STUDIES OF THERAPEUTIC EFFECT OF BETA RADIATION ON ONYCHOMYCOSIS FOR AN INNOVATIVE MODALITY OF TREATMENT

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This thesis is submitted in fulfilment of the requirements for the degree of Doctorate of Philosophy (PhD) under the faculty of Post-Graduate Medical Sciences & Research, University of Dhaka. This work has been carried out at the Centre for Nuclear Medicine and Ultrasound, Dhaka Medical College (DMC) Campus, Bangladesh Atomic Energy Commission (BAEC), Department of Skin & VD, Department of Microbiology, Dhaka Medical College from 1998 to 2002. This is an original and innovative type of work and to the best of my knowledge it has not yet been done anywhere in Bangladesh.

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Certification of thesis work

The thesis titled ***"STUDIES OF THERAPEUTIC EFFECT OF BETA RADIATION ON ONYCHOMYCOSIS FOR AN INNOVATIVE MODALITY OF TREATMENT"*** *is submitted by Dr. Shahana Afroz, . Reg.10/97–98, Re Reg.73/02-03 in fulfilment of the requirements for the degree of Doctorate of Philosophy (PhD) under the University of Dhaka. This is an original and innovative type of work and so far has not yet been done elsewhere in the country. The work is interesting to us and is being approved by:*

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DEDICATED TO

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for guiding me on the right path to earn Rizq-e-Halal;

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ABSTRACT

Onychomycosis is the most frequent cause of nail disease and the most prevalent type of dermatophytosis in Bangladesh. The humid and warm climate of this tropical country is congenial for the growth of fungi. Therapeutic limitations of conventional antimycotic agents in respect of low cure rates, high relapse rate, inherent side effects, long duration of treatment and high cost in treating onychomycosis have provided clear incentives to explore alternative forms of treatment procedure.

The primary aims of the present thesis work were (i) to use beta radiation as a curative therapy for Onychomycosis, optimisation of its dosages and to promote an innovative clinical development in the field of therapeutic application of nuclear medicine; (ii) to assess the efficacy of beta radiation either alone or in combination with conventional antifungal therapy; and (iii) to reduce the duration of drug exposure and cost of treatment for onychomycosis.

The present study is an open, randomised and controlled trial to verify the efficacy of beta radiation in patients with onychomycosis. Using the appropriate statistical formula, sample size of the study population was determined and in each group 92 patients were came out. With an assumption of patients drop out and for better statistical analysis, a total of 330 patients, who fulfilled the inclusion criterion having diagnosed to have onychomycosis clinically and mycologically were randomly allocated to enter in therapeutic regimen. Study population was randomised in three groups. Group – A (n –110) received griseofulvin orally 500mg once daily for 12-16 weeks; Group – B (n=110) received beta radiation, 500 rads bi-weekly for 3 weeks (total 2500 rads); and Group – C (n=110) received combined beta radiation (total 2500 rads in 3 weeks) and griseofulvin (500 mg daily for 6 weeks). Patients were followed up for 24 weeks. Efficacy of the treatment was evaluated in all 287 patients while 43 (13.03%) cases were dropped out from the initial allocation.

At the end of the follow up period (6 months after discontinuation of treatment) mycological cure rate was achieved 41 (42.70%), 36 (38.70%) and 65 (66.33%) in Group-A, Group-B and Group-C respectively. The mycological cure rate was highly significant (P=0.000) and considered to be the acceptable outcome of treatment. Clinical cure rate was considered as another way of assessment. Percentage of clinical cure rate was similar as mycological cure rate and equally significant (P=0.000). Recurrence rate of the disease was highest in griseofulvin-induced patients 21 (21.88%) and in beta radiation exposed patients was 14 (15.06%). This rate was least in combination therapy group of griseofulvin and beta radiation 4 (4.08%). Cure rate in Group – C is significantly higher than Group – A and B as well (P=0.000).

It can be concluded that in Group – C as the cure rate is highest, recurrent rate is the lowest, duration and cost of treatment are significantly less, this modality of treatment can be considered as the more acceptable procedure for management of onychomycosis in developing country like Bangladesh. Group – B (beta radiation only) can also be accepted in special occasions to replace Group – A (Antifungal).

This innovative treatment procedure could be introduced in other Nuclear Medicine Centres of the country with a view to expand the procedure, so that large number of patients could be beneficiary as end user.

CONTENTS

LOCATION OF FIGURES AND TABLES

ABBREVIATIONS

α – Alpha

β – Beta

γ – Gamma

AIDS – Acquired Immuno Deficiency Syndrome

BAEC – Bangladesh Atomic Energy Commission

BSMMU – Bangabandhu Sheikh Mujib Medical University

CNM&U – Centre for Nuclear Medicine and Ultrasound

DMCH – Dhaka Medical College & Hospital

DNA – Distal Nail Avulsion

DSA – Diazotised Sulphanilic Acid

DTM – Dermatophyte Test Medium

FDA – Food and Drug Administration

IAEA – International Atomic Energy Agency

IFCC – International Federation of Clinical Chemistry

IPH – Institute of Public Health

KOH – Potassium Hydroxide

NAS – National Academy of Sciences

RIT – Radioiodine Therapy

SDA – Sabourand Dextrose Agar

SLE – Systemic Lupus Erythomatosus

SSMC&MH – Sir Salimullah Medical College and Mitford Hospital

CHAPTER 1: INTRODUCTION & BACKGROUD

CHAPTER I: INTRODUCTION AND BACKGROUND

1.1 DERMATOPHYTOSIS

The skin constitutes the main site of recognizable fungal infections in human. The skin appendages – hair and nail are also vitally involved in these infections (Harry LA, 1998). These infections can be grouped into superficial and deep mycoses. The superficial fungal infection is termed *Dermatophytosis*. The *dermatophytes* are universally accepted as primary pathogens of previously healthy skin, nail and hair. *Dermatophytosis* is a clinical entity caused by at least three of the anamorph genera i-e. Trichophyton, Microsporum and Epidermophyton. In its restricted sense, *Dermatophytosis* is colonization by a dermatophytic fungus of the keratinized tissue of the human body *(Stokes & Ridgeway, 1987).* The terms *Ringworm* and *Tinea* are two synonyms of *Dermatophytosis*.

Depending on the part of the human body infected, *Dematophytosis* has certain distinctive features that are characteristic of the particular site. For this reason the *Tinea* is divided into the following types (Collins and Taylor, 1967, Hunter et al., 1981: Myrvik and Weiser, 1988; Mitchell, 1992):

(1) *Tinea capitis* (Scalp),
(2) *Tinea barbae* (Beard),
(3) *Tinea facici* (Face),
(4) *Tinea corporis* (Arms, Legs and Trunk),
(5) *Tinea manus* (Hands),
(6) *Tinea pedis* (Feet),
(7) *Tinea cruris* (Groin, perineum, perianal region) &
(8) *Tinea unguium/onychomycosis* (Nail).

1.2 GROSS ANATOMY OF NAIL

The nail is one of the appendices of the skin, the other name is ungues. It is the horny translucent plates of approximately triangular shape lying on the extensor surface of the distal segment of each digit.

The nail includes three major regions-

(1) Proximal root (radix).

(2) Exposed body of the nail.

(3) Distal border.

The thickness of matured nails varies from about 0.50 to 0.75 mm (Gray H, 1989; Forslind B, 1970; Hashimoto K, 1971).

1.3 ONYCHOMYCOSIS

Clinical impression indicates that there is a high prevalence of superficial mycoses (dermatophytosis) in Bangladesh (Chow MR & Haq MN, 1979). Among the dermatophytoses, the occurrence of onychomycosis was found to be the highest in comparison with the other varieties. In a mycological study fungus was isolated from body (88.2%), groin (94.6%), foot (84.6%) and nail (100%). (Alam, 1971) Scalp infection was not included in this study.

Onychomycosis is defined as the infection of nail by fungus and represents up to 30% of diagnosed superficial fungal infections (Harry L.A, 1998).

1.3.1 ETIOLOGY OF ONYCHOMYCOSIS

Universally recognized as etiologic agents are species of Epidermophyton, Trichophyton and rarely Microscrosporum fungi, but it may also be caused by other dermatophytes, yeasts and nondermatophytic moulds. Nondermatophytic moulds usually infect toe-nails and are rarely seen in finger-nails (Harry LA 1998; Scher RR 1999; Rippon JW, 1998). However, Dermatophytes remain by far the most common pathogen. In a large sample based study of more than 3,000 nails, it was found that 91% of fungal infections were caused by Dermatophytes, 6% by Candida and 3% by non-dermatophyte moulds (Summer Bell RC, Kane Jand K S, 1989). In another survey, the etiology of onychomycosis included dermatophytes (68%), yeasts (28%) and moulds (07%) (Galimberti RL et al., 1991; Clayton YM, Hay RJ, 1993; Ramani R et al., 1993).

1.3.2 PATHOGENESIS OF ONYCHOMYCOSIS

The natural history of dermatophyte infection is the same initially in all types of the disease. Colonization begins in the outer most layer of the skin and the ultimate outcome of the disease depends on host, strain, species variation of the fungus and anatomic site. (Clayton YM & Midgley G, 1985)

Infection in onychomycosis extends from the lateral nail fold. The fungus first invades the Keratin of the nail-bed and then extends to the lower surface of the nail plate. The most superficial part of the nail is rarely invaded. As the disease progresses, there is a disturbance of the nail bed, which results in the formation of soft keratin. The soft keratin, thus formed, accumulates as subungual debris

through which the fungus freely grows. Section of an infected nail shows horizontally aligned hyphae and arthrospores of dermatophytes between the lamellae. Inflammatory response is not common (Islam S, 1993).

1.3.3 CLINICAL FEATURES OF ONYCHOMYCOSIS

Frequently, the clinical appearance of onychomycosis caused by one species of fungus is indistinguishable from that caused by other species; however, there are various clinical clues that could allow one to speculate an organism of a certain species (Harry LA, 1998).

Clinical features found in a few of the species are being described here-under:

Clinical features of onychomycosis caused by *T. rubrum* -

According to the description of Harry LA (1998) onychomycosis caused by T.rubrum is a relatively deep infection of nail. Its onset is slow and insidious, with little inflammatory reaction. Disease usually starts at the distal corner of the nail and involves the junction of the nail and its bed with yellow discolouration and gradually it spreads through the entire nail. Beneath this discolouration, the nail plate becomes loose from the nail bed. Gradually the entire nail becomes brittle and breaks off, leaving an undermined remnant that is black and yellow from the dead nail and fungi that are present. Fingernails and toenails present a similar appearance. Skin of the toes or soles is also likely to be involved, with characteristic branny, scaling, erythemetous and well defined patches.

Clinical features of onychomycosis caused by *T. mentagrophytes:*

It is a superficial infection of nail (Harry LA, 1998). No parenchymal inflammation is noted. Infection generally begins with scaling and remains localized within a portion of the nail. The entire nail bed may be involved with single or multiple spots. *Shelly* and *Wood* (1982) showed that they are excellent 'hunting ground' for hyphae, lying well within the nail plate.

Clinical features of onychomycosis caused by *Candida albican*:

As described by Harry LA (1998), Elewski BE (1996) usually causes paronychea. Disease starts under the lateral and proximal nail fold and a small amount of pus may be expressed. Adjacent cuticle is pink, swollen and tender on pressure. Neighbouring portion of the nail becomes dark, ridged and separated from the bed. Later, the entire nail plate may be separated. Fingernail is more commonly infected than toenails. Nail beds are not friable and yellow like others.

In addition to the usual etiological species like ***Epidermophyton, Ttrichophyton and Microsporum***; non-dermatophytic fungi as well rarely cause onychomycosis – mainly in toenail and seldom in fingernail. Whatever is the species, clinical appearance of fungal infections is the same and not easily distinguishable.

1.3.4 CLASSIFICATION OF ONYCHOMYCOSIS

Harry LA (1998), Elewski BE (1996) and Zaias N (1980) have outlined four classic types of onychomycosis which are as follows:

Distal subungual onychomycosis: Primarily involves distal nail bed and the hyponychium with secondary involvement of the underside of the nail plate of finger and toe.

White superficial onychomycosis: Involves toe-nail plate on the surface. It is produced by *T. mentagrophytes* - species of *cephalosporium* and *Aspergillums* and *Fusariam oxysprorum* fungi.

1. **Proximal subungual onychomycoses:** Involves the nail plate mainly from the proximal nail fold. It is produced by *T. rubrum, T. megninii.*
2. **Candida Onychomychosis:** Involves the entire nail plate. It is due to *Candida albicans* and is seen only in patients with chronic mucocutaneous candidiasis.

1.3.5 DIAGNOSIS OF ONYCHOMYCOSIS

Diagnosis of onychomycosis is carried out by identification of fungal elements by direct microscopy and isolation of fungus by culture. However, failure to find the fungus by either method does not rule out a fungal cause (Elewski BE, 1996).

Microscopic examination: As described by Elewski BE (1996) and Harry LA (1998) very thin shavings are taken from the diseased portion of the nail and sample is divided into two parts. One part is placed on a glass slide. A drop of 40% solution of potassium hydroxide (KOH) with or without chlorazol black E dye is added to the sample. A cover slip is placed over the specimen and pressed down firmly. The excess fluid should be removed by touching the slides of the

cover slip with small square of blotting paper. Gentle heating is applied until the scales are thoroughly macerated. It is then ready for a microscopic study.

For interpretation – the mycelium may be seen under low power, but better observation of both hypae and spores is obtained by the use of the high dry objective with reduced illumination.

Culture: As described by Harry LA (1998), Elewski BE (1996), the remaining part of the sample was planted on Sbouraud's glucose agar or Mycosel agar and cultured at room temperature. Adequate growth for identification occurs in 5 to 14 days, depending on the kind of fungus.

Taplin, et al have devised a culture medium and Dermatophytes Test Medium (DTM), for the diagnosis of dermatophytosis (Tapline D et al., 1969). The medium inhibits growth of bacterial and saprophytic contaminants. The alkaline metabolites of the dermatophytes change the colour of the pH indicator in the medium from yellow to red, which distinguishes them from fungal contaminants and *Candida albicans*. If a dermatophyte is present, the medium will turn red. Saprophytes turn the medium green. *C. albicans* does not cause colour changes, but produces a typical yeast colony.

1.3.6 DIFFERENTIAL DIAGNOSIS OF ONYCHOMYCOSIS

Numerous affections of the nails make a firm diagnosis of onychomycosis difficult unless fungi are actually demonstrated; therefore, great care should be exercised in the performance of the microscopic fungal examination.

Harry LA (1998) has suggested following differential diagnoses for onychomycosis:

(i) Allergic contact dermatitis caused by nail polish is confronted mostly in case of female patients.

(ii) Recurrent contact articaria to foods or other sensitizers among kitchen workers mimics candidal paronychea.

(iii) Psoriasis is another important condition mimicking onychomycosis. However, it typically begins in the middle of the free edge of the nail rather than in a corner as occurs in onychomycosis.

(iv) Lichen planus – it is exceedingly difficult to differentiate and again presence of the fungus is essential. Both psoriasis and lichen planus usually show other areas of skin involvement.

In addition to the above stated conditions, a few other less important conditions should also be kept in mind – they are:

(v) Darier's disease

(vi) Reiter's disease

(vii) Hyperkeranotic scabies (Norwegian Scabies).

