Table No. 8 shows that the number of male patients is 24. The mean age is 50 years with a S.D. of 6.6.

In Table 9 is shown that the number of female patients is 8. The mean age is 50 years with a S.D. of 5.34.

Table 10 shows the age and sex distribution of all the 32 patients. There are 24 male patients and 8 female patients in the study giving a M.F. ratio of about 3:1. In the age group between 40 to 50 years there were 15 male patients (62.5%) and 37.5% (29.17+8.33) were above 50 years of age. There were 50% female patients between 40-50 years and 50% (4 patients) between 50-60 years. A total of 19 patients (59%) were between the age group of 40-50 years. 34.37% of the total patients were between 50 to 60 years age group. There were only 2 male patients above the age of 60 years.

Table 11 shows that 6 patients (18.75%) had education up to S.S.C. level, 3(9.38%) up to H.S.C. 7(21.87%) patients completed degree or equivalent and 16 patients (50%) had post-graduate qualification.

Table 12 shows that 7 patients (21.62%) were officers and 5 (15.62%) were clerks. Four patients (12.50%) were engineers 3 patients (9.37%) were physicians, 2(6.25%) were teachers and one was a writer.

Figure 8 shows the income distribution of all the patients. 81.25% had an average monthly income of Taka 2000-5000 only. 6.25% patients Taka 5000-8000 and the rest 12.50% had a monthly income of Taka 8000 and above.

TABLE : 8

AGE DISTRIBUTION OF MALE PATIENTS (n-24)

| AGE RANGE (YEAR) | NUMBER | MEAN WITH SD |
|---|---|---|
| 40 - 50 | 15 | - |
| 51 - 60 | 07 | 50 |
| 61 - 70 | 02 | ±6.6 |

TABLE : 9

AGE DISTRIBUTION OF FEMALE PATTIENTS (n-8)

| AGE RANGE (YEAR) | NUMBER | MEAN WITH SD |
|---|---|---|
| 40 - 50 | 04 | - |
| 51 - 60 | 04 | 50 |
| 61 - 70 | 00 | ±5.3 |

TABLE : 10

AGE AND SEX DISTRIBUTION OF TOTAL PATTIENTS (n-32)

| AGE RANGE | MALE PATIENTS | | FEMALE PATIENTS | | TOTAL | PERCENTAGE |
|---|---|---|---|---|---|---|
| (M) | (24) | (F) | (08) | (M+F) | | |
| In Years | NUMBER | % | NUMBER | % | No. of patients 32 | |
| 40-50 | 15 | 62.5 | 04 | 50 | 19 | 59.37 |
| 51-60 | 07 | 29.17 | 04 | 50 | 11 | 34.37 |
| 61-70 | 02 | 8.33 | 00 | | 02 | 6.26 |

TABLE NO. 11

EDUCATIONAL BACKGROUND OF PATIENTS (n-32)

| LEVEL OF EDUCATION | MALE | FEMAL | PERCENTAGE |
|---|---|---|---|
| 1. S.S.C. | 1 | 5 | 18.75 |
| 2. H.S.C. | 3 | 0 | 9.38 |
| 3. B.A.-equivalent | 4 | 3 | 21.87 |
| 4. M.A./equivalent | 14 | 2 | 50 |
| TOTAL | 22 | 10 | 100 |

TABLE 12

OCCUPATION OF PATIENTS (n=32)

| OCCUPATION | NUMBER OF PATIENTS | PERCENTAGE |
|---|---|---|
| 1. HOUSEWIFE | 7 | 21.87 |
| 2. BUSINESSMAN | 5 | 15.62 |
| 3. OFFICERS | 5 | 15.62 |
| 4. CLERKS | 5 | 15.62 |
| 5. TEACHERS | 2 | 6.25 |
| 6. ENGINEER | 4 | 12.50 |
| 7. PHYSICIAN | 3 | 9.37 |
| 8. WRITER | 1 | 3.15 |
| TOTAL | 32 | 100 |

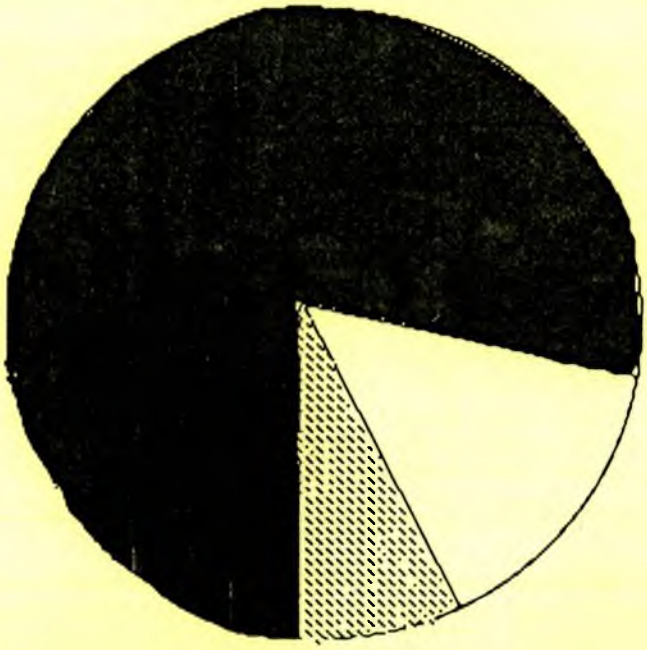


FIGURE-8 INCOME DISTRIBUTION OF THE PATIENTS

| INCOME PER MONTH | PER CENTAGE OF PATIENTS |
|---|---|
| TAKA 2000-5000 | 81.25% |
| TAKA 5000-8000 | 6.25% |
| TAKA 8000-ABOVE | 12.50% |

Table No. 13 shows the clinical presentation of all the thirty two patients. Thirty of them (93.75%) had neck pain, in addition eleven had low back pain also. There was restricted spinal movement in seventeen (53.21%) patients and radiation of pain to other areas in 26 (81.25%) patients. Two patients (6.25%) had tremor of the hands and there was thenar and hypothenar muscle wasting in four (12.25%) patients.

In Table 14 is shown that twenty three patients had symptoms for less than a year to a year. Five patients had symptoms for more than a year upto 2 years and 4 patients (12.50%) had sympoms for more than 2 years.

Table No. 15 shows the duration of the two main symptoms. A total numbers of thirteen patients were suffering from morning stiffness of which 61.54% were suffering for less than a year, 015.38% for 1 to 2 years and 23.08% for more than 2 years. Fifteen patients were suffering from limitation of movement. 66.67% were suffering for less than a year, 6.66% for 1 to 2 years and 26.67% for more than 2 years.

In Table No. 16 is shown the different areas to which pain is radiated. Pain is radiated to the occiput in four patients (9.30%), to the shoulder girdle in nine patients (20.93%) to the scapula in nine (20.93%). 39.54% patients (17) had radiation of pain to the arm and 9.30% had radiation to the chest.

The radiological features of the patients are shown in Table No. 17. Fourteen patients (43.7%) had mild degeneration, twelve patients (37.5%) had moderate changes and siz patients (18.75%) had severe degenerative changes.

TABLE NO. 13

CLINICAL REPRESENTATION OF PATIENTS

| CLINICAL FEATURES | NUMBER OF PATIENTS | PERCENTAGE |
|---|---|---|
| 1. NECK PAIN | 30 | 93.75 |
| 2. LOW BACK PAIN | 11 | 34.37 |
| 3. RESTRICETED MOVEMENT | 17 | 53.12 |
| 4. RADIATION OF PAIN | 26 | 81.25 |
| 5. TREMOR | 2 | 6.25 |
| 6. MUSCLE WASTING | 4 | 12.25 |

TREMOR : An involunrtary trembling.

MUSCLE WASTING : Gradual decay or diminution of bulk of muscle.

TABLE 14

DURATION OF ALL SYMPTOMS OF PATIENTS (n-32)

| DURATION OF SYMPTOMS (YEARS) | NO. OF PATIENTS | PERCENTAGE |
|---|---|---|
| LESS THAN 1 | 23 | 71.87 |
| 1 ——→2 | 05 | 15.63 |
| MORE THAN 2 | 04 | 12.50 |
| TOTAL | 32 | 100 |

TABLE NO. 15

DURATION OF TWO MAIN SYMPTOMS OF THE PATIENTS

| YEARS OF TWO MAIN SYMPTOMS OF THE PATIENTS | | | | |
|---|---|---|---|---|
| YEARS OF DURATION | SYMPTOMS | | | |
| | MORNING STIFFNESS | | LIMITATION OF MOVEMENT | |
| | NUMBER OF PATIENTS | % | NO. OF PATIENTS | % |
| LESS THAN 1 | 8 | 61.54 | 10 | 66.67 |
| 1 to 2 | 2 | 15.38 | 1 | 6.65 |
| MORE THAN 2 | 3 | 23.08 | 4 | 26.67 |
| TOTAL | 13 | | 15 | |

MORNING STIFFNESS: Rigidity of the joints for 15-30 minutes in the morning which occurs in patients with arthritis of related disorder.

LIMITATION OF MOVEMENT: Confinement of specific movement of the body.

TABLE NO. 16

RADATION OF PAIN TO DIFFERENT AREAS

| AREA INVLOVED | NUMBER OF PATEINTS | PERCENTAGE |
|---|---|---|
| OCCIPUT | 4 | 9.30 |
| SHOULDER GIRDLE | 9 | 20.93 |
| SCAPULA | 9 | 20.93 |
| ARM | 17 | 39.54 |
| CHEST | 4 | 9.30 |
| TOTAL | 43 | 100 |

OCCIPUT: BACK OF THE READ
SHOULDER GIRDLE: PART OF THE UPPER ARM AND TRUNK
SCAPULA : BACK OF THE SHOULDER

TABLE NO. 17

X-RAY FINDINGS OF 32 PATIENTS

| X-RAY DIAGNOSIS | NUMBER OF PATIENTS | % |
|---|---|---|
| MILD DEGENERATION | 14 | 43.75 |
| MODERATE DEGENERATION | 12 | 37.50 |
| SEVERE DEGENERATION | 6 | 18.75 |
| TOTAL | 32 | 100 |

MILD: Mild degenerative change seen in $C_5$ and $C_6$

MODERATE: Degenerative change seen in more than two vertebral.