1.3.7 TREATMENT OF ONYCHOMYCOSIS

It is considered by many authors that a full cure for onychomycosis may not always be possible; especially pedal onychomycosis is incurable (Gupta AK et al.,

1997). Local factors affecting treatment success include thickened, slow growing or traumatized nails; systemic factors include compromised immune status, diabetes, obesity, concurrent medications and peripheral vascular disease (Gupta A K et al., 1997).

In consideration of antifungal drugs, three things should be observed-

(i) Spectrum of activity of the antifungal agent.

(ii) Pharmacokinetic profile of the agent.

(iii) Clinical type of infection.

Additional considerations-

(i) Safety.

(ii) Compliance.

(iii) Cost.

Conventional methods of treatment for onychomycosis includes

(i) Medical treatment.

(ii) Surgical treatment.

Medical treatment

Griseofulvin has been conventionally accepted as an antifungal drug for many years. Till today, it is a viable therapeutic option in many cases, especially in resource-poor countries like Bangladesh (Daniel CR, 1996).

Griseofulvin – Dose 350-750mg / daily orally with meal

4 – 6 months for fingernail infection.

10 – 18 months for toenail infection.

Success rates are –

15 – 30% for toenail.

50 – 70% for fingernail.

Nail disease caused by *Candida albicans* usually cannot be cured by applying griseofulvin (Harry L A, 1998; Crislip MA et al., 1989)

Advent of new drugs produces new approaches in therapy. After many studies, the newer antifungal drugs are being shown to be more efficacious. (Gupta AK et al., 1997; Galimberti R et al., 1996).

Common drugs are- (a) Itraconazole

(b) Terbinafine

(c) Fluconazole

These drugs are broad spectrum for antifungal activity; quickly appear in the nail plate within days of starting of oral therapy and incorporate in the nail matrix (Richard BO, 1996).

(i) New drugs ensure reduced duration of therapy in comparison with griseofulvin, the older medicine.

(ii) They display a low risk to benefit ratio, are safer than Ketoconazole and much more effective.

(iii) Very expensive specially in context of poor countries.

Commonly accepted doses of newer medicines (Gupta et al., 1997).

Terbinafine – 250 mg daily orally for fingernail – 6 weeks, toenail – 12 weeks.

Itraconazole – 200 mg twice daily orally for 1 week each month for fingernail – 2 months and for toenail – 3 months.

Fluconazole – 150 – 300 mg/once in a week orally for 6 –12 months. Less data have been published on Fluconazole.

Surgical Treatment

When routine therapeutic treatment in onychomycosis including oral and local antifungal drug has failed, surgical intervention is then considered to be the next option. The operation is called as Distal Nail Avulsion (DNA) - (Baran R, Haneke E., 1987; Clark RE, Tope WD, 1994; Scher RK, 1989; Albom MJ, 1997; Baran R, 1981; Haneke E, Baran R, 1994).

This is the most commonly performed nail operation in which the nail plate, which is a physiological cover of the matrix and the nail bed, is separated from them.

Indication of operation:

(i) Therapeutic:-

Onychomycosis

Ingrown nails

Onychogryphosis

Onychodystrophies

Nail bed defects

Subungual or periungual warts

(ii) Diagnostic –

Nail bed / nail matrix biopsy

Complications

Pain, bleeding, infection, necrosis, trauma to matrix and nail bed giving rise to nail deformities and dystrophies.

Advantages

(i) Simple procedure.

(ii) Can be used as an adjunct to antifungal.

(iii) Useful for facilitating nail bed biopsy.

Disadvantages

(i) It is an invasive technique.

(ii) New nail grows slowly, so it has to be protected from injury.

(iii) Possible damage to nail matrix with resultant dystrophies.

Prophylaxis

Few prophylactic measures are helpful in preventing onychomycosis.

i. As the disease frequently starts on the feet, the patient should be advised to dry the toes thoroughly after bath.

ii. The use of a good antiseptic powder between the toes is strongly advised for susceptible persons.

iii. Plain talcum cornstarch may be dusted into socks and shoes.

1.4 ONYCHOMYCOSIS IN BANGLADESH

Onychomycosis induces a serious public health problem in both developed and developing countries. Its toll in term of sufferings, disability, psychological trauma and economic loss is much greater than generally realized (Al Doory, 1997). Although onychomycosis may not be generally fatal but it can be very troublesome and in many circumstances, may even be incapacitating.

The Bangladeshi population is vulnerable to fungal infection because of a variety of factors. They include: moist climate, excessive population density, over crowding, poverty, malnutrition, poor hygienic conditions and ignorance. Unfortunately, extensive research has not yet been carried out on this disease and its treatment response. Although many mycological works including corneal., oesophageal and respiratory fungi have been reported in Bangladesh (Islam S 1993), only 6 studies have so far been carried out in the erstwhile East Pakistan and also in Bangladesh which investigated the prevalence of dermatophytosis among Bangladeshi population (Alam S A, 1971; Chowdhury M R, 1979; Islam

S, 1993; Chowdhury M Z, 1994; Haq E, 1994). There had been only one research on Onychomycosis among them. Moreover, no assessment has so far been made on the conventional treatment procedure and its sequel for onychomycosis.

1.5 LOW RADIATION TREATMENT FOR ONYCHOMYCOSIS - PROBABLE NEW FRONTIERS

Treatment for infection caused by dermatophytes creates many problems but ridding the nails of fungus (onychomycosis) still remains an extremely difficult task. It is usually less responsive to conventionally used anti-fungal drug, griseofulvin which needs at least 6 – 12 months to cure the disease with high incidence of recurrence and sometimes requires invasive surgical removal of the nail. In addition to this, anti-fungal treatment in onychomycosis has some inherent limitations like dermatophytid in case of overuse of topical anti-fungal chemicals. Oral therapy limits its own use because of high cost, prolonged drug exposure and high risk benefit consideration as there is a risk of liver damage as a complication (1:10,000). All these mostly advocate against use of this medication in onychomycosis (Harry L. A, Richard B. O, William D. J., 1990). With a view to overcome the stated limitations, a different treatment modality may be introduced.

The proposed treatment modality involves the application of nuclear radiation, specially beta radiation (soft or hard beta). It is to be noted that sporadic uses of beta radiation as antimycotic agent for onychomycosis have already been practiced elsewhere in the world (Meinhof W, 1965). In Bangladesh, the same utilization has been observed with similar sequel of success (Chowdhury S. J.,

1996 personal communications). It was in practice in older nuclear medicine centres in Dhaka, Rajshahi and Chittagong. However, all those applications were non-specific, non-systematic and inadequate. Moreover, no specific and systematic follow-up was done. However, by personal communication, possibilities of encouraging results with this beta radiation were obtained from old users and future success of this therapy procedure for onychomycosis was assumed.

The proposed new treatment modality uses beta radiation, which is absolutely a local application in the site of lesion only with negligible tissue penetration without any systemic involvement. This portable beta source device is a simple 1.2 cm diameter (approximately) applicator of Strontium-^{90} source providing required beta rays and specially made suitable for application in nail bed only.

This proposed new anti-fungal radiation treatment modality would almost certainly be cost effective. One source costs about US $7500.00 with 28 years of half-life with recommended active use for at least 10 years. It was observed that in 1995 a total of 2198 patients with fungal infection attended in DMCH for treatments of which 325 patients were of Onychomycosis (Shahidullah M, 1995 personal communication). The costs of beta source for one year is about Taka 30,000 i.e. if this source is used for only 325 patients in a year, the cost per dose per patient stands at Tk.18.00 only, assuming a total of 5 doses per patient and thus the treatment cost of beta radiation will be very low. If organizing the referral from other treatment centres could increase number of patients the corresponding treatment cost would be reduced further.

1.6 SOURCES OF RADIATION IN NUCLEAR MEDICINE

Regarding radionuclide therapy in the Nuclear Medicine practice, we basically concentrate on three types of ionising radiations i.e. Alpha (α), Beta (β) and Gamma (γ) radiations.

Alpha ray: The alpha particle (α, 4/2α, or 4/2 He) is a helium nucleus consisting of 2 protons and 2 neutrons (Early PJ and Sodee DB, 1995). It is a heavy charged highly energetic particle with very short range even in air (5 cm). It has discrete energy level. Practically alpha has no penetration in any media other than air without acceleration. Use of alpha radiation as a source of therapy is not yet universal. Rather it is confined to certain very advanced and highly sophisticated laboratories, mainly in developed countries like USA, Germany and Japan. It is used as therapy for specific areas like, soft tissue sarcoma, superficial lesions, etc. High specific ionisation is an advantage while at the end of their Bragg peaks ionisation fragments causes unwanted dose build up which is considered to be a disadvantage. So due to involvement of high cost, advanced technology and relatively less encouraging results in most of the cases, alpha radiation is not widely used as external radiation therapy.

Beta ray: Although the range of beta particles or electrons is very short, it has penetration capability to certain extent in medium other than air, especially soft tissue with a maximum range of approximately 1 – 11 mm (Silberstein EB and Taylor A , Jr., 1995). Thus β- particle can be used as external therapy source for at least soft tissue lesions.

Gamma ray: Gamma rays carry no electrical charge; therefore, they are not subjected to forces of attraction or repulsion as alpha, beta and positron particle. Unlike these particles Gamma rays are the only emission from an unstable nucleus that are members of the electromagnetic spectrum (Early P J, Sodee D B, 1995). Gamma rays have high penetration capability in any medium and can pass large distances before undergoing any interaction. Therefore, gamma radiation having energies used in nuclear medicine cannot be used as a therapy source for superficial soft tissue lesion, which in fact, provides radiation to deeper sites. As a result, gamma rays are excluded from the therapeutic use for superficial organs. However, this gamma radiation has been predominantly used for diagnostic purposes in nuclear medicine practices.

1.7 ROLE OF BETA RADIATION AS A THERAPY IN NUCLEAR MEDICINE

Beta radiation is a particle emitted from the nucleus of an atom undergoing disintegration carrying a unit negative (negatron β^-) or positive (positron β^+) charge (Early P J, Sodee D B, 1995; Rocha AFG, Harbart JC, 1978).

Beta decay: Radioactive decay by beta emission is a process in which essentially, a neutron in the nucleus is transformed into a proton and an electron. Schematically, the process is $n \dashrightarrow p + e^- + \nu$

The electron (e^-) and the neutrino (ν) are ejected from the nucleus and carry away the energy released in the process as kinetic energy. The electron is called a Beta particle. The neutrino is a particle having practically zero or insignificant mass ($<1/2000\ m_0$, m_0 = electron rest mass) and no charge. It undergoes virtually

no interactions with matter and therefore is undetectable. β^- emission may be represented in standard nuclear notation as -

$$^{A}_{Z}X \text{ ----------> } ^{A}_{Z+1}Y +$$

This is an isobaric decay mode i.e., the parent and daughter are different element but have the same mass. Radioactive decay processes are often represented by a decay scheme. Likewise fig-1 shows a beta decay scheme for ^{90}Sr.

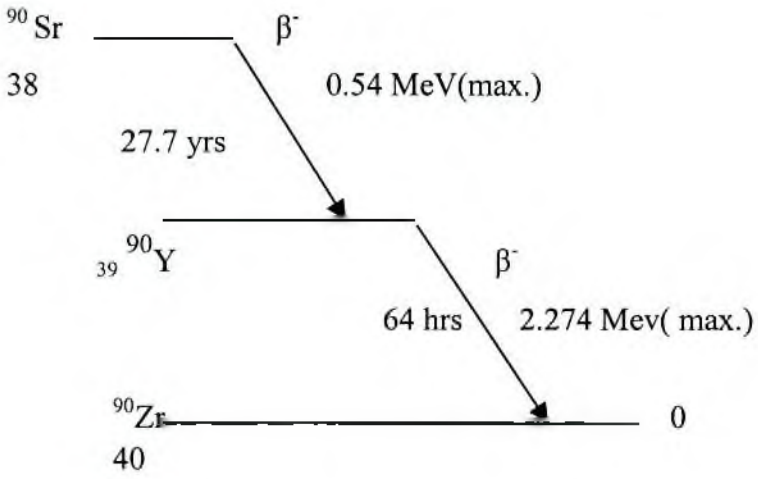


Fig.1: Decay scheme of ^{90}Sr

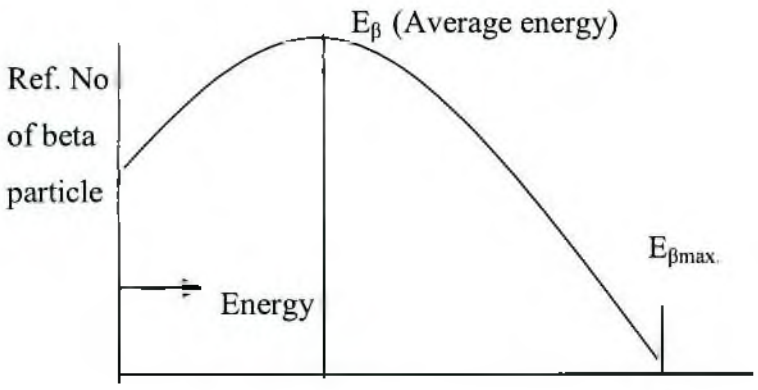


Fig-2: Energy spectrum of beta particle.

Average energy of the beta particle is usually at 1/3rd of the maximum energy. Beta radiation is the most important therapeutic tool in the field of therapeutic nuclear medicine. Application of Beta radiation is proven to produce change of both physical and chemical properties in cellular level, which ultimately results, in irreparable mitotic damage thus preventing reproduction. (NAS, 1990) In fact the effect of beta radiation is observed in molecular level with extent to DNA damage. (Minhof W, 1965) By interacting with DNA it causes sublethal or lethal damage (Minhof N, 1965; Phillip TL, 1991).

Nuclear Medicine really began in 1936 with the application of pure beta emitter, P-32 in Leukaemia by Lawrence and his co-workers. (Lawrence J H, 1340) In 1942, Hertz and Roberts used radioiodine for treating Graves' disease. (Hertz and Roberts, 1942) Almost simultaneously, Hamilton and Lawrence also reported on the therapeutic possibilities of radio phosphorus and radio iodine (Hamilton and Lawrence, 1942). Till today, we have been safely applying beta radiation to the eye in postoperative pterigyum cases with considerable success rate. Beta ray originated from I-131 is a unique treatment in hyperthyroidism and differentiated thyroid cancer where radiotherapy and chemotherapy are not useful (Klein I, Becker DV and Levey GS, 1994).

Beta radiation in combination with antithyroid drug is another well-accepted method of treatment for Graves' Hyperthyroidism with almost hundred percent efficiency (M N Maisey and I. Gosplman, 1991)

While diagnostic Nuclear Medicine has made dramatic advancement, therapeutic nuclear medicine has somewhat fallen behind. In fact, the number of radionuclide therapeutic procedures has declined. One reason is the notable advances made in radiotherapy and chemotherapy. These two areas have been the subject of considerably more extensive research and development, as well as, financial support than has radionuclide therapy albeit nuclear medicine therapy is a non-invasive and less hazardous, comparatively less expensive and most effective modality of treatment (Lale RD, 1984).

Nuclear medicine therapy is safe because it involves massively charged particles having less penetration range in human tissue (about 1 – 11 mm)- (Silberstein, 1993). This radiation hazard is lower in comparison to diagnostic radiology and X-ray therapy or conventional radiotherapy, which was previously used for superficial skin diseases. Beta radiation is applied locally at the site of lesion with negligible tissue penetration without any systemic involvement. Beta radiation is considered as the innocent one among the radiation family due to its inherent acceptable qualities – which are as follows:

- It is a charged particle.
- Low penetration (high LET).
- High local absorption dose.
- No systemic involvement, no burden on liver.
- Very low cost.
- Easy to apply.
- Portable applicator.
- Easy availability.
- Long shelf-life.
- Non-invasive.
- Minimal or no side effect.
- No drug interaction
- Self limiting.
- Sparing of uninvolved tissue.
- No hospitalisation is needed.
- Other systemic disease does not interfere in radiation procedure.

1.8 MODE OF ACTION OF BETA RADIATION IN DESTRUCTION OF DISEASE TISSUE

Biological effect of radiation depends on the dose, type of radiation and observed endpoint. Certain effects occur relatively fast while others may take years to become evident the radical produced by a charged particle tract in a biological system may all be reacted within a period of 10^{-3} sec. Some biochemical

processes are altered almost immediately (in less than about 1 sec). Cell division may be affected in a matter of hours (Turner JE, 1986).

Biological effects may be resulted from direct and indirect action of radiation. Direct biological effects are produced by the initial action of radiation itself while indirect are caused by the later chemical action of free radicals and other radiation products. An example of a direct effect is a strand break in DNA caused by an ionization in the molecule itself while indirect effect is the strand break that results when an OH radical attacks a DNA sugar at a later time (~ 10^{-11} and ~10^{-9} sec).

In our present study, the β-radiation is directly ionising radiation and thus it is reasonably assumed that the β-particles in the fungi molecule cause ionisation, which ultimately damaged by the action of ionization and thus fungal infection were being controlled.

1.9 RADIATION IN DERMATOLOGY

The use of ionising radiation in dermatological practice has been well recognized for many years.

Radiotherapy is an integral part of dermatological therapy and skin specialists are mostly familiar with modern radio therapeutic indications and radiation techniques (Goldschmildt H, 1990).

The use of ionising radiation in dermatological therapy markedly decreased owing to the development of more efficient medications and to the increased awareness of potential genetic and somatic hazards of radiation (Goldschmildt H, 1975).

Today, dermatological X-ray therapy should be considered only when results from radiation therapy could be expected to be superior to those of other methods. Ionising radiation should not be administered in children or pregnant women. Careful selection of patients and rigorous application of radiation protection measures are essential to reduce radiation hazards to a minimum (Goldschmildt H, 1990).

A wide variety of radiation methods are available for various types of neoplasm involving different organs (Goldschmildt H, Sherwin WK, 1980).

RADIATION METHODS

Therapy	Sources of Synonyms	Energy kV	TSD (cm)	Wavelength (A) (average)	HVT	D½ (mm tissue) for avg penetration
Mega voltage	Betatron, particle accelerators	>1000	80	0.001	>10mm Pb	200
Super voltage	Gamma ray Telecurie sources	400-800	50-80	0.03	5-10 mm Pb	80-110
Orthovoltage	Deep x-ray Conventional x-ray	200-400	50-80	0.14	2-4mm Cu	50-80
Half-deep	Intermediate	110-130	30	0.1	4 mm Al	30
Contact	Ultra short distant Chaoul	50-60	1.5-3.0	0.8	2-4 mm Al	4-30
Superficial x-ray	Low voltage Std. x-ray Pyrex- window unit	60-100	15-30	0.5	0.7-2.0 mm Al	7-10
Soft x-ray	Beryllium-window unit	20-100	10-30	0.15	0.1-2.0 mm Al	1-20
Grenz ray	Ultra soft Super soft	5-20	10-15	2	0.03 mm Al	0.2-0.8

(Goldschmidt H et al., 1985)

Traditionally, the most commonly used radiation units in dermatology were:

- Low-voltage superficial X-ray machine with Pyrex windows (60 to 100 KV).
- More recently Beryllium –windowed X-ray units have become available for soft X-ray therapy (20 to 100 KV), which is ideally suited for all dermatological problems.
- Ultra soft greanz rays (5 – 20 KV) are very useful for superficial skin diseases.
- Domonkos had been the pioneer in the use of the ultra soft distance, low voltage, contact therapy technique (Chaoal), which offered several advantages in the treatment of skin cancer (Domonkos, 1965).
- Contact therapy units (50 – 60 KV) have not been commercially available for several years and are now superseded by Soft X-ray machine.

1.9.1 RADIATION THERAPY IN BENIGN SKIN DISORDERS

Indications for radiation therapy in benign skin disorders have been approved to a small list of dermatoses. With few exceptions, radiation therapy should be considered only after other therapeutic methods have failed and when active treatment seems essential for the well being of the patient. This philosophy is in agreement with the recommendations of the National Academy of Sciences (NAS), which were endorsed by the food and drug administration. (FDA, 1977) Limitation of the total fractionated dose to 1000 cGy for x-rays (5000cGy for

Grenz rays) and the application of meticulous radiation protection measures are essential.

Application of radiation is practiced under following benign conditions (Goldschmidt, 1975):

- Lymphadenosis benign cutis.
- Psoriasis.
- Eczematous plaques.
- Pruritus ani.
- Pruritus vulvae (Hollanded, 1968).
- Langerhans cell and other aspects of the cutaneous immune system (Lindel of B et al. 1986).
- Keloid (Bonasree C Deka, et al., 1987).

1.9.2 ADVANTAGES AND DISADVANTAGES OF RADIATION THERAPY

Radiation therapies have many advantages and disadvantages. (Joldschmidth, 1975) They are as follows:

Advantages of radiation treatment-

- Most important advantage of radiation therapy over surgical methods is that radiation is non-invasive, self-limiting and causes preservation of uninvolved tissue as much as possible.

- Recurrence of surgically treated (residual tissue) can be successfully irradiated.
- Irradiation is a simple office procedure and no hospitalisation is needed.
- Patients in poor health can often be treated with ionising radiation with fewer complications and less stress than surgical method.
- Advantage over medical therapy – no systemic involvement and no burden on liver.

Disadvantages of radiation treatment-

- Multiple visits are needed, usually 5 – 25 treatments.
- Usual radiation squeals - like.

Worsens surgical scar, permanent alopecia, various skin reactions, usual fear and apprehension regarding cancer formation.

1.9.3 SUCCESS RATE OF RADIATION THERAPY IN DERMATOLOGICAL CASES

Most reports in the literature indicate that cure rate is 95% (90% to 100%) following radiotherapy of a complicated catarous cancer (Goldsmith and Sherwin, 1980).

Five years' cure rate published by Bert and associates of the New York Skin and Cancer group for five hundred histology proven basal cell carcinoma was 93% (Bert RS, et al., 1968).

1.10 CONCERN AND APPREHENSION REGARDING HARMFUL EFFECTS OF RADIATION

There is a lot of public concern and apprehension regarding the harmful effects of radiation. Beta ray application is a well-documented safe way of radiation therapy. However, physicians involved in Nuclear Medicine procedure need to address the fears of patients and their families, particularly with respect to cancer induction (Lale RD, 1984).

The Nuclear Medicine Physicians should rigorously employ techniques by which exposure to radiation can be kept to a minimum level, consistent with obtaining the therapeutic benefits desired. Hopefully public education will restore the proper perspective on this subject (John Willium et al., 1996).

However, proper treatment, prevention and thereby, management of onychomycosis are seemed essential in our country. Considering all prevailing limitations of conventional antifungal therapy, it is desirable, therefore, that a study should be undertaken to investigate the treatment response and thus to establish a new way of treatment for this disease which may be considered as an innovation.

1.11 RATIONALE

- Bangladesh is one of the developing countries reported to have a high prevalence of fungal infection / onychomycosis.
- The high humidity and warm climate of this tropical country is congenial for the growth of fungi.
- Fungus causes permanent disfiguration of nail, which may create serious cosmetic problems.
- Therapeutic limitations of conventional antimycotic agents in respect of low cure rate, high relapse rate, inherent side effects, long duration of treatment and high cost in treating onychomycosis have provided clear incentives to explore alternative forms of treatment procedure.
- The present study aims to arrive at a treatment procedure with capability of overcoming the aforesaid limitations of conventional therapy and would be non-invasive and non-systemic involvement of therapy as well.
- Sporadic uses of beta radiation as antimycotic agent for onychomycosis have already been practiced elsewhere in the world (Meinhof W, 1965).

- In Bangladesh, its utilization has been observed with similar sequel of success (Chowdhury S.J, 1996 personal communications).

  Older nuclear medicine centres like, Dhaka, Chittagong & Rajshahi were in utilization of beta radiation and all those applications were non-specific, non-systematic and inadequate. Moreover, no specific and systematic follow-up was done. However, by personal communication, possibilities of encouraging results with this beta radiation were obtained from old users and future success of this therapy procedure for onychomycosis was assumed.

1.12 HYPOTHESES

In this thesis, the following hypotheses were considered:

- Therapeutic application of beta radiation on onychomycosis is an innovative modality of treatment.
- This therapeutic modality is less time consuming and cost effective.

1.13 OBJECTIVES

The prime objectives of the present study are:

1. To use beta radiation as a curative therapy in Onychomycosis, optimisation of its dosages and to promote an innovative clinical development in the field of therapeutic application of Nuclear Medicine.

2. To study the efficacy of beta radiation as a replacement or supplement to the conventional anti-fungal drugs in patients with Onychomycosis.

3. To reduce the duration of drug exposure and cost of prevailing medical therapy in the management of Onychomycosis.

CHAPTER 2: LITERATURE REVIEW

2.2 HISTORICAL REVIEW

Dermatophytes have plagued mankind from prehistoric times to the present. One hundred and twenty-five years ago, a physician, Robert Remak observed fungus cells in favic crusts (Kisch B, 1954). Onychomycosis, one of the varieties of dermatophytes is the most common and visible variety of dermatological conditions and described from the earliest historical times (Feulard H, 1961; Gates W, 1939; Rosenthal T, 1961). References to what were undoubtedly onychomycosis and their treatments are found in the ancient writings and herbals. Ringworm, Tinea or dermatophytosis has been recognized as a definite clinical entity since the ancient times in India. The Greeks named the disease "herpes" – a term which still persists. The Romans associated the lesions with insects and named the disease "*Tinea*" meaning any small insect larva. This name is retained in the clinical terminology of the disease. The English word, ringworm, then, is a combination of the meaning of the Greek and Latin terms.

In 1920, Hopkins and Benham began scientific study of medical mycology, which is the source of the study of nail fungus. The Laboratory of Columbia University was one of the first to systematically study fungi involved in disease. Rodha Benham is considered to be the founder of modern medical mycology (Benham RW, 1953; Ajello L, 1968; Sanyal N, 1969; Rippon JW, 1988).

2.3 PREVALENCE OF DISEASE

Although the exact prevalence is unknown, several authors in different studies have reported a high prevalence of onychomycosis. This is said to be the most

frequent cause of nail disease and represents 30% of all mycotic infections of the skin (Richard BO et al., 1997).

Onychomycosis accounts for up to 50% of all nail diseases and affects 2% to 18% or more of the world's population (Christensen NM, Chiritesca ME, Scher RK 1996, Elewski BE, 1996, Elewski BE, 1998). Cases of onychomycosis represent up to 30% of diagnosed superficial fungal infections (Midgly G, 1994; Meinhof W, 1980, Achten G, Wanet Rouard J, 1980, Andrew J, Achten G, 1987).

A recent survey in the United Kingdom suggests that 2.71% of the population is affected by onychomycosis (Roberts DT, 1992).

In the United States, about one half of all patients reporting to dermatologists' office for nail disorders have onychomycosis. A few studies showed 13.6% incidence of onychomycosis in a population of northeast Ohio (Elewski BE and Charif MA, 1997; Elewski BE and Roderick, 1999; Ghanham MA et al. In press; Andre J, 1987).

Sanyal M (1969) and Alam SA (1971) reported that onychomycosis is the most prevalent type of dermatophytosis in India and Bangladesh respectively and it is the third common type of dermatophytic infection in both the countries.

The prevalence of fungal infections of the skin and nails has increased remarkably in recent years. In a number of new surveys found dermatophytic onychomycosis to occur in about 9% of the study population. The prevalence may approach

approximately 50% in individuals over 70 years of age (Elewski BE, 1999; Heikkila H and Stubb S, 1995; Elewski BE and Charif MA, 1997).

The etiological prevalence as a cause of onychomycosis includes dermatophytes (68 %), yeasts (28 %), and moulds (7%) - (Galimberti RL et al., 1991; Clayton YM, Hay RJ, 1993; and Ramani R et al., 1993). Dermatophytes are by far the most common nail pathogens accounting for 80% to 90% of all skin infections. It is most frequently encountered, at least in temperate zones (Clayton YM, 1992; Heneke E, 1991; Zaug M, 1992). Infection caused by moulds reported as a major factor in nail disease in tropical and subtropical countries. (Kotrajaras R, Chongsathien S. Rojanavanich V, et al., 1988; Gugnani HC, Oyeka CA, 1989 and Kombila M et al., 1990) A high prevalence of Candida infections of the nails has been reported both in temperate and tropical countries like Saudi Arabia (Al Sogar SM, Moawad MK, Al-Humaidan YM,1991), India (Banerjee U, Sethi M, Pasricha JS, 1989) and Thailand (Taylor RL, Kotrajaras R, Jotisanicasa V, 1968).

Toe-nail infection is several times, even four times more common than finger-nails infection and is generally more difficult to treat because of the slow rate of its growth. (Elewski BE, Charif MA., 1997; Robert DT, 1994) In the finger-nails, Candida is the most frequent cause of infection (Galimberti RL et al., 1991; Clayton YM Hay RJ, 1993; Ramani R et al., 1993 and Scher RK, 1994).

However, numerous other conditions such as psoriasis and lichen planner can mimic onychomycosis. (Basak PJ, Scher RK and Ricci AR, 1990; Daniel CR III,1995)

2.4 PREDISPOSING FACTORS FOR ONYCHOMYCOSIS

Many predisposing factors are responsible for the incidence of onychomycosis (Daniel CR, Lawson LA, 1987; Daniel CM, Daniel MP and Daniel CR, 1995)

Table – 1: Predisposing factors for Onychomycosis

Pre existing Tinea pedis
Advancing age
Trauma
Exogenous heat/moisture
Hyperhidrosis
Immunosuppression
Possibly postmenopausal status (women)
Hereditary factors.

Pre existing Tinea pedis is an important factor, particularly in case of distal subungual onychomycosis. Increasing age is another factor of onychomycosis, however, uncommonly it occurs in children. Trauma specially associated with the wearing of occlusive footwear; exogenous heat and moisture; hyperhidrosis; immunosuppression (wide spread use of antibiotic and immunosuppressive therapies) and hereditary factors (e.g., a T cell defect or difference in keratin structure), are all very positive factors for occurrence of onychomycosis.

In addition, onychomycosis is more common in postmenopausal women than in pre menopausal women. One may speculate that this is possibly due to loss of the protective effects of oestrogen (Daniel C R, Lawson L A., 1987; Daniel C R, Norton L A, Scher R K., 1992).

Despite the fact that fungal nail infections are not contagious in strict sense, those persons who are genetically predisposed will more commonly acquire such infections. The people who are not exposed however, to any of the above predisposing factors, are more likely to remain uninfected. In addition, a good number of onychomycosis are more resistant to treatment than others. Such patients may initially respond to therapy, but commonly relapse (Daniel CR, Jackson MD, 1996).