SEVERE: Other signs like osteophyte, spur formation and

liping are seen along with degenerative change in more than two vertebral.

DEGENERATIVE CHANGE: Dimunation of bony density of vertebral shown by radioluscent area at the upper or lower anterior angle of the vertebral. These changes are due to stress of wear and tear on the vertebral.

In figure 9 is shown the serum vitamin A level of the patients who participated in the single blind study. Before intake of the drugs the patients who had an average serum value of 0.43 umol/L had an increase of their serum level to 0.82 umol/L after the trial. The average serum vitamin A level was 1.00 umol/L after intake in those patients whose mean serum A level was 0.85 umol/L initially. The patients having a mean serum vitamin A level of 1.4 umol/L before intake had an average serum vitamin A level of 1.5 umol/L after intake. The overall mean value of serum vitamin A level was 0.70±o.39 umol/L before intake. The mean serum vitamin A level of all the patients increased to only 0.90±0.06 umol/L after intake of the exmperimental drugs. The rise was not statistically very significant ($P<0.5$).

In contrast to vitamin A, the mean serum vitamin E level increased from 3.20±1.09 umol/L to 13.93±6.29 umol/L, a shown in Figure 10, during the therapy. The rise was about 400% and highly significant ($P<0.005$). The patients with serum level of 2 umol/L had an increase of serum vitamin E level to 14 umol/L of those patients whose serum level was initially 3.5 umol/L. After three weeks of drug intake the serum level rose to 18 umol/L in those who had 4.5 umol/L before intake.

The next Figure 11 depicts the symptoms and percentage of the patients. Before intake of the experimental drugs in the single blind trial, 91.6% were suffering from neck pain, 66.6% from restricted spinal movement and radiation of pain. 58.3% and 33.3% of the patients were suffering from low back pain and muscle wasting respectively. After the trial all the patients were relieved except 33.3% who were still suffering from muscle wasting.

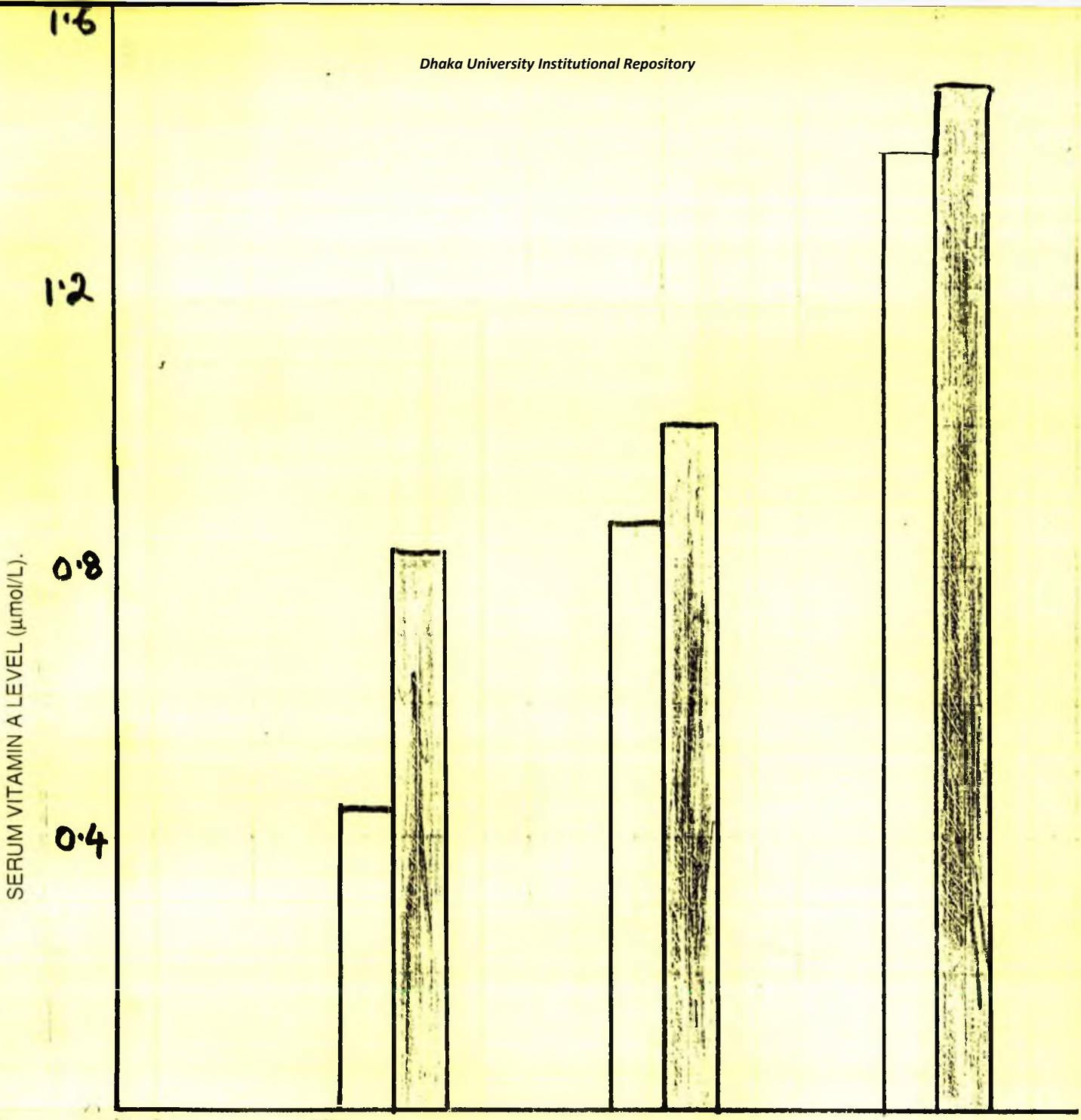



FIGURE 9: SERUM VITAMIN A LEVEL (µmol/L) IN PATIENTS BEFORE AND AFTER INTAKE OF DRUGS IN SINGLE BLIND TRAL.

BEFORE INTAKE MEAN = 0.70±0.39.

AFTER INTAKE MEAN = 0.95±0.60

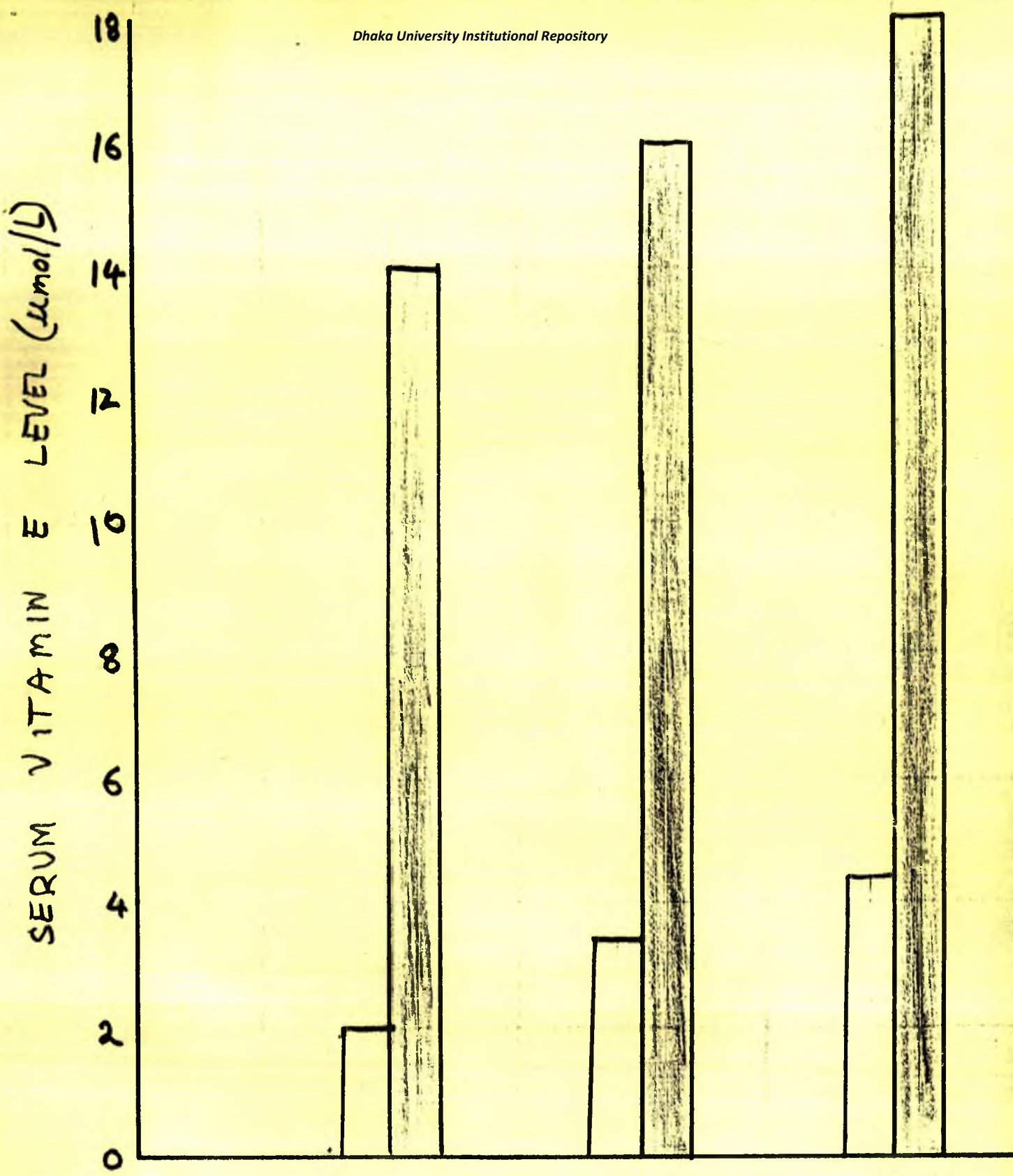



FIGURE 10: SERUM VITAMIN E LEVEL (µmol/L) IN PATIENTS BEFORE AND AFTER INTAKE OF DRUGS IN SINGLE BLIND TRIAL

BEFORE INTAKE MEAN 3.20±1.09.