In developed countries like United States, dramatically high frequency of onychomycosis has been observed in recent years. The possible predisposing factors are aging of population, high incidence of diabetes mellitus, greater use of immunosuppressive and antibiotic agents and AIDS epidemic (Chiritesen MM, Chititesca ME, Scher RK, 1997; Elewski BE, Charif MA, 1996).

However, although different authors used different statistics, they pointed to the humid climate as the main predisposing factor (Clayton YM, 1993).

2.5 ONYCHOMYCOSIS IS A FAMILIAL AND COMMUNAL DISEASE

It is the personal experience of the authors and that of others that infection including the nails and soles of the feet by T. rubrum and possibly other dermatophytes may present more commonly in families and in communal areas such as public swimming pools. An understanding of the familial and communal factors might prompt the patient to take members to reduce reinfection. These might include hygienic issues, keeping feet dry, the use of 100% cotton socks, appropriate footwear that enables feet to 'breathe', judicious use of the foot

powders and topical antifungal cream or shampoo and possibly treating other affected family members and friends (English MD, 1957; Gupta A K et al., in press; Zaias N et al., 1996).

2.6 ONYCHOMYCOSES HAS EMOTIONAL AND PSYCHOLOGICAL CONSEQUENCES

Far from merely a cosmetic issue, onychomycosis may have emotional and psychological consequences for the patients (Chiritescu MM, Chiritescu ME, Scher RK, 1996; Scher RK, 1994). The condition may be associated with significant pain and discomfort; in severe cases, onychomycosis could lead to disfigurement and loss of dexterity and mobility.

From a recent quality of life, study by Lubeck et al. (1993) cited by Drake LA et al. (1998) has demonstrated that onychomycosis can impose significant psychological and social limitations. In this study, patients with fungal nail disease reported to avoiding intimate and social occasions for fear of exposing their disfigured nails and experienced difficulties with work – related activities requiring them to use their fingers or toe for long periods. All these, might make a sensitive patient feel somewhat socially isolated (Scher RK, 1999).

2.7 ONYCHOMYCOSIS IN IMMUNO DEFIFCIENCY

There are many mucocutaneous changes or specific skin diseases associated with human immuno deficiency virus infection (Dover JS, 1992; Cockrell CJ, 1990). It is now established that tinea pedis and onychomycosis are more frequent in HIV infected patients (Smith KJ, 1994; Domparartin D, 1990). Researchers confirm

that onychomycosis affects 1/3rd of HIV infected patients. The frequency of onychomycosis in a control group of a study conducted in the Ohio was around 14% (Bernard C et al 1998; Elewski BE, Charif MA, 1997).

Around 13% of patients under Iodine–131 therapies due to Grave's disease turned into hypothyroid state and around 90% of those patients were affected by onychomycosis along with other skin mycoses. International Atomic Energy Agency (IAEA) concluded the cause as immuno deficiency (IAEA CRP Project No.7876, 1995).

2.8 TRADITIONAL MANAGEMENT OF ONYCHOMYCOSIS

The general therapeutic measures for onychomycosis consist of elimination of predisposing factors, removal of infected keratin, and the use of antifungal (Galimberti RL,1980).

The traditional management of onychomycosis includes mechanical, chemical and surgical approaches as well as topical and oral antifungal medications (Daniel CR, 1996). Alternative to the systemic management of onychomycosis include topical and surgical treatments. Traditionally topical agents used as monotherapy for onychomycosis are only able to inhibit the growth of fungal nail infections; clinical and mycological cures have recently been observed after treatment with some of the newer preparations. In contrast, surgical treatment almost always needs to be used in conjunction with either topical or systemic antifungal therapy (Philip R, Cohen and Richard K, Scher, 1994).

2.9 NON PHARMACOLOGICAL APPROACHES FOR MANAGEMENT OF ONYCHOMYCOSIS

Various non pharmacological approaches have been used to manage nail fungal infections including (Daniel CR, 1996)-

(a) Buffing or filling of the nails.

(b) Nail avulsion or debridement.

(c) The use of surgery or various chemicals and solutions to remove nail plate.

2.10 LIMITATIONS OF DIFFERENT TREATMENT PROCEDURES IN CONTROL OF NAIL FUNGUS

Every treatment system has its own limitations as delineated below (non-exhaustive) - (Daniel CR, 1996).

Removal of Nail Plate

(a) No legitimate rationale for removing the nail plate in patients with simple onychomycosis.

(b) The procedure is uncomfortable and probably does not reduce the likelihood of relapse.

(c) Permanently alter the shape of nail unit and lead persistent nonattachment of nail plate to nail bed (onycholysis) and increase the likelihood of ingrown nail.

(d) No control study showing the benefit of nail plate removal in patients with onychomycosis.

Chemical Removal of Nails

(a) This method is only reserved for patients with onychogryphosis or very thick nails or painful nail who cannot go for surgery.

(b) Unfortunately, these methods do not 'cure' the infections, but only keep the worst aspects of the disease under control (Daniel CR, 1996).

Topical Antifungal Therapy

Numerous topical antifungal agents (creams, lotions, solutions, powders and spray) have been used for the empiric management of onychomycosis.

(a) It is widely agreed that these preparations are largely ineffective, even when used in conjunction with nail avulsion.

(b) The results are generally disappointing, particularly when there is moderate to marked infection.

(c) Experience with the newer topical solutions is inconclusive and definitely less efficacious (Haria M, Bryson HM, 1995; Cohen PR, Scher RK, 1994; Gupta AK, Scher RK and Doncker PD, 1997; Gupta AK, 1997).

Traditional Oral Antimycotic Agents

Oral antifungal agents have been used for the treatment of onychomycosis for almost half a century. Two oral antifungal drugs – traditionally comprised the mainstay of the treatment of onychomycosis are -

(i) Griseofulvin (ii) Ketoconazole

Major limitations of the treatment with Griseofulvin or Ketokonazole are –

(i) Long duration of therapy required (Robert DT 1994; Richard A, Arres – Estrada J, Picrard – Franchimont C. 1993; Scher RK 1990).

(ii) Clinical and mycological cure rates are low (Davis RR, Everall JD, Hamilton E 1967; Frain – Bell W, Riddell RW, Stevension CI et al., 1960).

(iii) There is a greater than 75% probability that the patient will relapse within 2 years (Davis RR, Everall JD, Hamilton E, 1967).

(iv) The potential for significant side effects is another problem, particularly with Ketoconazole (Davies RR, 1980; Dollry C, 1991, Janssen PAJ; Symoens JE, 1983; Hay RJ, 1993).

Table – 2: Side effects associated with the use of Griseofulvin
(Physician's Desk Reference 1995, Gupta AK, 1994)

Common –	Headache, nausea.
Less common –	GI disturbances (e.g. vomiting, diarrhoea) Dermatological reactions (e.g. photosensitivity, pruritus, urticaria, exacerbation of SLE, and others). Other reactions (e.g. fatigue, fever, menstrual irregularities, possible reduction in oral contraceptive efficacy).
Rare –	Changes in laboratory values (e.g. neutropenia, granulocytopenia, monocytosis, liver abnormalities, albuminuria, proteinuria, cylindruria).

Table – 3: Side effects associated with the use of Ketoconazole

(Physician's Desk Reference, 1995; Gupta AK, 1994; Janssen PAJ, Symoens JE, 1983)

Potential side effect –	Hepatotoxicity (1/10000 or less but life threatening if occurs).
Less common –	Headache, Nausea, Pruritus, Urticaria allergic reaction.
Rare –	Suppression of testosterone or adrenal cortico steroid function.

2.11 NEWER ANTIFUNGAL AGENTS

There are three new antifungal agents like, itraconazole, terbinafine and fluconazole. These agents are being increasingly considered for treatment of onychomycosis. There are several good reviews on this topic. (Elewski BE 1994; Gupta AK, Sauder DN, Shear NH 1994; Gupta AK, Shear NH, 1994; Gupta AK, Shear NH 1996; Gupta AK, Shear NH, Sauder DN, 1993; Hay RJ, 1993; Hay RJ, 1992; Lesher JL, 1992). Itraconazole is approved in United States for the treatment of dermatophytic onychomycosis and terbinafine in Canada for this condition. The third agent, fluconazole, has not been approved for the treatment of onychomycosis. (Gupta AK, Scher RK and Doncker PD 1997)

Although these newer agents are promising in the treatment of fungal nail disease, they have their inherent limitations. They include potential adverse effects, drug interactions, high cost and compliance of patient. All the authors, however, are apparently unanimous on the aspects of: (a) cost perspective of these drugs,

which is exorbitant, especially in the context of poorly developing countries, like Bangladesh; (b) Need for periodic expensive laboratory monitoring.

Side effects associated with Itraconazole - Sporanx[R], Alcantara R, Garibay JM, 1988; Chiriteseu MM, 1996 have described side effects relevant to itraconazole. Common side effects are gastrointestinal disturbances, headache. On rare occasion nausea, pruritus, rhinitis, rash and dyspepsia are evidenced. Dizziness, fatigue, fear, impotence, decreased libido and malaise are other associated side effects.

Side effects associated with Fluconazole - In a study of 4,000 patients the incidence of side effect was 16%. (Chiriteseu MM, 1996; Elewski BE, 1998) Commonest side effects of fluconazole are gastrointestinal disturbances, headache. Other effects include hepatotoxicity (Diflucan[R]), Steven Johnson syndrome with AIDS (Osterloh IH, 1992), rash, abnormal liver function tests and drug interaction. (Bickers DR, 1994)

Side effects associated with Terbinafine - Lamisil[R]; Villars VV, Jones TC, 1992; Stricker BH, De-Jong PM, Schreuder F, et al 1992; Kovacs MJ, Alshammari S, et al 1994 have described the following side effects. Headache is the commonest type of side effects. Gastrointestinal disturbance (diarrhoea, dyspepsia, nausea) and skin (rash, urticaria, pruritus) are other varieties of side effects. Rare types of side effects include fatigue, inability to concentrate, leg pain, back pain, taste disturbance and transient hypoglycaemia.

2.12 CONTROVERSY / RESERVATION REGARDING THE TREATMENT OF ONYCHOMYCOSIS

Onychomycosis, particularly of the toe-nails, is a therapeutic challenge. Experience with topical antifungal therapy alone has been uniformly disappointing (Assaf RR, Elewski BE & Cleveland MD, 1996).

There is a wide variation of opinion regarding the treatment of onychomycosis with a spectrum of views across various physicians, centres and countries. Some people remain indecisive as to whether or not to go for treatment of onychomycosis (Rashid A, 2002). An unsatisfactory treatment outcome is the failure to achieve the desired result. An international survey showed that treatment strategies differed based on practices, as well as approaches adopted by different treating hospitals, patients' preferences, severity of disease, experience of the physicians, other associated medical conditions, socio-cultural aspects, prolonged duration and cost of treatment.

Until recently, the treatment of onychomycosis was discouraging because of the: relatively disappointing success rate, the need of prolonged therapy, high relapse rate and regular expensive laboratory monitoring necessary with traditional oral antifungal agents, like Griseofulvin and Ketoconazole etc. (Robert DT 1994).

2.13 IDEAL ANTIFUNGAL AGENTS

Broad spectrum, oral antifungal drugs are the most effective agents amiable for the treatment of moderate to severe onychomycosis. An "ideal" oral antifungal

agent would embrace the properties (not exhaustive) portrayed in the Table below:-

Table— 4: Properties of an "ideal" oral antifungal agent used for the treatment of onychomycosis (Richard B Odom, 1996).

Favourable nail kinetics
Incorporated into nail matrix
Diffuses through nail bed
High clinical cure rate.
High mycological cure rate.
Low incidence of relapse
Effective when used for short-term therapy
Low incidence of side effects
Few drugs interactions
Cost effective

2.14 ROLE OF SURFACE APPLICATOR IN NUCLEAR MEDICINE (Sr-90)

Marie and Pierre Curie used radium as a therapeutic agent in the treatment of cancer since its discovery in 1898. Radium disintegrates with a half-life of 1622 years to form radon. In 98.8% of its disintegration, one alpha particle is ejected with energy 4.79MeV. In the rest of the disintegration, a lower energy alpha ray followed by a gamma ray is produced.

This radium product series $^{214}_{83}$Bi decays by beta emission into an isotope of polonium accompanied by 8 prominent gamma lines with many weaker lines. Because of the strong gamma lines with energies up to 2.2MeV, having high

penetration capability, the radiation from this product series of radium is used for therapeutical treatment purposes.

When this radium is used in the treatment of cancer, all the alpha and beta particles are arranged to stop by the case (0.5mm Pt.) surrounding the radium salt, and gamma rays contribute to the biological effects. Hence, radium applicator is not suitable for providing treatment to the patients having superficial lesions. Beta ray applicators, on the other hand, are found useful for the treatment of superficial lesions (Johns H E and Cunninghum J R, 1978).

For many years, beta ray plaques were made using radium. Radium was sealed into a shallow metal box, the back and sides of which were thick enough to cut off the beta rays while the front surface was made very thin to allow the beta particles to come out. At this surface, 95% of the ionisation is due to beta particles and the rest 5% is due to the gamma rays. At a depth of 5 mm, most of the effect is due to gamma rays. Hence, such applicators were found not satisfactory for treating superficial lesions because of the presence of penetrating gamma components. Radon, on the other hand, which has also been used for many years, is sealed in a glass bulb with enough thin walls to allow beta rays to come out. However, since it is extremely difficult to produce glass bulb with identical wall thickness, the beta ray dose from such applicators is hard to predict. Because of this manufacturing difficulties and also the presence of unwanted gamma rays, radon applicators are found not particularly suitable for treating superficial lesions (Friedman, M., and Lewis, L.G 1949).

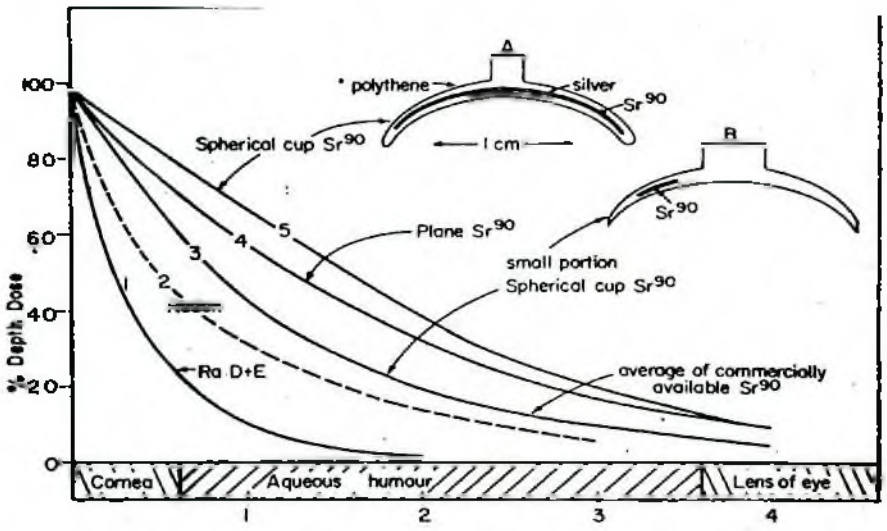
In 1952, ^{90}Sr, a long-lived fission fragment, became available for beta ray applicator. ^{90}Sr decays with half-life of about 27.7 yrs into ^{90}Y, which in turn decays with a half-life of 64 hrs into ^{90}Zr. The maximum beta energy from ^{90}Sr is 0.54 MeV while the ^{90}Y produces penetrating beta particles with maximum energy of 2.27 MeV. ^{90}Sr, foil – bonded in silver, is covered with polythene plastic to a thickness of about 0.5mm which is sufficient to stop the low energy beta particles from ^{90}Sr, is considered quite satisfactory for treatment of superficial lesions with almost uniform dose-deliver system. The back of the foil is usually covered with a layer of silver to absorb all the radiations from coming back to the operator (Sinclair, W.K and Trott, N.G 1956; Friedell, H.L., Tomas, C.I., and Krohmer, J.S, 1954). Thus ^{90}Sr is considered as suitable surface applicator.