AFTER INTAKE MEAN 13.93±6.29

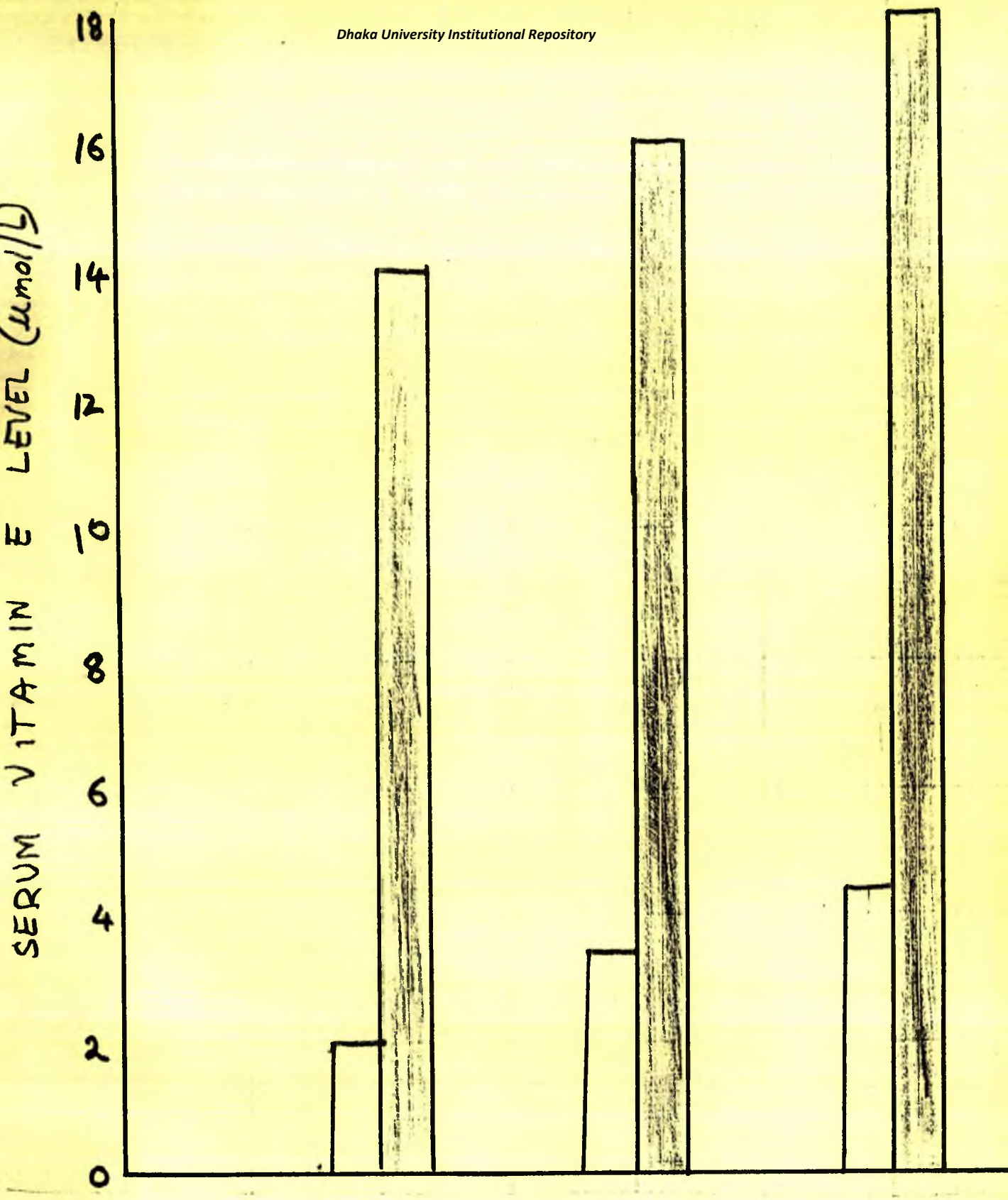



FIGURE 10: SERUM VITAMIN E LEVEL (µmol/L) IN PATIENTS BEFORE AND AFTER INTAKE OF DRUGS IN SINGLE BLIND TRIAL

BEFORE INTAKE MEAN 3.20±1.09.

AFTER INTAKE MEAN 13.93±6.29

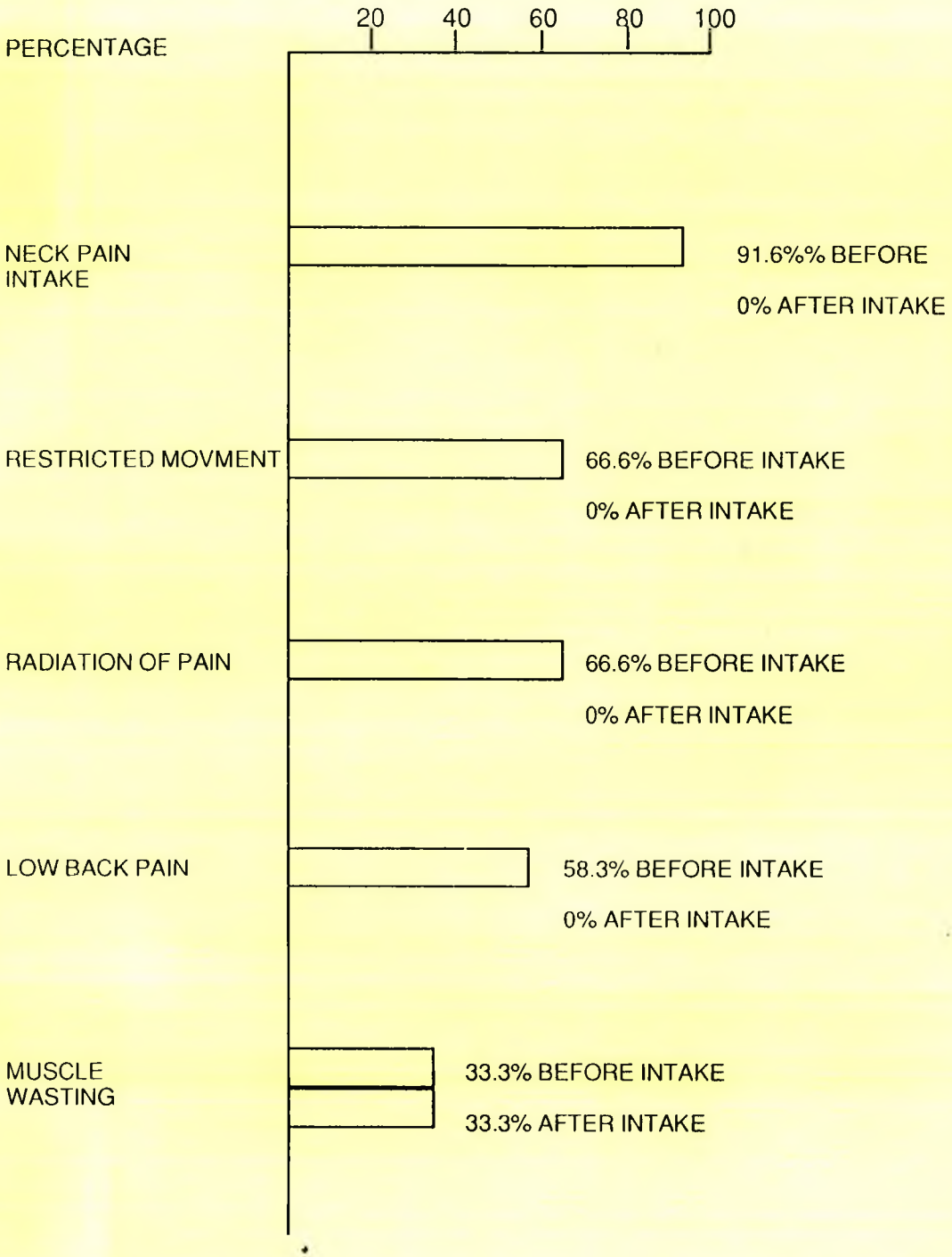


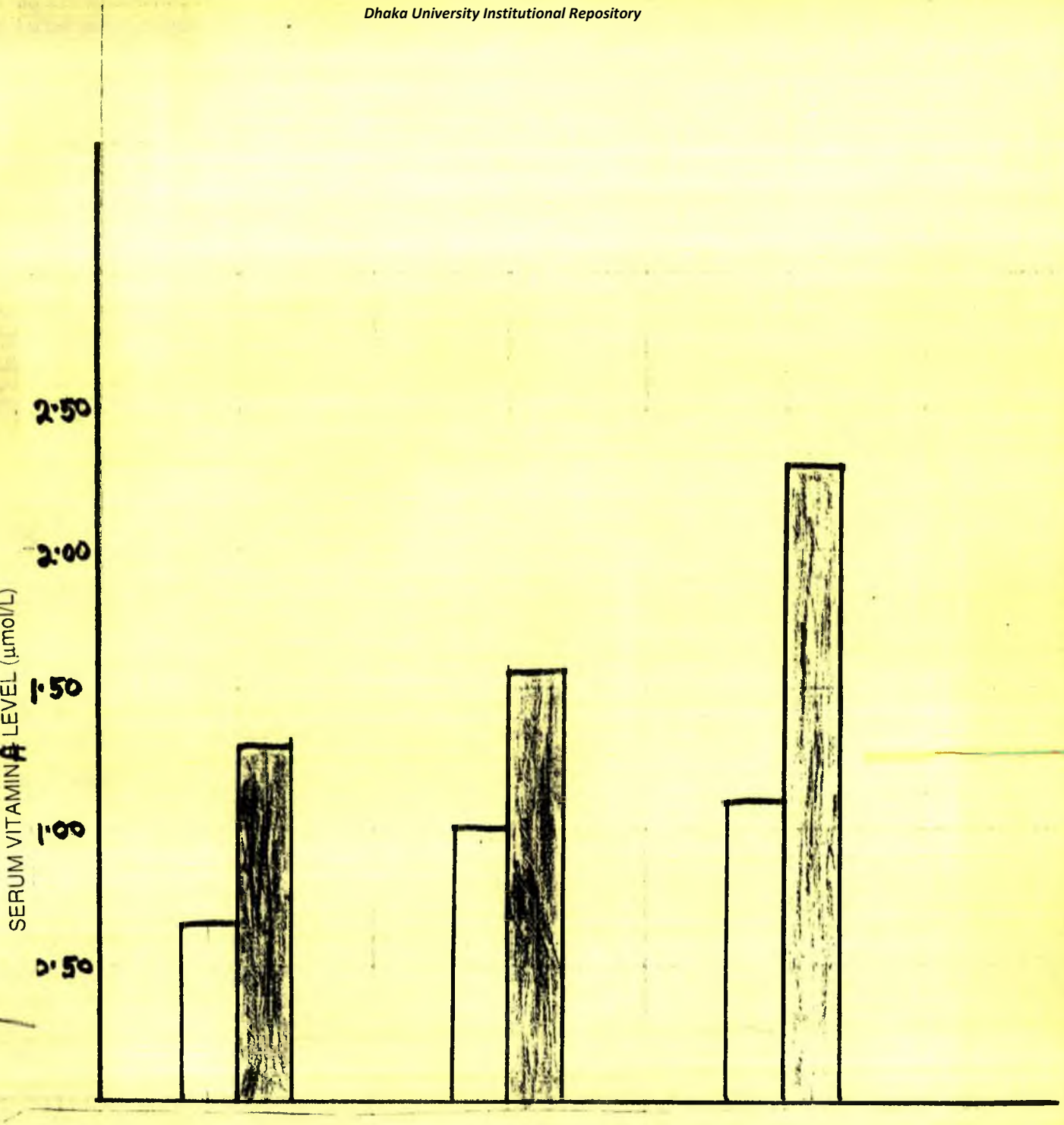

FIGURE 11 : SYMPTOMS AND PERCENTAGE OF PATIENTS BEFORE AND AFTER INTAKE OF DRUGS IN THE SINGLE BLIND TRIAL.

In Figure 12 is shown that before intake of sample No. 1 containing vitamin A, the mean serum vitamin A level was 0.87±0.28 umol/L The mean serum vitamin A level was 1.67±0.6 umol/L after intake ($P<0.1$) for three weeks. The patients with serum level of 0.60 umol/L had arise in serum level to 1.26 umol/L. Before intake of the drugs, the patients who had an average serum value of 1.00 umol/L had an increase of their serum level to 1.55 umol/L. The patients whose serum level was 1.2 umol/L initially had an increased value of 2.3 umol/L.

Figure 13 show that the difference of mean serum vitamin E level before and after intake of sample containing vitamin A only is not at all significant. The mean serum vitamin E level was 10.44±4.6 umol/L and after intake the mean serum level was 10.10±4.3 umol/L.

The intensity of pain in the patients in the double blind trial who took sample containing vitamin A is shown in Table No. 18. The intensity of pain ranged from mild to severe in 6 patients before intake. After intake, the intensity of pain remained the same in 4 patients, in one patient it rose from mild to moderate and in another it rose from moderate to severe intensity.

Figure No. 14 represents a bar chart showing some symptoms of the patients before and after intake of sample No. 1 containing vitamin A. 83.3% patients were suffering from neck pain an 33.3% from low back pain. There was restricted spinal movement in 66.6% patients. Even after intake of the sample, the patients were still suffering.




SERUM VITAMIN A LEVEL (µmol/L) IN PATIENTS (n=6) BEFORE AND AFTER INTAKE OF SAMPLE CONTAINING VITAMIN A.

BEFORE INTAKE MEAN 0.87±0.28.

AFTER INTAKE MEAN 1.67±0.60

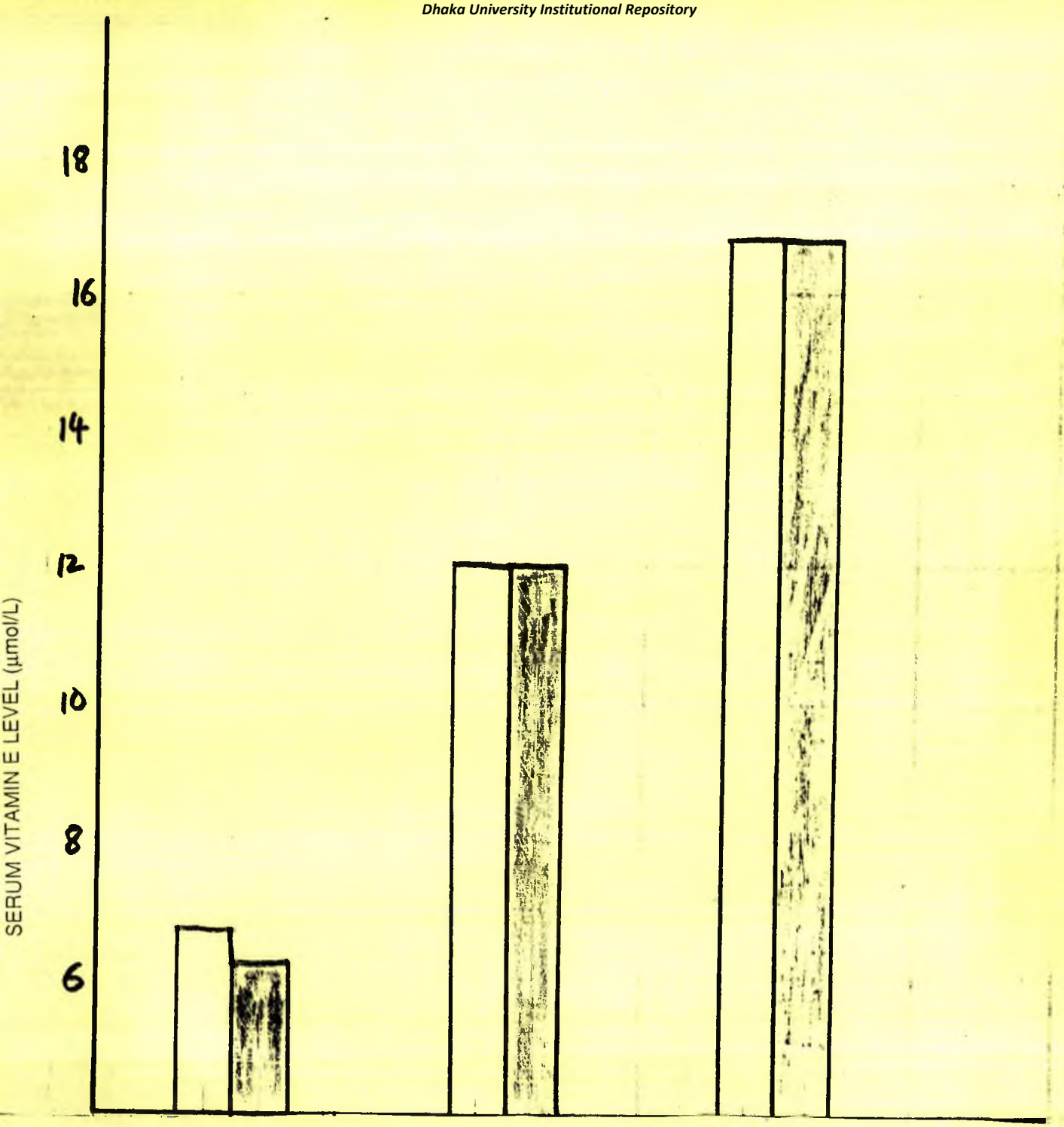



FIGURE 13: SERUM VITAMIN E LEVEL (µmol/L) IN PATIENTS (n=6) BEFORE AND AFTER INTAKE OF SAMPLE CONTAINING VITAMIN A.

BEFORE INTAKE MEAN 10.14±4.6.

AFTER INTAKE MEAN 10.10±4.3

TABLE NO -18

INTENSITY PAIN IN PATIENTS BEFQRE AND AFTER INTAKE OF SAMPLE NO 1 CONTAINING VITAMIN A.

| NO. | INTENSITY QF PAIN | |
|---|---|---|
| | BEFQRE INTAKE | AFTER INTAKE |
| 1. | + + | + + |
| 2. | + | + + |
| 3. | + + | + + |
| 4. | + + | + + |
| 5. | + + + | + + + |
| 6. | + + | + + + |

+ = mild intensity of pain.

++=moderate intensity of pain.

+++= Severe intensity of pain.