2.15 DEPTH DOSE CHARACTERISTICS OF BETA APPLICATOR

Beta ray applicators are mainly useful for the treatment of superficial lesions such as in eye and in other superficial organs.

^{90}Sr – a long lived fission fragment is in common use as beta applicators.

^{90}Sr decays to ^{90}Y with a half life about 27.7 years, which in turn decays to ^{90}Zr with a half life of about 64 hrs. The maximum beta energy from ^{90}Sr is 0.54 MeV, while the ^{90}Y produces penetrating beta particles with maximum energy 2.27 MeV. A group at the Royal Marsden Hospital are considered pioneer for the development of the ^{90}Sr beta applicators for treatment of superficial lesions. A summary of their dose measurements is shown in fig- below (H.E.Johns, 1978).

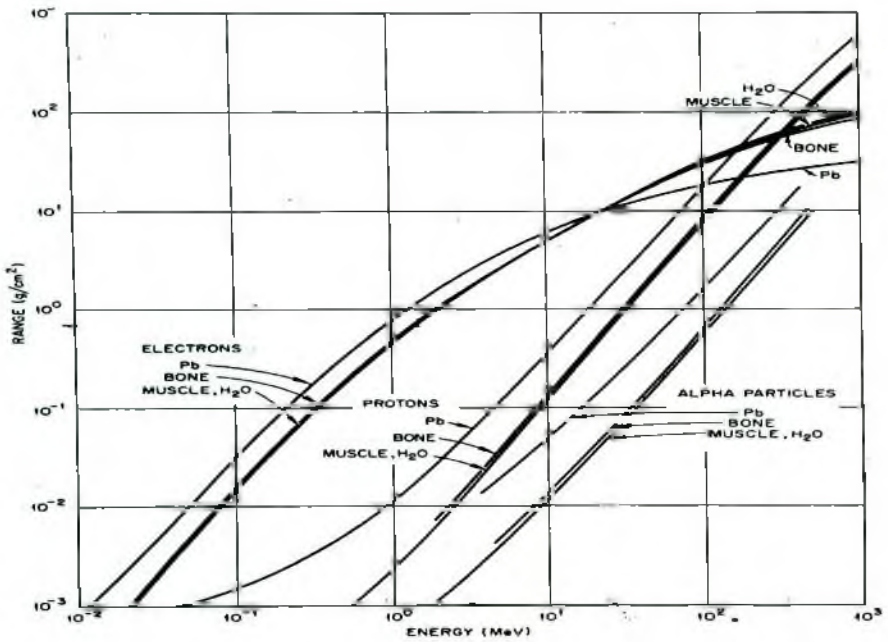


Here, curves 3 and 4 give the percentage depth dose for small and large spherical cup applicators while 5 give % depth dose for large plane applicators. These applicators are developed from ^{90}Sr. [Courtesy of H E Johns. The Physics of Radiology. 3rd Ed.]

Fig.3: Depth dose characteristics of beta applicator

2.16 RANGE OF BETA PARTICLE FROM ^{90}SR SURFACE APPLICATOR

In the present study, we used a plane circular ^{90}Sr surface applicator as beta source. The energy 0.54 MeV from ^{90}Sr is assumed to be absorbed in the source encapsulation materials while the energy 2.27 MeV from its daughter product ^{90}Y is responsible for providing necessary dose to the area covered by the applicator. The range of this beta particle can be estimated from the fig - give below:

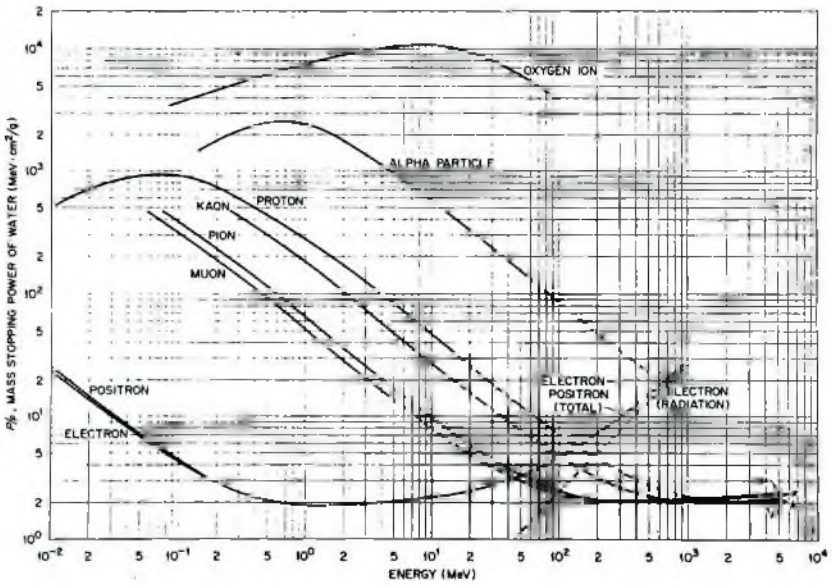


Range of protons, alpha particles and electrons (Beta particles) in water, expressed in g/cm^2 [Courtesy of James E Turner. Atoms, Radiation, and Radiation Protection. 1st Ed]

Fig.4: Range of beta particle from ^{90}Sr surface applicator.

From the curve, we see that beta particle from ^{90}Y with maximum energy 2.27 MeV may have the range of about 0.95 cm in bone, muscle or water.

This also correlates with range determined from mass stopping power of water for beta particles as shown in fig.5 below.

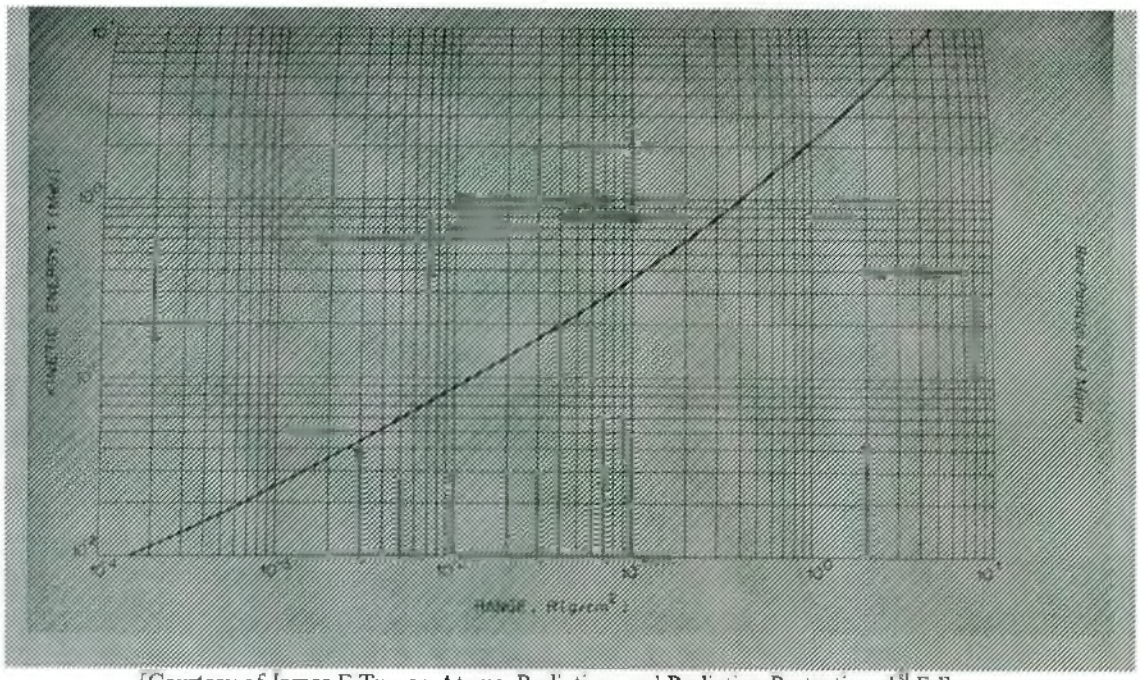


Mass stopping power of water in MeV-cm^2 /g for various heavy charged particles and beta particles.[Courtesy of James E Turner. Atoms, Radiation, and Radiation Protection, 1st Ed]

Fig.5: Mass stopping power of water for charged particles.

From the curve it is observed that a beta particle with max. Energy 2.27 MeV might have a range of about 1 cm in water.

From beta particle range –energy curve fig.6 for materials of low atomic numbers it can also be seen that the range of beta particle from $^{90}Sr - ^{90}Y$ decay system, the range of 2.27 MeV beta particle may have the range about 1.02 cm.



[Courtesy of James E Turner. Atoms, Radiation, and Radiation Protection. 1st Ed]

Fig.6: Beta-particles range-energy curve for materials of low atomic number.

2.17 PENETRATION OF BETA RADIATION IN NAIL

From the analysis of the above three graphs, it can reasonably be estimated that the range of penetrating beta particle from $^{90}Sr - ^{90}Y$ decay system could be about 0.95 – 1.02 cm in nail like materials assuming it as the constituent part of human body ($1 g/cm^3$) having density, which is slightly more than soft tissue but less than bony materials. So nail bed can easily be irradiated by beta ray as one selected in the present study.

2.18 SIMILAR WORK PERFORMED ELSEWHERE IN NUCLEAR RADIATION THERAPY

The present study is an innovative type of work, so exactly same instance is not available. However, followings are certain work which appears to be similar in nature have been described for strengthening the application of Nuclear Medicine radiations and to establish its limited risk.

Long term studies after radioiodine therapy (RIT) for Hyperthyroidism showed that radioiodine treatment has no increased risk of Carcinoma, Leukaemia, Infertility and teratogenic effect in pregnancy (Rivkees et al., 1988).

In a very large population based study, it showed that there is no absolute risk of cancers of the small bowel and thyroid after RIT for hyperthyroidism (Franklyn et at 1999).

Radiation exposure to gonads from RIT for Graves' disease is 0.8 - 1.4 rem, and this is similar to the exposure from a barium enema or intravenous pyelogram (Farrer and Toft, 1991).

Any reduction in fertility could not be seen in patients receiving I-131 for hyperthyroidism (Singer et al., 1995).

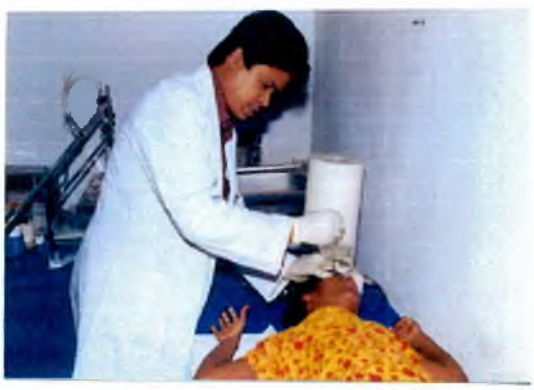


Fig 7: Application of beta radiation in post surgery pterigyum .

Beta radiation originated from Strontium applicator in post surgery pterigyum cases and other corneal diseases are in use with its prolong existence, which is still in practice in our country, specially in Nuclear Medicine centres and elsewhere in the world. Its use is safe with no reported adverse effect for sensitive part like, lens of the eyes (Lederman M, 1956; Dr. V Baucham, Head, Department of Nuclear Medicine, Chulalongkorn Hospital, Bangkok, Thailand, personal communication).

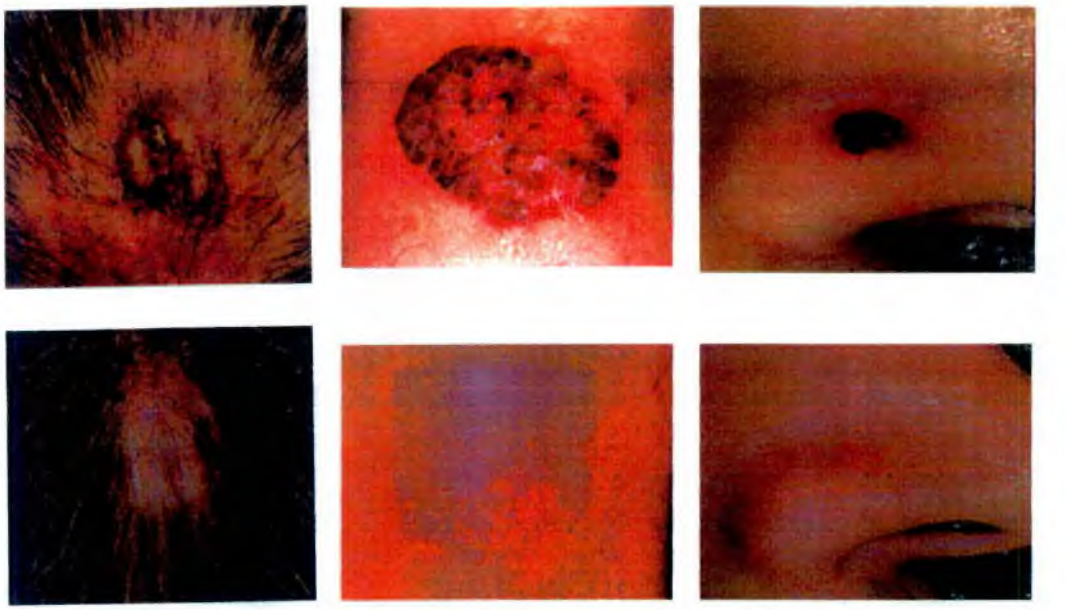
Radiation therapy to arrest the overgrowth of scar (Keloid) after surgery has been found to be useful with no known side effects (Van Den Brenk and Minty, 1960; Greer and Vickers, 1970).. It has been established that radiation therapy alone can be of certain help in the very recent Keloids, which are still cellular, well vascularised and growing (Edsmyr et al., 1974).

A wide variety of radiation sources that have been used in different radiotherapies till now are: radium needle implantation, 50 to 150 KVp X-ray, electron beam, etc. The less penetrating strontium – 90 beta ray is another alternative method of treatment for relieving symptoms and preventing regrowth of Keloid (Bonasree et al 1987).

A new study shows that the use of beta radiation may prevent repeat blockages in heart vessels following angioplasty as a nuclear stent. More than 75% of all 7,00,000 patients per year in USA receive stents. Beta radiation is highly localized form of radiation and the treatment is called "Nuclear Brackytherapy"; takes little

time with minimal radiation exposure and found that it reduces approximately 66% repeat blockages (Laskey and Sunthalaringam, 2000).

A very new development of utilization of beta radiation was observed in the field of skin cancer treatment. A radioactive patch containing holmium-166 (Ho^{166}) has been developed for skin cancer treatment and successfully applied for Squamous cell carcinoma of skin, Basal cell carcinoma, Bowen's disease etc. Contrary to the other conventional radiation therapies beta radiation seems to be more convenient and recommended to replace conventional surgery and external radiation therapy for skin cancer (Kyung BP, et al., 1999).



Pre and post treatment (3 mo) squamous cell carcinoma of scalp

Pre and post treatment (3 mo) Bowen's disease

Pre and post treatment (3 mo) Basal cell carcinoma

Fig. 8: Pre and post beta radiation treatment of Squamous cell carcinoma of scalp, Bowen's disease and Basal cell carcinoma.

2.19 IMPORTANCE OF PATIENT COUNSELLING

It is important to remember that the causative pathogens of onychomycosis are ubiquitous and hard to avoid. In addition, fungal infections are contagious to some extent, recalcitrant by nature, and often recurring. Without appropriate patient education regarding prophylactic measures to prevent recurrence, the disease will often return (Epstein E, 1998; Gupta AK, 1997).

Before treating fungal nail infections, it is essential to confirm the diagnosis. Potassium hydroxide (KOH) preparation and culture are the best method to this end. Numerous conditions (psoriasis, Darier's disease, lichen planus, chronic mucocutaneous candidiasis) and nondermatophyte pathogens (Candida, Scopulariopsis, Fusarium) can imitate a dermatophytes-related fungal nail infection. It is also important to counsel the patient in advance about the length of therapy (namely, the use of traditional antifungal agents will require many months of therapy), possible side effects, the expenses involved, laboratory tests that may be required, proper nail care, and the fact that no treatment is 100% effective.

In addition, patient should be provided with some general advice for good nail hygiene. (Table – 5)

Table – 5: General recommendations for nail care in patients with onychomycosis

Keep nails short
File down hypertrophic nails
Avoid trauma / irritants
Cotton and vinyl gloves for wet work
Heavy cotton gloves for dry work
Never use same instruments on both infected and uninfected nails
Wear properly fitting shoes
Good support
Wide toe box
Avoid higher heals and narrow toed shows
Take one's own instruments to the nail/beat salon
Use antifungal shoe / foot powder daily.
Keep feet cool but dry.

CHAPTER 3: PATIENTS AND METHODS

CHAPTER 3: PATIENTS AND METHODS

3.1 STUDY DESIGN

This study was an open, randomised, therapeutic trial to compare the therapeutic efficacy of beta radiation with the conventional antifungal treatment for dermatophytic onychomycosis. It was not possible to perform a double- blind study; a placebo-controlled double-blind study deemed likely to cause patient's compliance problem.

3.2 STUDY POPULATION

Patients with clinical suspicion of onychomycosis, who attended out patient department of Skin & VD of Dhaka Medical College Hospital and Sir Salimullah Medical College hospital were enrolled in the study, as were patients referred by private practitioners and those who came through personal communications. Patients with age above 12 years were taken irrespective of sex.

3.3 SAMPLE SIZE

Sample size of the study was determined using the appropriate statistical procedure. The outcome of treatment depends on (a) cure; and (b) duration of treatment to achieve success. The following formula was used considering the expected percentage of success as outcome on new treatment group.

$$n = \frac{P_1, q_1 + P_2 q_2}{(P_2 - P_1)^2} \text{ X } f(\alpha, \beta)$$

Where,

n = Required number of patients in each treatment group.

P_1 = Percentage of success (30%) expected on standard treatment group, i-e. anti-fungal.

P_2 = Percentage of success (50%) in the other treatment group i-e. β radiation therapy.

α = Level of significance (0.05).

β = Type II error (0.2) i-e. (1-β) or power of the test at 80% to detect a difference of magnitude (P_2-P_1)

f = Constant for fixed values of α & β, i-e. For α = 0.05 and β = 0.2, f=7.9

Using the above formula, the required sample size arrived at is 92. Hence, keeping consistency with the formula, 110 patients were taken in each treatment group with an assumption of patients' dropout and for better statistical significance.

[This study also intends to compare the results between treatment plan C (Beta radiation + Anti-fungal drugs) and treatment plan A (Anti-fungal drugs). Assuming 60% cure rate in treatment plan C, the sample size in each group becomes 89.

Thus, the higher sample size was selected considering the treatment difference between treatment plan A & B].

3.4 SELECTION OF PATIENTS

A total of 330 patients who tested positive for nail fungus by direct microscopy and/or culture, were selected from the study population (As suggested by literature, Jolly HW et al., 1983).

i) Patients below 12 years age,

ii) Pregnant women,

iii) Receiving oral contraceptives (susceptible agent for hepatotoxicity)

iv) Patients with hepatocellular dysfunction or porphyrin (Contraindication to Griseofulvin intake) were excluded from the study population.

These precautions were taken with a view to avoid radiation exposure to minor children, radiation hazard to foetus, possible risk of birth defect by griseofulvin (Physician's Desk Reference, 1995; Gupta AK, Sauder DN, Shear NH, 1994; Brodell RT, Elewski BE, 1995) and to prevent further risk of liver damage, etc. (Jolly H W et al., 1983; Harry LA, Richard BO, William DJ, 1998).

All the patients were divided into three groups before commencing treatment with beta radiation and anti-fungal drug on the basis of randomised allocation. In-group A, patients were given anti-fungal drug alone; in group B they were given beta radiation only and the group C was provided with a combination therapy of anti-fungal drug and beta radiation. Group – A was considered to be a control group.

3.5 PLACE OF STUDY

The study was carried out at Centre for Nuclear Medicine & Ultrasound (CNM&U), DMCH, and Dhaka. It is the first and oldest Nuclear Medicine Department in Bangladesh (Erstwhile East Pakistan). This department is known for therapeutic application of nuclear medicine to thyroid cancer, thyrotoxicosis, beta radiation to pterygium, bone pain palliation in metastatic bone disease, radiation synovectomy, post surgery Keloid ablation and certain others. Along the way, the centre has already earned good reputation regarding its radiation dosages, extent of its effectiveness and rate of success in application of therapeutic nuclear medicine, which are directly related to the present study. It is worth pointing that, this centre is well recognized and licensed by the competent authority of Bangladesh Atomic Energy Commission (BAEC) to undertake these types of studies.

3.6 PREPARATION OF PATIENTS AND INVESTIGATIVE WORK PLAN

For each patient, complete clinical history and physical examination were performed prior to inclusion of the subject into the study population under 'patients screening programme' (Drake L A et al., 1996). Before selecting the patient, the treatment procedure was elaborately described to the patient and consent was obtained from all patients or as appropriate, the patients guardian *(Appendix – I consent form).* Drug history and other necessary information and findings were recorded in the pre designed data sheet to capture a wide range of relevant information *(Appendix – II).*

Economic condition of the patients was ascertained by interviewing them regarding their monthly income from all possible sources. Patients who had previously used anti-fungal therapy had to observe at least a 4 weeks drug-free period prior to the entry into the study protocol. No antifungal or corticosteroid drugs other than the test medication was to be used during the study.

Each patient had undergone the following investigations.

(i) Mycological tests:

Isolation, identification and characterisation of the specific fungus by-

a. Direct microscopy and

b. Culture.

(ii) Blood chemistry & haematopoietic function test:

Liver function test, random blood sugar, renal function test and complete blood count using the **a) Standard Biochemical techniques** and **b) Microscopy respectively.**

3.6.1 MYCOLOGICAL METHODS

Specimen collection:

Nails with infection were washed with 70% alcohol to remove surface contaminants. Then the scraping was obtained using a sterile scalpel blade. The materials were collected from the active border of lesions. The superficial layers

of the nail were removed and scraping was collected from deeper layers in sterile dry petri dishes.

Microscopic examination of the specimens

A 40% potassium hydroxide (KOH) solution was used to digest the keratin surrounding the fungi for better visualization of hyphae and spores. A small quantity of the collected specimen was placed on a clean microscopic glass slide and covered with a cover slip.

Cleaning of the specimens was also speeded up by gently heating the preparation over the pilot flame of a Bunsen burner, taking care to prevent drying. The slides were then placed in petri dishes, together with dampened pieces of filter paper to prevent the preparation from drying out at room temperature. After one hour, the prepared slides were examined under microscope using 10 X and 40 X objectives with the condenser iris diaphragm closed sufficiently to give good contrast. Branching hyphae and arthrospores were searched in nail specimens.

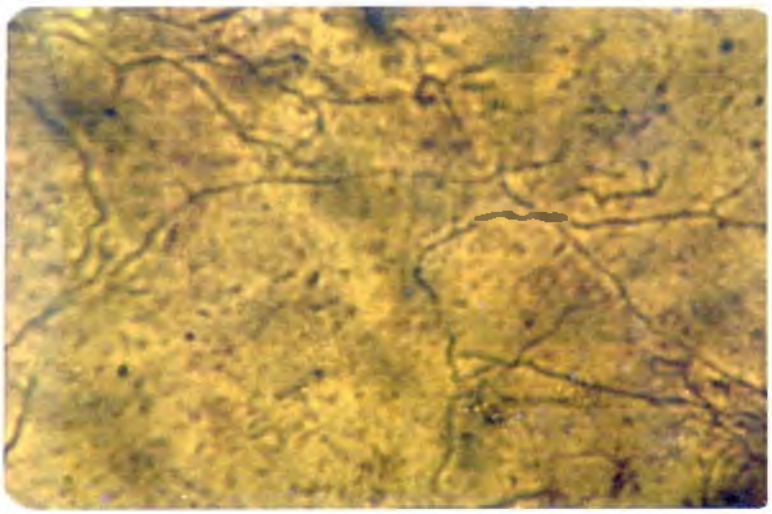


Fig: 9 Dermotaophytic hyphae in 40% KOH preparation as seen with the 40 X objective.

Interpretation

The demonstration of hyphae or arthospores or both were taken as positive while their absence were recorded as negative by direct microscopic examination.

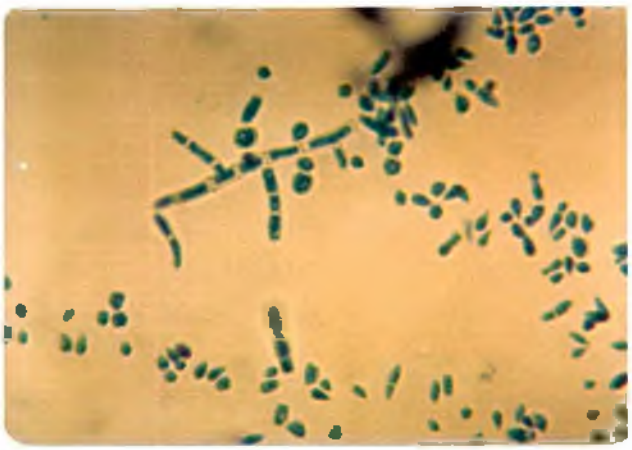


Fig. 10: Microscopic colonial morphology of T.rubrum, showing elongated macroconidium and numerous tear dropshaped micro conidia borne single on hyphae (X750).

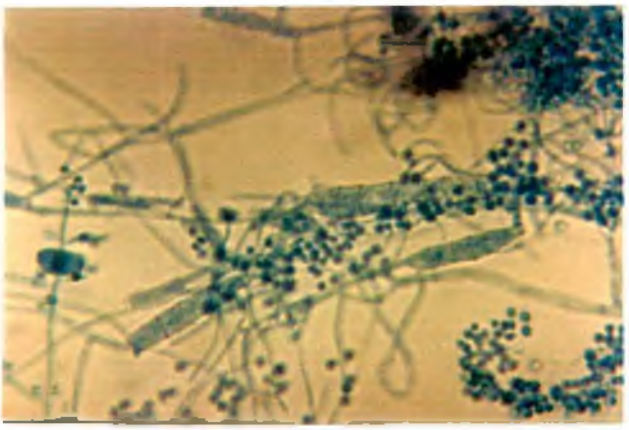


Fig. 11: Microscopic colonial morphology of T.mentagrophyte showing numerous microconidia in grape-like clusters. Several elongated (cylindrical) multiseptate macroconidia and spiral hyphae(X650).

Culture of the specimen

Specimens were cultured for isolation and identification of ? Onychomycosis / ? Dermatophytes.

(a) Primary isolation media

Tube cultures were done for primary isolation of dermatophytes. Fungobiotic agar (Appendix II1) and Sabourand's dextrose agar (SDA) with thiamine (*Appendix IV* were made as primary culture. Fungobiotic agar media containing Chloramphenicol and cycloheximide inhibits the growth of bacteria and saprophytic fungi, respectively.

(b) Subculture:

Plate cultures were done for subcultures and Sabouraud's dextrose agar *(Appendix –V)* was used for the purpose.

Inoculation

Small nail fragments were inoculated on the slanted surface of media with sterile straight wire loop.

Temperature and environment of inoculation:

The tubes containing fungobiotic agar were incubated at room temperature and the tubes containing SDA with Thiamine were incubated at room temperature as well.

Observation of Culture:

The incubated tubes were then examined on every alternate day for four weeks to see, if there was any growth. The tubes, which did not show any evidence of

growth even after four weeks, were discarded. When growth was found present, subcultures were done aseptically from the periphery of the colony on SDA in petri dishes. The inoculated plates were then incubated at room temperature for the study of gross and microscopic morphology of the colony.

Stock Culture

Preservation of stock cultures were maintained accordingly to Beneke and Rogers (1970), in screw capped tubes containing SDA slant at 5-10°C in a refrigerator. Subcultures were prepared every 3 months.

Identification of fungi

The species of dermatophytes grown in subcultures were identified on the basis of their colonial characteristics and gross microscopic morphology using normal saline preparation.

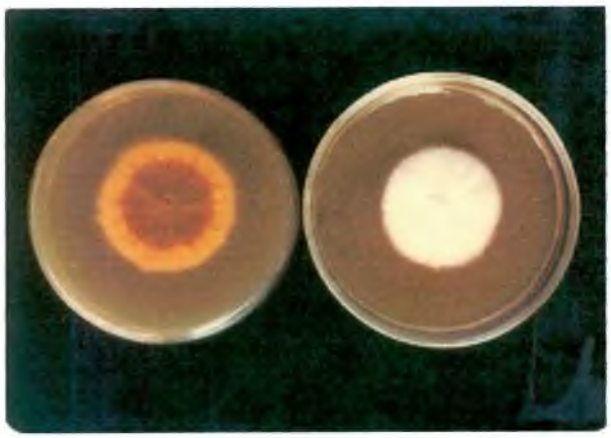


Fig.12: Gross colonial characteristics of T.rubrum surface (right) white and fluffy. Reverse site (left) red.

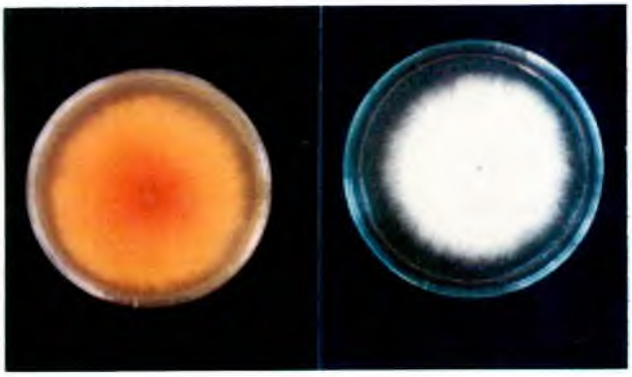


Fig.13: Gross colonial characteristics of T.mentagrophyte. Surface (right) white, flat and cottony. Reverse site (left) slightly yellowish.

Blood Chemistry and Haematopoietic function test

Internationally accepted standard biochemical methods were used to determine biochemical and haematological parameters of the study population. The following studies were performed:

1) Blood glucose
2) S. Bilirubin
3) SGPT
4) Blood Urea
5) S.Creatinine
6) Haemoglobin estimation and
7) Complete blood count: WBC total count and Differential count. Platelet count.