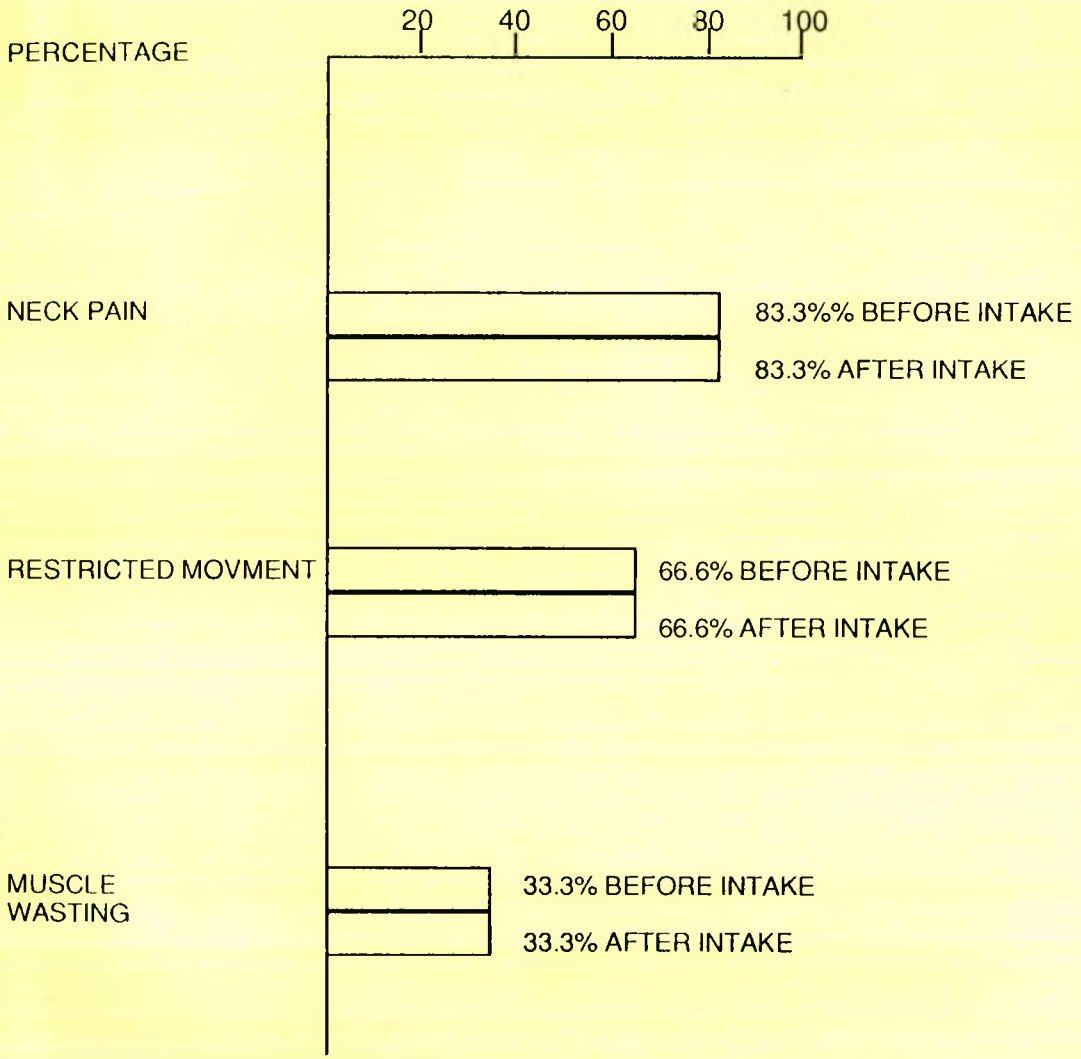



FIGURE 14 : SYMPTOMS AND PERCENTAGE OF PATIENTS BEFORE AND AFTER INTAKE OF OF SAMPLE CONTAINING VITAMIN A.

In Figure No. 15 is shown the serum vitamin A level of the patients who took sample containing both vitamins A and E. Patients whose mean serum vitamin A level was 0.80 umol/L intially had an increased value to 1.70 umol/L. The mean serum level was 1.76 umol/L after intake in those who had 1.0 umol/L before intake. In those, who had an serum value of 1.24 umol/L before intake had a rise to 2.2 umol/L after. The mean serum vitamin A level was 1.09±0.30 umol/L of all the patients before intake. After intake the average vitamin A level rose to 4.61 umol/L ($P<0.2$).

The Figure No. 16 shows the serum vitamin E level of the patients taking the sample containing vitamin A and E. Before intake the average serum vitamin E was 4.06±1.97 umol/L which rose to 17.14±6.81 umol/L. The rise was over 4000% and was statistically significant ($P<0.005$). The patients who were initially 2 umol/L had increased to 20 umol/L there level of vitamin E. Those who had a mean value of 4 umol/L rose to 23 umol/L. The mean serum level was 24 umol/L after intake in those patients who had 6 umol/L before intake.

Table No. 19 shows that after intake of sample No. 2 (i. e. vitamin A and E), all the patients were completely relieved of their pain after the therapy.

In Figure No. 17 is shown the symptoms and percentage of patients who took sample containing vitamin A and E by a bar chart. All the patients were suffering from neck pain before intake. 66.6% were suffering from restricted movement, 16.6% from low back pain and 16.6% from radiation of pain before intake of the drugs. All the patients were relieved after intake of the sample.

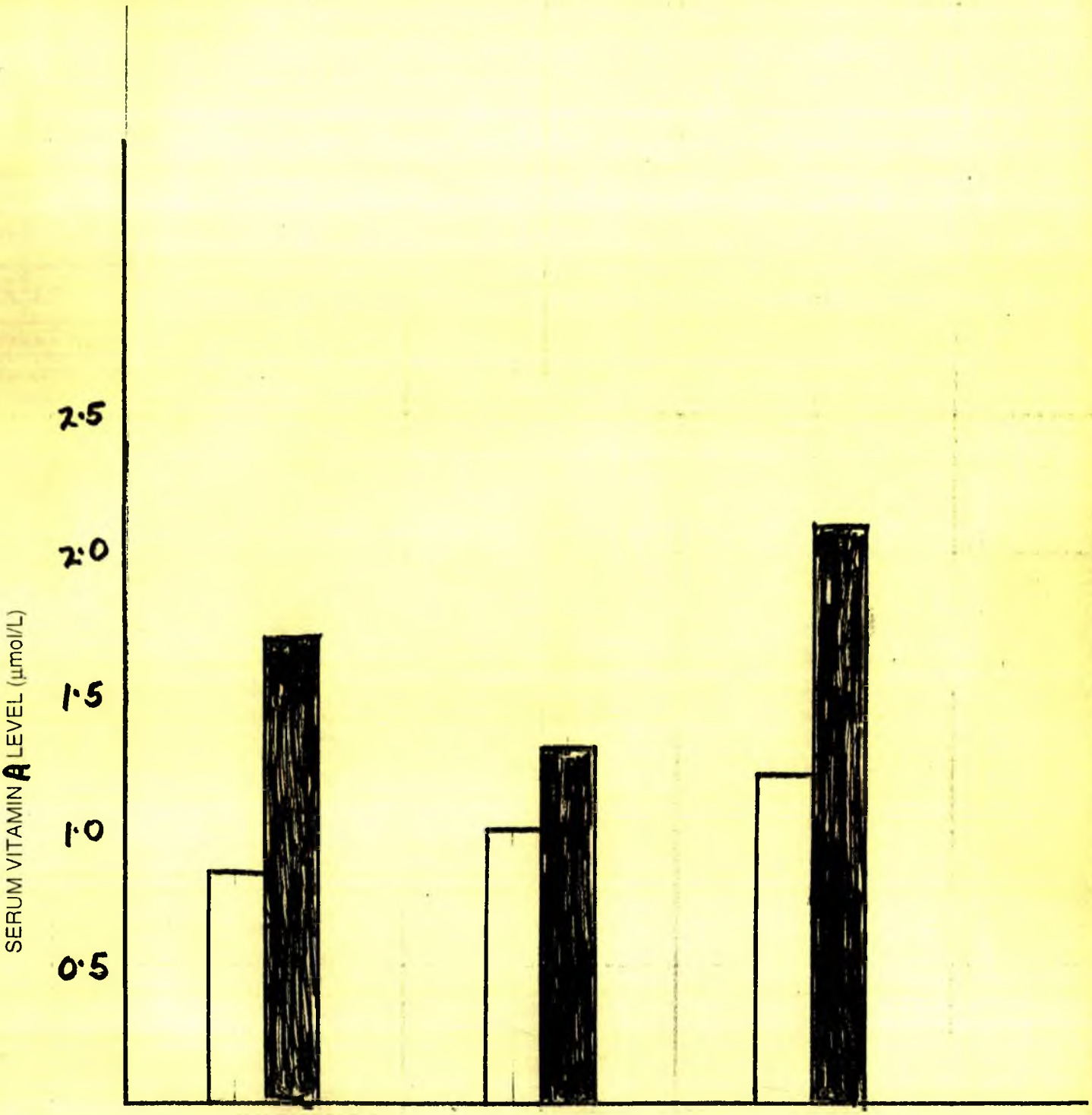



FIGURE 15: SERUM VITAMIN A LEVEL (µmol/L) IN PATIENTS (n=6) BEFORE AND AFTER INTAKE OF SAMPLE CONTAINING VITAMIN A AND E.

BEFORE INTAKE MEAN 1.09±0.30.