3.6.2 BLOOD CHEMISTRY

BLOOD GLUCOSE

Enzymatic colorimetric method without deproteinisation (Human/Brham and Trinder 1972, Teuscher and Richterich 1971)

Principle

The level of glucose was determined after enzymatic oxidation in presence of glucose oxidise. The hydrogen peroxide reacts under catalysis of peroxides with phenol and 4-aminophenazone to a red violet **quinoneimine(sp)** dye as indicator.

Sample materials

Serum, Plasma

Normal Values

(Fasting) 75 – 115 mg/dl or 4.2 – 6 mmol/L

(Random) up to 140 mg/dl 0r up to 7.8 mmol/L

SERUM BILIRUBIN (TOTAL)

Photometric Test for Direct (D) and Total (T) Bilirubin, modified method. (Jendrassik, L. and Grof P.1983, Bergh V and Muller A 1961 Human, Cat-No. 10740)

Principle

Bilirubin reacts with diazotised sulphanilic acid (DSA) to form a red azo dye. The absorbance of this dye at 654 mm is directly proportional to the bilirubin

concentration in the sample. Water soluble bilirubin glucuronides reacts directly with DSA whereas the albumin conjugated indirect bilirubin will only react with DSA in presence of an accelerator: total bilirubin = direct + indirect bilirubin.

Sample Material

Fresh haemolysis free serum, EDTA – or heparinized plasma.

Normal Values

Adults: Upto 1.0mg/dl, 17.1μmol/L

SGPT (ALT)

Colorimetric method (Reitman 1967, Shmidt and Schmidt 1973)

Human, Cat. No. 12012 Complete test kit. (Clin. Chem. Acta 1980, Wallnofer et al. 1974, Thefeld, W. et al. 1974)

Method

Kinetic method for the determination of ALT activity according to the recommendations of the Expert Panel of the IFCC (International Federation of Clinical Chemistry).

Reaction principle

2-oxoglutarate + L-alanine GPT L-glutamate + Pyruvate.

Pyruvate+NADH+H^+LDH L-alanine + NAD^+

Sample Materials

Fresh serum

Normal Values

Adults: Up to 12 U/L

BLOOD UREA

Urease – Berthelot colorimetric method without deproteinisation. (Fawcett, JK and Scott JE, 1967; Chaney, AL and Narbach Al., 1962)

Principle

Urease in serum is hydrolysed to ammonia in the presence of urease. The ammonia is then measured photometrically by Berthelot's reaction.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} \text{Ammonia} + CO_2$$

Sample materials

Serum, Heparinized or EDTA plasma

Normal Values

Serum	10 – 55 mg/dl 1.7 – 9.1 mmol/L

SERUM CREATININE

Jeffe colorimetric method without deproteinisation method (Henry RJ; Cannon DC; Winkelmin JW; 1960; Chaney AL and Marbach AL., 1962)

Principle

Creatinine in alkaline medium reacts with picrate to form a coloured complex. The rate of formation of the complex is measured.

Sample materials

Serum or plasma

Normal Values

Serum	Males	0.6 – 1.1 mg/dl 53 – 97 µmol/L
	Females	0.5 – 0.9 mg/dl 44 – 80 µmol/L

3.6.3 HAEMATOLOGICAL TESTS

Haemoglobin Estimation

Photometric Colorimetric Test for Determination of Haemoglobin in Blood.

Haemoglobin Cyanide Method

Human, Cat No. 10751

Principle (ICSH 1967, Vankamper and Zijlstra 1961)

Haemoglobin reacts with potassium hexacyanoferrate (III) and potassium cyanide to form a coloured complex haemoglobin-cyanide with an absorbance maximum of 540 mm. The absorbance of the complex is directly proportional to the haemoglobin concentration.

Sample Material

Whole blood

Normal Values (Wintrobe 1956)

	mmol/L	g/dl
Males	8.7 – 11.2	14 – 18
Females	7.5 – 9.9	12 – 16

OTHER HAEMATOLOGICAL TESTS

(i) WBC total count, (ii) WBC differential count and (iii) Platelet count (Khaleque and Mamun 1995)

(i) WBC Total Count

Venous or capillary blood was collected in WBC diluting fluid. After following the standard procedure total count of WBC was counted by-

Improved Neubauer counting chamber. Total WBC = N X 50/cm when dilution is 1 in 20.

(ii) WBC Differential Count

A film was prepared from a drop of peripheral or venous blood on a slide and the film was stained with a Romanowsky's stain eg. Leishman Stain.

The differential count, number and presence of abnormal cell were detected.

(iii) Platelet Count

Free flowing blood was drawn from the patient. It was then diluted with platelet diluting fluid. Following standard procedures the Platelet count was taken using the Improved Neubauer counting chamber.

3.7 DETERMINATION OF LETHAL DOSE OF BETA RADIATION

Before patient's nails were irradiated, a lethal/effective dose of beta radiation was determined. This was done by using an in- vitro method where radiations in different doses were applied on surface culture of fungi.

REQUISITES

Primary culture:

i) Fungobiotic Agar Media

Sub-culture:

i) Subouraud's Dextrose Agar Media

ii) Malt extract Agar media

The primary stock culture was grown on fungobiotic agar media where fungal colonies were developed and subcultures were prepared from those. This stalk culture was used to inoculate Sabouraud's dextrose agar media on petridishes. Six such petridishes were inoculated for culture.

One fungal species was subcultured as one focus / spot in the centre of a medium sized petridish. Growth of fungus was observed every day. Starting from 3rd day of inoculation beta radiation was applied in different doses and at various durations.

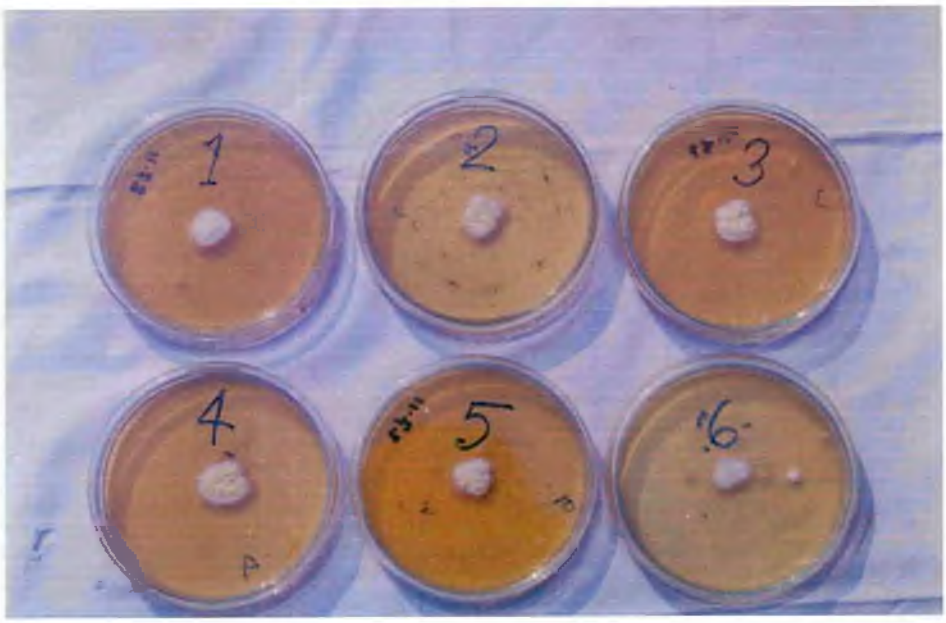


Fig.14: Surface culture of fungi

Fig.15: Invitro procedure for optimisation of beta radiation dose for onychomycosis

Fig.16: Irradiated petri dishes containing fungus culture

Petridish-1
Dose 500 Rads-
Two fractions / week
5 applications
Time/Fr = 4 min 27 sec

Petridish – 2
Dose 1000 Rads
Two fractions / week
3 applications
Time/Fr = 8 min 54 sec

Petridish – 3
Dose 1500 Rads
Two fractions / week
2 applications
Time/Fr = 13 min 21 sec

Petridish – 4
Dose 1000 Rads
One fractions / week
3 applications
Time/Fr = 8 min 54 sec

Petridish – 5
Dose 500 Rads
One fraction / week
5 applications
Time/Fr = 4 min 27 sec

Petridish-6
Control
No radiation exposure was applied.

For observations of the results of irradiation and to determine the lethal / effective dose of beta ray the following procedure was followed-

From the irradiated petridishes (1 – 5) and also from un irradiated petridish (6) (40%) KOH - slides and culture tubes were prepared by using sterile scalpel blade from centre and area respectively. Microscopic and culture examinations were performed. Examinations were carried on 4^{th}, 6^{th} and 8^{th} weeks. The obtained results were considered as:

Positive – Presence of septate hyphae, spherical spores in clusters and chain form.

Negative – Absence of hyphae and spores.

Table – 6: Post-irradiation Period (Microscopic finding)

Doses of Irradiation in respective slides	**Time of Examinations after completion of irradiation**		
	4^{th} Week	6^{th} Week	8^{th} Week
	Irradiated area	**Irradiated area**	**Irradiated area**
Slide – I: 2500 rads	Positive	Negative	Negative
Slide – II: 3000 rads	Positive	Positive	Negative
Slide – III: 3000 rads	Positive	Negative	Negative
Slide – IV: 3000 rads	Positive	Positive	Negative
Slide – V: 2500 rads	Positive	Positive	Negative
	Unirradiated	**Unirradiated**	**Unirradiated**
Slide – VI: 0 rads	Positive	Positive	Positive

In table – 6, slide – I shows the result of microscopic examination of KOH preparation from plate 1, slide – II shows that of plate 2 and so on up to plate 6.

Table – 7: Post-irradiation period (Culture finding)

Doses of Irradiation in respective slides	Time of Examinations after completion of irradiation		
	4^{th} Week	6^{th} Week	8^{th} Week
	Irradiated area	Irradiated area	Irradiated area
Slide – I	Positive	Negative	Negative
Slide – II	Positive	Positive	Negative
Slide – III	Positive	Negative	Negative
Slide – IV	Positive	Positive	Negative
Slide – V	Positive	Positive	Negative
Slide – VI	Positive	Positive	Positive

In table – 7, slide – I shows the result of culture findings from plate 1, slide – II shows that of plate 2 and so on up to plate 6.

Slide 1 and slide 3 showed same results. Results from slide 1 were taken as the acceptable lethal dose rate for (1) convenient and less exposure of radiation on a single day. (2) Simple office procedure (Ref)

3.8 THERAPEUTIC REGIMEN

Patient population was randomised in 3 groups to decide the treatment regimen.

In-group A – Anti-fungal drug was prescribed. Tablet Fulcinex (Griseofulvin, the conventionally used drug) was given. Dose – 500mg/day by mouth for 12 – 16 weeks.

A total of 110 patients in this group were considered as control.

In-group B – Beta radiation alone was considered for 110 patients.

For Beta radiation, the Beta applicator was used. It is a $^{90}Sr/^{90}Y$ Source device having initial dose rate of 2.05 rads/sec.

Fig.17: Beta Applicator (^{90}Sr) employed for the present study.

DOSIMETRY FOR Sr-90/Y-90 FOR APPLICATION IN FUNGAL INFEECTION SOURCES SPECIFICATION

Sr-90/Y-90

ENERGY Sr-90..... 0.546 MeV.

Y-90..... 2.274 MeV

ACTIVITY: 1022.60 MBq ON 13.10.1999

OVERALL DIMENSION: 30 X 15 mm

ACTIVE DIAMETER: 15 mm

DOSE ON 13.01.1998 WAS 2.0492cGy/Sec.

CALIBRATION OF DOSE RATE AT ANY TIME IS:

D = D_o exp – (0.693t/28.5)

D_o = Dose rate at the time t = 0 (i-e. ref. date)

D = Dose rate at t = t

T = Time period between initial measurement and date of measurement required.

Dose rate on 18.10.2001 is 112.20 rads / min.

Time for dose of 500 rads is about 4.456 mins.

A total dose of 2500 rads was given in fractions (at least 5 fraction).

The average dose per fraction was 500 rads in biweekly scheme.

PREPARATION OF SITE AND APLICATION OF BETA RADIATION FOR INFECTED NAIL

i) Area of nail site to be irradiated was cleaned with antiseptic.

ii) In case of thick nails it was filed to make thin.

iii) Nail site was measured to ensure that the beta applicator covers the desired area.

In-group C: After giving complete 5 fractions of Beta radiation, Tablet Fulcinex (Griseofulvin) was given in the dose of 500 mg/day for 6 weeks.

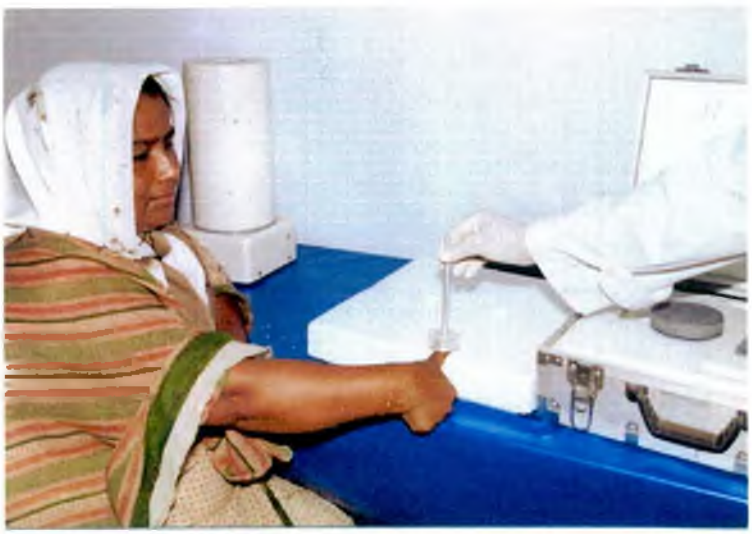


Fig. 18: Application of beta radiation in a case of fingernail onychomycosis

3.9 ADJUNCTIVE THERAPY

When paronychea was found as an associated condition, before giving pre-determined treatment for onychomycosis, giving antibiotic and anti-inflammatory drugs was practiced in the present study to control paronychea and thus to avoid beta irradiation at ulcerated condition. In few severe conditions local antibiotic was also applied.

3.10 FOLLOW UP

Patients of the study population were followed up at every 6 weeks for approx.6 months in post therapy state. The following parameters were considered for follow up.

i. Clinical evaluation.
ii. Direct microscopy.
iii. Culture.
iv. Biochemical and haematological parameters.

3.11 CLINICAL AND MYCOLOGICAL ASSESSMENT MEASURES

Patients were assessed at baseline (pre-treatment visit) and 24 weeks (for ultimate out come); follow-up assessments were made at approx. weeks 6, 12, 18 and 24.

At each visit, they were evaluated for clinical signs and symptoms and specimen were taken for KOH examination and culture.

Lesions were rated (on a four point scale) for decolourisation, scaling, vesiculation, inflammation, paronychea, exudation and tenderness. The four-point scale ranged from None, Mild, Moderate and Severe involvement.

The clinical response, based on rating of lesions was assessed as clinical improvement (as No, Mild, Moderate and Marked improvement) at every visit.

Side effect observed or reported and sign of recurrence (deteriorated) were also recorded at every visit.

The criterion as mentioned below were determined following the other studies (Richard BO, 1997; Jolly HW, 1983)

(I) Clinical success → A global evaluation of 'cleared' or "markedly improved" for the first time, any time during the study.