AFTER INTAKE MEAN 4.61±0.40

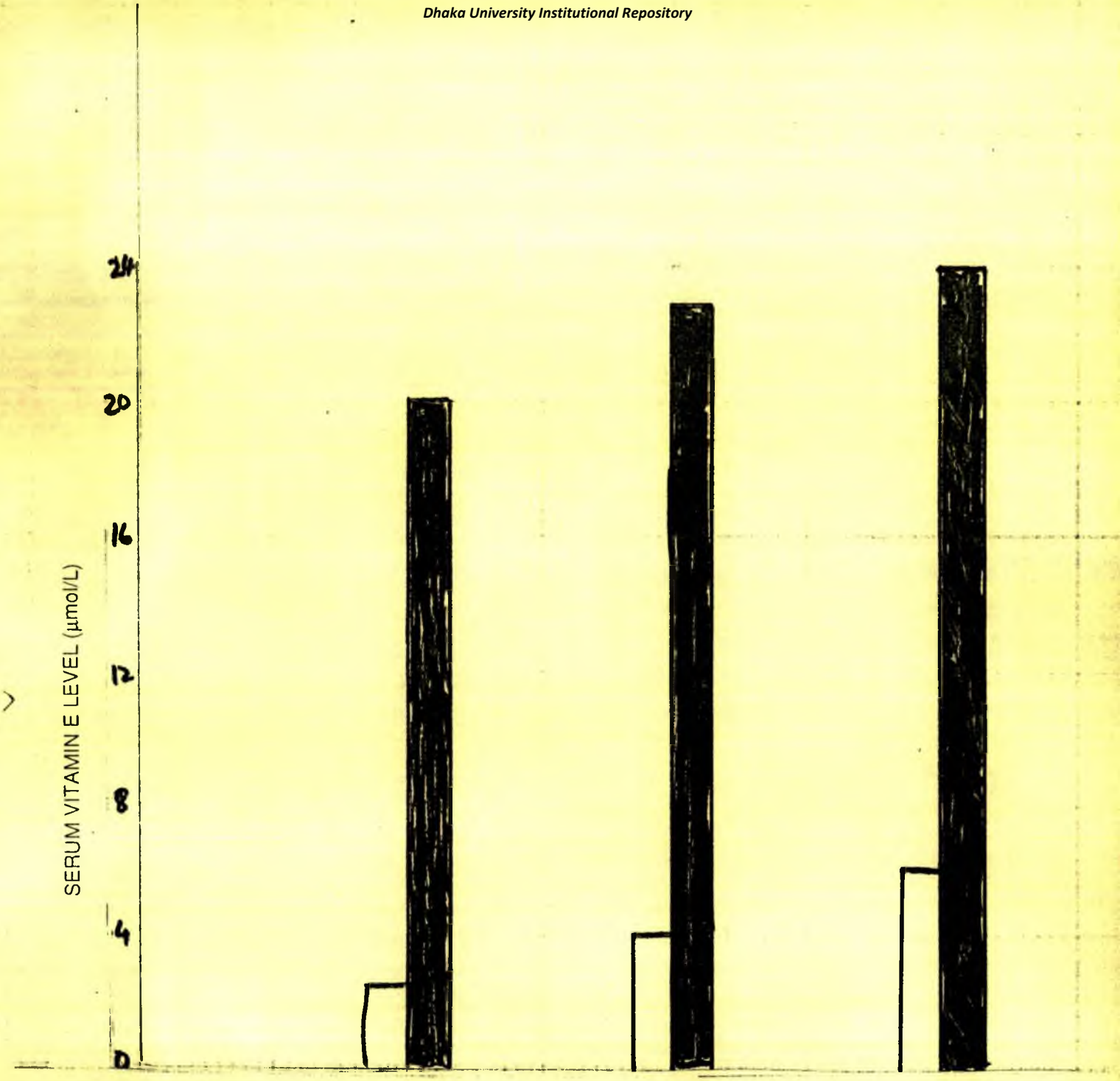



FIGURE 16 : SERUM VITAMIN E LEVEL (µmol/L) IN PATIENTS (n = 6) BEFORE AND AFTER INTAKE OF SAMPLE CONTAINING VITAMIN A AND E.

BEFORE INTAKE MEAN 4.06±1.92.

AFTER INTAKE MEAN 17.14±6.81

TABLE NO -19

INTENSITY OF PAIN OF PATIFINTS (N=6) BEFORE AND AFTER INTAKE OF SAMPLE NO 2 CONTAINING UITAMIN A AND E.

| | INTENSITY OF PAIN | |
|---|---|---|
| NO. | BEFQRE INTAKE | AFTER INTAKE |
| 1. | + + + | Complete relief |
| 2. | + + | Complete relief |
| 3. | + + | Complete relief |
| 4. | + + + | Complete relief |
| 5. | + + | Complete relief |
| 6. | + + | Complete relief. |

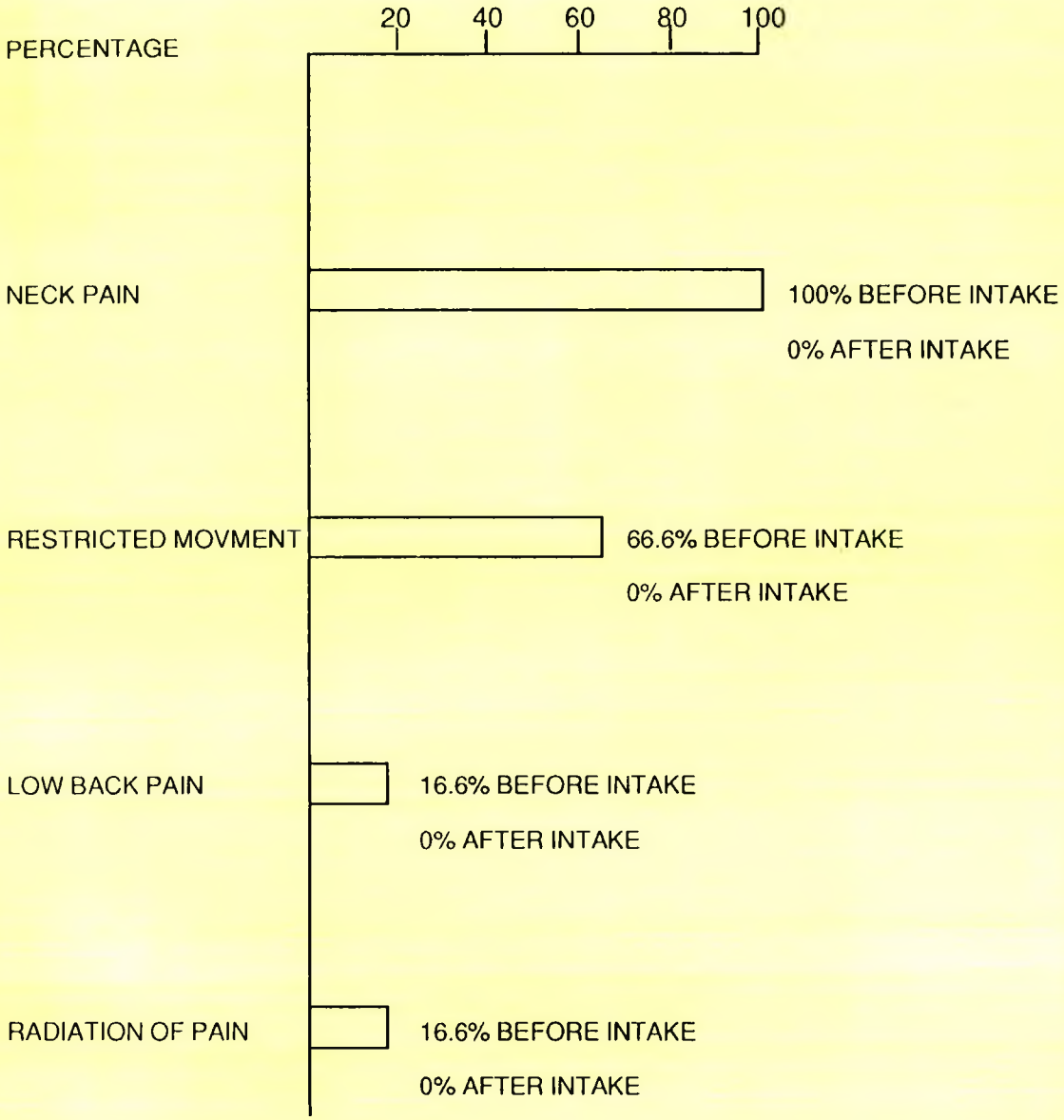



FIGURE 17 : SYMPTOMS AND PERCENTAGE OF PATIENTS BEFORE AND AFTER INTAKE OF SAMPLE CONTAINING VITAMIN A AND E.

Figure No. 18 shows that after three weeks of drug intake. Sample No. 3 containing vitamin E only, the rise in mean serum level of titamin A is not significant.

Figure No. 19 shows the serum vitamin E level of the patients taking sample containing vitamin E for three weeks. The patients whose mean level was intially 2.2 umol/L had an increase to 11 umol/L. Those who had initially 4 umol/L had a rise upto 24 umol/L. In those whose mean serum value was 9.5 umol/L before, had an increase. to 26 umol/L after intake. This rise was 3.5 fold altogather and was statistinally significant ($P<0.01$).

The clinical features of the patients taking sample No. 3 (i.c. vitamin E) are depicted in Table 20. It was found that same as sample No. 2, Sample No. 3 also resulted in complete relief of pain of all patients irrespective of whether they had moderate to severe pain before the therapy.

Fig. 20 respresents a bar chart showing the symptoms of patients berfore and after intake of Sample No. 3. 87.5% patients were suffering from neck pain, 12.5% from low back pain 25% from restricted spinal movement and 12.5% from low back pain 25% from restricted spinal movement and 12.5% from radiation of pain before the therapy. None were suffering from these symptoms after the intake of Sample No. 3.

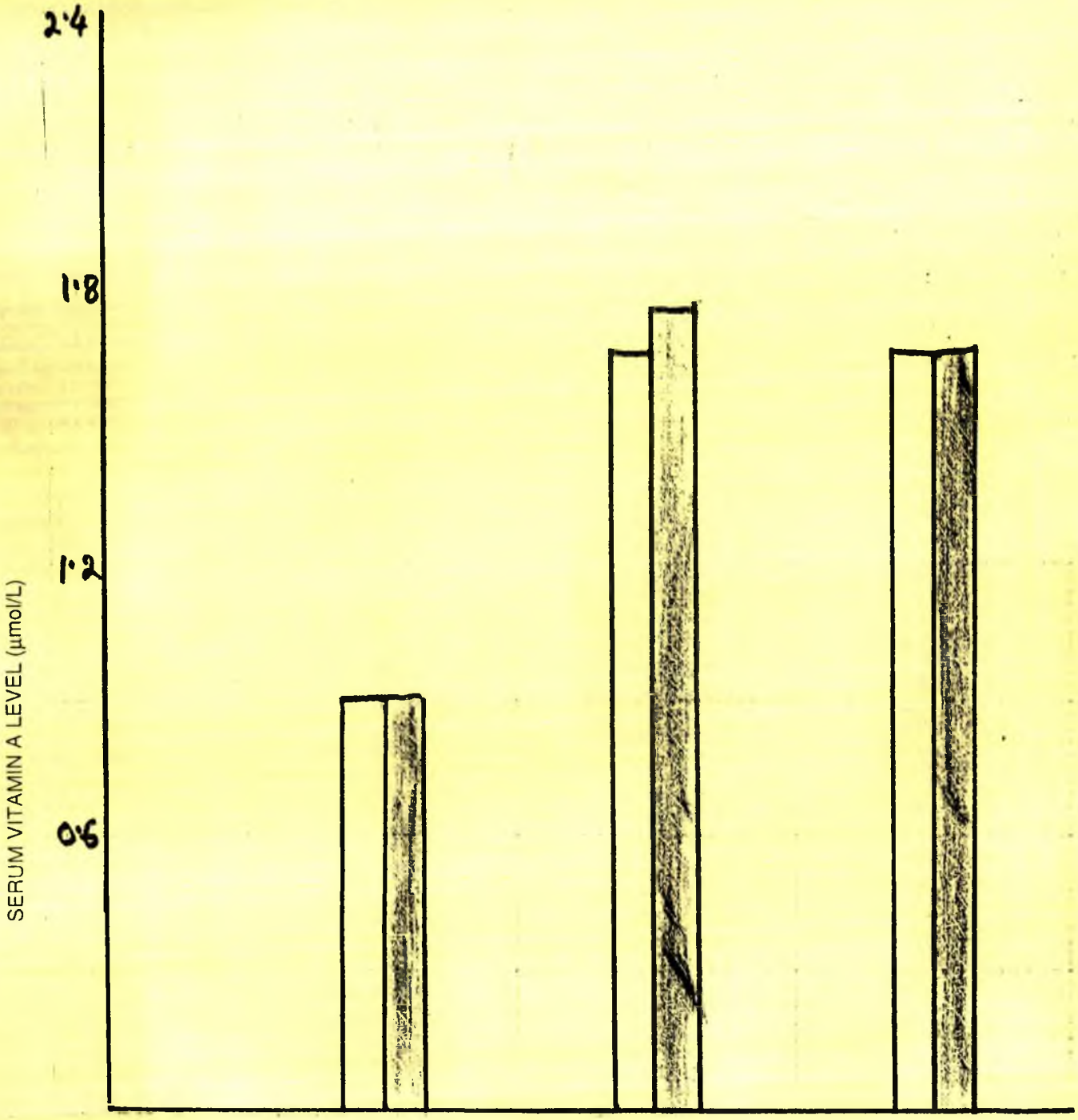



FIGURE 18: SERUM VITAMIN A LEVEL (µmol/L) IN PATIENTS (n=8) BEFORE AND AFTER INTAKE OF SAMPLE CONTAINING VITAMIN E ONLY

BEFORE INTAKE MEAN 1.38±0.43.

AFTER INTAKE MEAN 1.40±0.51

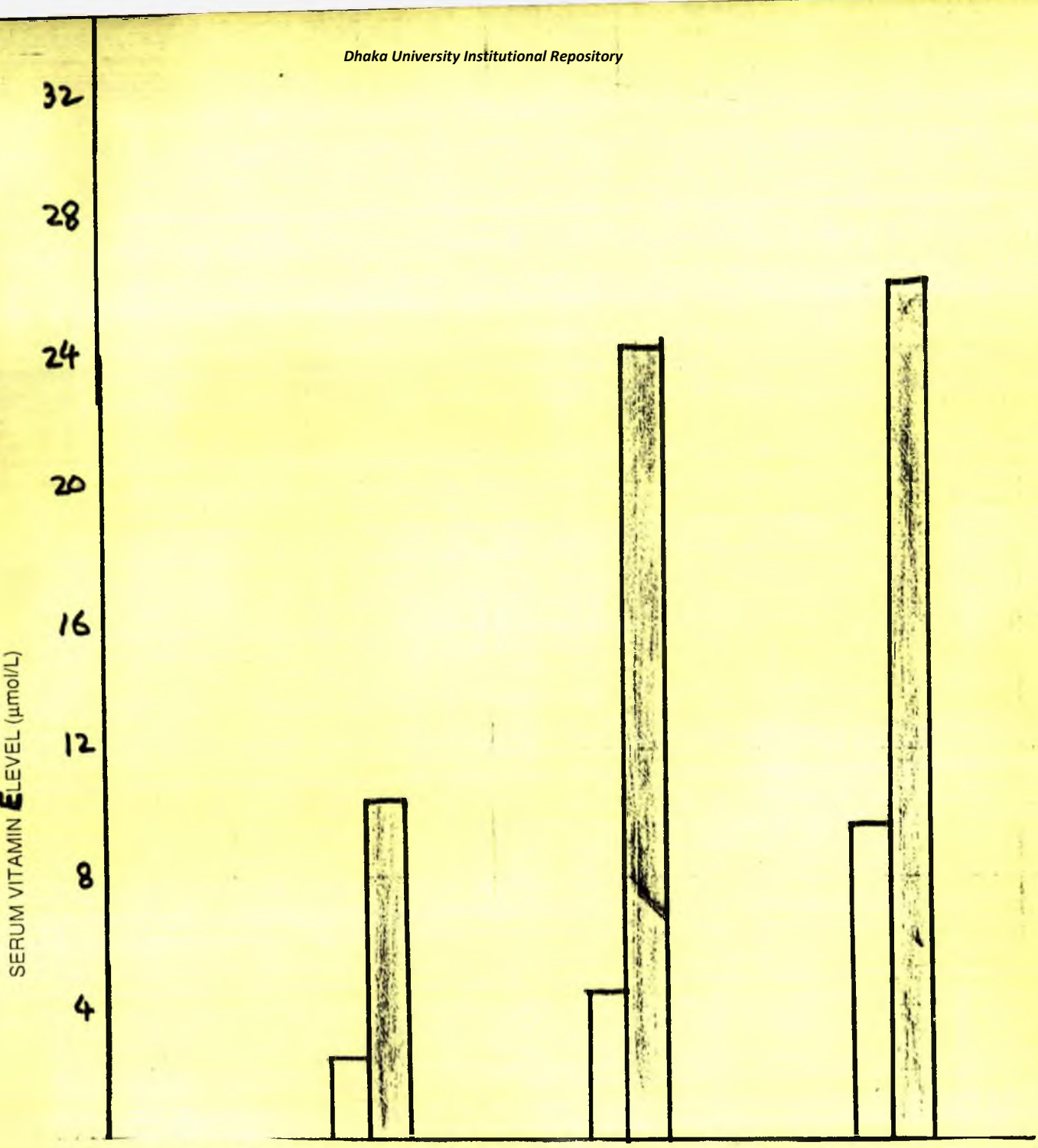



FIGURE 19: SERUM VITAMIN E LEVEL (µmol/L) IN PATIENTS (n=8) BEFORE AND AFTER INTAKE OF SAMPLE CONTAINING VITAMIN E ONLY

BEFORE INTAKE MEAN 5.80±3.23

AFTER INTAKE MEAN 20.43±11.53

TABLE NO-20

INTBNSITY OF PAIN IN PATIENTS (N=8) BEFORE AND AFTER INTAKE OF SAMPLE NO 3 CONTAINING VITAMIN E.

| | INTENSITY OF PAIN | |
|---|---|---|
| NO. | BEFQRE INTAKE | AFTER INTAKE |
| 1. | + + | Complete relief |
| 2. | + + + | Complete relief |
| 3. | + + | Complete relief |
| 4. | + + | Complete relief |
| 5. | + + | Complete relief |
| 6. | + + | Complete relief |
| 7. | + + | Complete relief |
| 8. | + + + | Complete relief. |

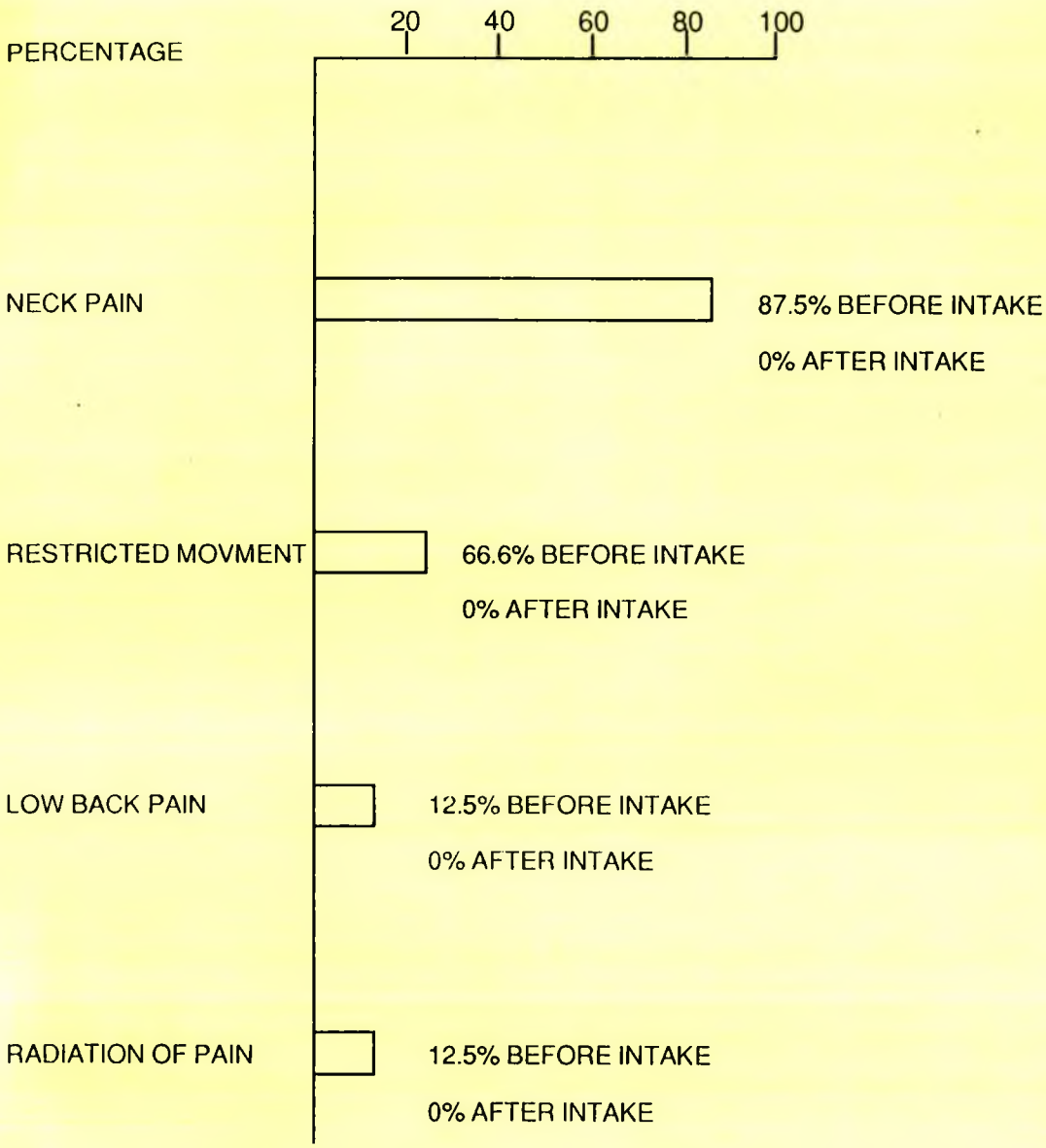



FIGURE 20 : SYMPTOMS AND PERCENTAGE OF PATIENTS (n=8) BEFORE AND AFTER INTAKE OF SAMPLE CONTAINING VITAMIN E.

## VI. DISCUSSION:

The present study gives a clear indication that vitamin E has a remedial action against spondylosis.

The mean age of the patients included in the study was 50±6.6 years. We did not find any patients below forty years of age.. Furthermore our study shows that 59.4% of the patients were from the age group 40-50 years, 34.4% were in the age group 51-60 years and 6.2% were aged more than that. This therefore shows that the disease spondylosis is one of old age occuring after the fortieth year. This agrees with Garett and Ahmad [6] who observed that inpairments of the spine ranked third after heart disease in patients aged 45 to 64 years and also with Ghusid[105] who recorded spondylosis as occurring in the mid fifties in both sexes.

We had twenty four male patients but only eight female patients. Schmorl and Junghans[s] also observed a higher prevalence of the disease in the males (87%) than in females (74%). This observed sex difference in prevalence of spondylosis could be due to the fact that because of socio-economic reasons and traditional beliefs, females tend to attend medical facilities less than the male patients.

The educational background of the patients show 50% of the patients had a Masters or equivalent degree. Out of the remaining 50%, 21.90% held a bachelorsor equivalent degree, 9.4 had finished the Higher Secondary Certificate (H.S.C.) examination and 18.7% had the Secondary School Certificate (S.S.C.) degree. All the patients were thus more or less educated. They also appeared to be quite health conscious.

It could be seen from the income distribution of our study that 81.3% of the patients had monthly income between Taka 2,000 and Taka 5,000 and the remainder had monthly income above this amount. Even though their income appears more than the average per capita income in the country it is possible that because of lack of nutritional awareness particularly with regard to the vegetable oils and other foods rich in vitamin A and Vitamin E, the patients were suffering from the degenerative disease that is knowns to arise from

chronic lack of anti-oxidants in the body. This agrees with the findings of Martin[31] who suggested that degeneration in ageing is not only due to formation of free radicals in the tissues but also due to lack of antioxidants.

Our study revealed that 93.8% of the patients presented with the cardinal clinical features[29] of neck pain on movement. Eleven of the patients suffered from low back pain as well. It could be that they were suffering from lumber disc degeneration in addition to cervical disc degeneration. As already mentioned earlier, this is a disease occurring in old age, probably due to constant wear and tear of the vertebral joints. It might be that due to more use of the neck (nodding, tolking) and the back (sitting, walking), the cervical and lumber vertebral joints are most affected.

There was restricted range of movement in 53% patients which might be due to osteoarthritic change in the joints. Radiation of pain occurred in the shoulder or upper arm or fingers in 81.37 of the patients (Table 13). This pain might be occurring due to compression of a nerve root supplying that specific dermatome by cervical disc protusion [29] on forminal encroachment by osteophytes.

At the time of the study about $\frac{3}{4}$ths (71.8%) of the patients were suffering for nearly a year (Table 14) and the rest more than that. Since it is a chronic disease of insiduous onset it could be taht the sufferings were not that marked or they had been getting medical attention off and on. It would also be that patients attended a physician only during the acute phese of the disease and once he got used to it, he started getting irregular medical attention.

That more than 60% of the patients were suffering from morning stiffness and limitation of activity for a year also indicated the chronic nature of the disease.

Seventeen patients (39.5%) had radiation of pain (Table 16) to the arm and nearly 21% felt the radiation of pain to the shoulder girdle and scapula. The shoulder and the arms are the most used parts of the body whatever may be the occupation of the patients and these being innvervated by nerves from $C_{6-7}$ tend to become subject to degenerative changes more than the rest of the body.

From the roentographic features it was observed that 43.8% had mild, 37.5% had moderate, and 18.3% had severe degeneration. These X-rays confirmed our diagnosis (Table 17).

In the single blind study which was begun with twelve patients to test the efficacy of vitamis A and E it was observed that the mean serum vitamin A of the patients who participated in the trial was 0.70±0/.0.4 umol/L before intake of the drugs. This value is within the normal range of 0.53 to 2 umol/L. After intake of vitamin A (50,000 IU daily), the mean serum vitamin A level rose to 0.95±I0.6 umol/L (Fig 9). The rise however was not statistically significant ($P<0.5$). The reason for this low rise in vitamin A level is not clear but low level of retinol binding protein (RBP)[98] could be responsible for this. A reason for low RBP level in the patients of the present study could be low protein intake.

It was observed that the serum vitamin E (Fig 10) before intake of the drugs was 3.0±1.09 umol/L. This level was thus much lower than that normal value of 11.61 to 44 umol/L. Since even a very low plasme tocopherol level for a long time show very little deficiency [80] symptoms as was proved by the Elgin project, the patients were able to lead a more on less normal life. After intake of vitamin E (100 mg daily) the average serum vitamin E level increased to 13.93±6.3 umol/L.The rise was highly significant ($P<0.005$).

In the single blind study, more than 90% of the patients were suffering from neck pain and more than 50% from low back pain as well. Before intake of the drugs, about $\frac{2}{3}$rds (66%) of teh patients were suffering from restricted spinal movement and radiation of pain. After the trial with vitamin E all patients were found to have recovered completely from the symptoms they had before the study began. This proves the efficacy of vitamin E as the experimental drug for which purpose the single blind trial was undertaken. The patients who were suffering from muscle wasting did not respond to vitamin E administration because once there is wasting of the muscles due to nerve degeneration, muscles do not regenerate.

After the efficacy of the drugs (vitamin E) was seen in the single blind studt, a randomised double blind study was carried out with twenty patients in which neither the patients nor the investigators were aware of the type of drugf being given.

Out of these twenty patients, six were given vitamin A (sample No. 1) (at a dose of 50,000 IU daily) six patients received a mixture of vitamins A and E (sample no 3) at the dose of 50,000 IU and 100 mg daily respectively and the remaining eight patients were given vitamin E (sample No 3) at a dose of 100 mg daily. All the patients were asked to take their drugs every day for three weeks. It was fortunate that all twenty patients of this double blind study turned up for the second examination and therefore effect of the drugs could be examined fully and the results obtained might be taken as satisfactory.

In the patients taking the sample containing vitamin A only (Fig 12) the average serum vitamin A level increased from 0.87± 0.9 umol/L to 1.67±0.6 umol/L. This change was signaificant. On the other hand, the mean serum vitamin E level remained unchanged at the level of 10.1±4.3 umol/L.

Patients taking this sample for three weeks had no relief what so ever in spite of having an increased average serum vitamin A level in fact there was an in crease in the intensity of pain in two patients of this group (Table 18). These patients were not also relieved of the other symptoms too (Fig 14) such as restricted spinal movement.

The serum vitamin E level before intake of sample no 2, containing both vitamin A and E averaged 4.06±1.97 umol/L. After intake the average serum vitamin E level rose to 17.14±6.81 umol/L (Fig 15). The difference was highly significant ($P<0.005$). The mean serum vitamin A level also changed from 1.09±0.30 umol/L to 4.61±1.61 umol/L Unlikethe patients taking sample no 1. all patients receiving sample no 2 were completely relieved of their pain, regardless of whether they had moderate or severe pain before the study (Table 19) Fig 17).

In patients who recieved, sample no 3 containing vitamin E only, the average serum vitamin E level rose from 5.8±3.2 umol/L to 20.43±11.53 umol/L with obviously no change in vitamin A level (Fig 18). All eight patients receiving this drug were completely relieved of their pain (Table 20) and other symptoms as well (Fig 19).

However, there were patients who already had normal vitamin E level in their plasma (13.25±2 umol/L) and upon administration of vitamin E for three weeks (100 mg/day) there was no or little change in this level. But untill interestingly, the patients reported to be relieved of their pain. Relief of pain in these patients

with no concomitant rise in their plasma vitamin E level can not be explained at this time. Pain is a symptom in response to motor nerve stimulation in the pain perception pathway and in human it is a relative term varying widely from person to person. Because we did not have any devise to measure the intensity of pain of the patients we had to depend on the patients regarding the intensity of the pain they were complaining. Therefore it could be plausible that the so-called relief of these patients from pain upon adminsitration of a drug could just be a psychological phenomenon.

From the results and the foregoing discussion, it thus appears that the patients suffering from spondylosis had serum vitamin E level below normal and the patients in whom the serum vitamin E level rose up to the normal value after three weeks of vitamin E intake there was relief of pain. The serum vitamin A level of these patients were observed to be well within the normal value [48] and this vitamin did no seem to have any effect whatsoever on the symptoms.

P. T. O

## VII. CONCLUDING REMARKS

During the process of free radical reaction that takes place in spondylosis, termination or inactivation of these free radicals is obviously of great benefit, and naturally this is the mechanism by which normal cells protect themselves from free radical injury. In biological system, termination is achieved by several means. Out of all the exogenous or endogenous antioxidants vitamin E is by far the most inportant which either block the initiation of free radical formation or inactivate (i.e. scavenge) the free radicals.

Many early investigators had already recognised that tocopherols could function as an antioxidant in vitro. Later, when peroxide residues were detected in the adipose tissues of vitamin E deficient animals, it was proposed [29] that vitamin E also functioned as an invivo antioxidant.

Vitamin E inadequacy has been seen in many biochemical and pathological conditions in which there is alteration in membrane structure and function by free radical attack.

Pathologically it was seen that degeneration that occures in spondylosis may be due to free radical damage of the proteoglycans of the intervertebral discs. It may be that vitamin E given to the patients in our study prevents further degeneration of the intervertebral discs, so that patients got relief from pain. Since the serum vitamin E level of these patients were low according to normal value, they suffered from the degenerative effects due to lack of antioxidants. When the serum vitamin E level of the patients in our study were within the normal level, they were relieved of pain.

Because of the small number of patients examined the present study may be considered as a pilot study. A large scale study comprising of a sizeable number of patients (hundreds) should be initiated on the basis of the findings of the present study. Also because tocotrienols are now claimed to be 40-60% more potent antioxidant[106] than the tocopherols (both known as vitamin E), the study should be done, if possible with tocotrienols, or at least with a mixture of the two.

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