(II) Mycological success → A negative KOH and culture anytime during the study.

(III) Overall success → Simultaneous clinical and mycological success.

(IV) Clinical relapse → Deterioration in the global evaluation after a patient had achieved clinical success.

(V) Mycological relapse → Conversion of KOH or culture to positive after both had been negative.

In our study mycological success, mycological failure and mycological relapse criterion were mainly followed.

Laboratory safety tests were performed at each 6th week consecutive visits for 24 weeks to monitor treatment safety. All adverse effects were recorded throughout the study period.

3.12 STATISTICAL ANALYSIS

Information obtained from the patients, were entered into microcomputer using Microsoft Word program. Data were cleaned for out-of-range errors and inconsistency.

Statistical analysis was carried out using commercially available software package, SPSS (Statistical Package for the Social Sciences).

Unpaired student's 't' test was used for comparison with the three treatment groups of patients. Statistical significance was evaluated with 'p' values. (Table 8, 9, 10, 11, 12, 13, 22, 23)

Chi-square test was extensively used for comparison between different treatment groups. (Table 14, 16, 19, 20, 21, 26, 27, 28, 29, 30)

CHAPTER 4: RESULTS

CHAPTER 4: RESULTS

This open, parallel group studies reported in the present thesis work were performed to assess the efficacy of nuclear radiation in the treatment of onychomycosis. Three hundred and thirty patients were selected on random allocation. The study population were divided into three groups such as: Group – A, Group – B and Group – C. In each group the number of patients was 110. The treatment strategy for each group was different. Group – A was treated with antifungal drug, Group-B with beta radiation while Group – C was treated with combination of antifungal drug and beta radiation. In each group, the patients' inclusion was on randomised basis. Group – A was considered as the control group. During the final analysis of the results patients who attended all four visits for follow up were included and finally analysed. In Group – A, 14 (12.73%) patients, in Group – B, 17 (15.45%) and in Group – C, 12 (10.90%) patients were dropped from the study. Thus, we got 287 patients after 4th follow up for final evaluation.

An age distribution containing the study population of all three-treatment groups was described in Table – 8.

Table - 8: Age distribution in different groups of patients under study

(n=287)

		P value[a]	
		Group B	Group C
	Age (years) (Mean±SD)	35.52±9.91	38.41±12.59
Group – A	36.41±11.48	0.558	0.242
Group – B	35.52±9.91		0.073

[a]Unpaired Student's 't' test,

The mean age in years (mean ± SD) for Group – A (n=96) was 36.41 ± 11.48, for Group-B (n=93) was 35.52 ± 9.91 and for Group-C (n=98) it was 38.41 ± 12.59. The age difference in different treatment groups was not statistically significant (P=0.346). This age distribution was also presented in bar chart as in fig-1 in decade pattern.

Fig–1: Indicates the highest number of the disease occurred in between 31 – 40 years and lowest frequency was found in 61 – 70 years.

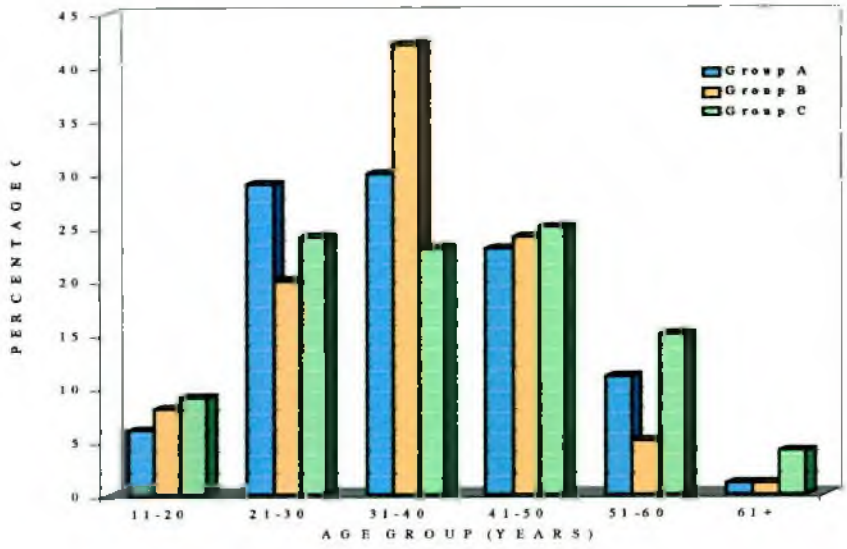


Fig. 19: Decade wise age distribution of study patients

Height and weight distribution of the study population (n=287) was described in table – 12. In Group – A mean height was 159.13cm ± 7.20, in-group – B, 160.23cm ± 9.23 while in-group – C it was 158.65cm ± 7.04. Average weight observed in patients was 60.49kg ± 8.76 in Group – A, 60.95kg ± 12.40 in-group B and 59.49 kg ± 9.79 in-group – C. On statistical analysis no significant

difference was observed in different patient groups in regard of height and weight (P=0.447).

Table – 9: Distribution of patient's height and weight
(n=287)

		P value[a]	
		Group B	Group C
	Height (cm) (Mean±SD)	160.23±9.23	158.65±7.04
Group – A	159.13±7.20	0.346	0.634
Group – B	160.23±9.23		0.173
	Weight (kg) (Mean±SD)	60.49±8.76	59.49±9.79
Group – A	60.49±8.76	0.762	0.447
Group – B	60.95±12.40		0.357

[a]Unpaired Student's 't' test

Table – 10 indicates economic status of study population. It was based on family income (in Taka) from all possible sources.

The average income with 1SD in Group – A was 9665.00 ± 12265, Group – B 9121.00 ± 9970.38 and in Group – C: 8248.00 ± 8584.15 respectively.

Table–10: Economic status of study population (n=287)

		P value[a]	
		Group B	Group C
	Family income (Taka) (Mean±SD)	9121.00±9970.38	8248.00±8584.15
Group – A	9665.00±12265.94	0.731	0.345
Group – B	9121.00±9970.38		0.508

[a]Unpaired Student's 't' test

The observed variation in income was statistically not significant. It is to be noted that most of the subjects in our study population were from low and middle-income group.

Duration of the signs and symptoms of past illness was as shown in table – 11.

Table – 11: Past history of onychomycosis in different groups of patients (n=287)

		P value[a]	
		Group B (n=26)	Group C (n=55)
	Duration of symptoms(years) (Mean±SD)	3.10±1.39	5.19±4.87
Group – A (n=15)	3.80±1.10	0.097	0.278
Group – B (n=26)	3.10±1.39		0.035*

[a]Unpaired Student's 't' test, *Significant at P<0.05

It was observed that the mean duration with ISD was 3.80 ± 1.10, 3.10 ± 1.39 and 5.19 ± 4.87 in Group A, B and C respectively. The observed mean difference in duration of past history between group A and B is not significant while the difference in between group C and B was statistically significant (P< 0.05).

The distribution of drug history was as shown in table –12.

Table – 12: Antifungal drug history in different groups of patients (n=287)

		P value[a]	
		Group B (n=9)	Group C (n=28)
	Duration of drug use(months) (Mean±SD)	2.00±1.94	2.36±4.62
Group – A (n=9)	4.67±2.00	0.011*	0.157
Group – B (n=9)	2.00±1.94		0.824

[a]Unpaired Student's 't' test, *Significant at P<0.05

From table – 12 it was seen that in-group A the patients drug history covered a period of 4.6 ± 2.00 in Group B it was 2 ± 1.94 while in-group C it was 2.36 ± 4.62. The difference of the duration of drug intake was found significant between group A and B (P=0.011) while the difference was not significant between group A & C or B and C respectively.

Duration of signs and symptoms of present illness was as shown in table – 13.

Table – 13: Duration of sign and symptoms of the present illness in the population under study (n=287)

		P value[a]	
		Group B	Group C
	Duration of sign and symptoms (months) (Mean±SD)	11.27±36.50	11.38±22.38
Group A	5.92±5.45	0.149	0.019*
Group B	11.27±36.50		0.980

[a]Unpaired Student's 't' test, *Significant at P<0.05

From this table - 13 it showed that the duration of present illness in patients of Group – A was 5.92 ± 5.45, in Group – B 11.27 ± 36.50 and in Group – C 11.38 ±22 respectively.

Duration of present illness between group A and C was significant (P<0.05) while in groups A and B (P=0.149) and B & C were not significant.

Demographic data in table – 14, out of 287 patients, 125 (43.6%) were male and 162 (56.4%) were female representing the sex distribution in the present study population. In group A, group B and group C the male was 41.7%, 44.08% and 44.89% respectively while the female population was 58.3%, 55.92% and 55.11% respectively. Difference between total male and female is not significant (P=0.149). Although difference between Group A & B are significant (P<0.05).

The education characteristics were well represented in the given table -14 where illiterate patient was considered as one having no alphabetical knowledge and others with alphabetical knowledge were considered literate with respective grades. In that respect, 19 (6.62) patients were illiterate while 268 (93.38%) patients were literate. Significantly higher percentage of literate patients was accumulated for treatment (P<0.01) in the present study.

Table – 14: Demographic characteristics of the study population

Parameters	Group A (n=96)		Group B (n=93)		Group C (n=98)		Total (n=287)	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Sex								
Male	40	(41.70)	41	(44.08)	44	(44.89)	125	(43.55)
Female	56	(58.30)	52	(55.92)	54	(55.11)	162	(56.45)
Chi-square test: $X^2 = 0.190$, df = 2, P=0.910								
Education								
Illiterate	4	(4.16)	3	(3.25)	12	(12.24)	19	(6.62)
Class I-V	20	(20.84)	13	(13.97)	13	(13.27)	46	(16.02)
Class VI-X	18	(18.75)	27	(29.03)	13	(13.27)	58	(20.22)
Class X+	54	(56.25)	50	(53.75)	60	(61.22)	164	(57.14)
Chi-square test: $X^2 = 17.090$, df = 6, P=0.009**								
Occupation								
Student	6	(6.25)	4	(4.30)	9	(9.18)	18	(6.27)
Housewife	41	(42.71)	33	(35.48)	33	(33.68)	107	(37.28)
Service holder	30	(31.25)	44	(47.32)	44	(44.90)	118	(41.14)
Business	14	(14.58)	6	(6.45)	7	(7.14)	28	(9.75)
Manual Labourer	5	(5.21)	6	(6.45)	5	(5.10)	16	(5.56)
Chi-square test: $X^2 = 11.082$, df = 8, P=0.197								
Residence								
Slum	43	(44.79)	43	(46.24)	44	(44.89)	130	(45.29)
Building	53	(55.21)	50	(53.76)	54	(54.11)	157	(54.71)
Chi-square test: $X^2 = 0.027$, df = 2, P=0.987								
Family history								
Positive	45	(46.87)	45	(48.38)	57	(58.16)	146	(50.87)
Negative	51	(53.13)	48	(51.62)	41	(41.84)	141	(49.13)
Chi-square test: $X^2 = 2.961$, df = 2, P=0.228								
Past history								
Positive	14	(14.58)	22	(23.66)	54	(55.10)	89	(31.01)
Negative	82	(85.42)	71	(76.34)	44	(44.90)	198	(68.99)
Chi-square test: $X^2 = 40.932$, df = 2, P=0.000*** (P<0.001)								
History of recent medication								
Present	20	(20.83)	41	(44.08)	17	(17.34)	80	(27.87)
Absent	76	(79.17)	52	(55.92)	81	(82.66)	207	(72.13)
Chi-square test: $X^2 = 21.726$, df = 2, P=0.000*** (P<0.001)								

**Significant at P<0.01, ***Significant at P<0.001

Regarding the occupation, the highest percentage was found in service holder group (41.14%), while second group was housewife (37.28%), then the businessman comes (9.75%) and thereafter the student group (6.27%) and the last one was the manual labourer (5.56%). Statistically no significant difference was observed among them (P=0.197).

With respect to residence, 130 (45.29%) of the study population belong to slum dwellers and the rest 157 (54.71%) belongs to the buildings. Difference was also not significant (P=0.987).

Family history for the disease was positive in 146 (50.87%) and negative in 147 (49.13%). The findings of difference were not significant (P=0.228).

Past history of the disease itself was positive in 89 (31.01%) and negative in 198 (68.99%), which was highly significant on statistical analysis (P<0.001).

History of recent antifungal medication was positive in 80 (27.87%) and negative in 207 (72.13%), which was statistically significant (P<0.001).

Table – 15: Chief complaints presented in different groups of patients

Complaints	Group A (n=96)		Group B (n=93)		Group C (n=98)		Total (n=287)	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Pain	82	(85.00)	85	(91.00)	85	(86.73)	252	(87.80)
Brownish-yellow discoloration of nail	36	(38.00)	19	(20.43)	43	(43.88)	98	(34.15)
Black-grey discoloration of nail	47	(48.95)	67	(72.04)	47	(47.95)	161	(56.09)
Bright-white discoloration of nail	12	(12.50)	7	(7.52)	6	(6.12)	25	(8.71)
Destruction of nail	0		1	(1.07)	0		1	(0.35)
Scaling of nail	19	(19.97)	26	(27.95)	15	(15.30)	63	(21.95)
Pus formation	0		12	(12.90)	3	(3.06)	15	(5.23)
History of trauma	6	(6.25)	12	(12.90)	12	(12.24)	30	(10.45)
Itching	17	(17.70)	9	(9.67)	9	(9.18)	35	(12.19)
Thickening of nail	0		0		5	(5.10)	5	(1.74)

Out of 287 patients 87.80% of them had pain, 34.15% of the patients had brownish yellow discolouration of infected sites and nails with bright white discolouration were 8.71%. Destruction of nail was only in 0.35%, scaling of nail was 21.95 % while pus formation in infected sites was about 5.23%. Patients with history of trauma were 10.45%, itching of infected sites was 12.19% while thickening of nails was about 1.74%.

Table – 16: Site of involvement in different groups of patients (n=287)

Sites	Group A (n=96) No.	(%)	Group B (n=93) No.	(%)	Group C (n=98) No.	(%)	Total (n=287) No.	(%)
Single fingernail	43	(44.79)	43	(43.24)	19	(19.38)	105	(36.58)
Multiple fingernail	33	(34.37)	12	(12.90)	27	(27.55)	72	(25.08)
Single toenail	21	(21.87)	14	(15.05)	12	(12.25)	47	(16.37)
Multiple toenail	3	(3.12)	16	(17.20)	22	(22.45)	41	(14.28)
Single fingernail plus single toenail	0		12	(12.90)	6	(6.12)	18	(6.27)
Single fingernail plus multiple toenail	0		3	(3.22)	11	(11.22)	14	(4.87)
Multiple fingernail plus multiple toenail	0		0		3	(3.06)	3	(1.04)

$X^2 = 69.235$, df = 12, P=0.000*** (significant at P<0.001).

Out of 287 patients, there were 105 patients (36.58%) had single fingernail involvement while multiple fingernails were involved in 72 (25.08%) patients, single toenail was involved in 47 (16.37%) cases, multiple toenails were in 41 (14.28%), single finger and single toenail in 18 (6.27%) patients, single finger and multiple toenails in 14 (4.87%) and multiple finger and multiple toenails in 3 (1.04%) patients only.

Single fingernail involvement was found to be the highest in percentage while multiple fingers and multiple toenails involvement was found as the lowest one.

The percentages of fingernails involvement appear to be more than that of toenails. These findings were statistically significant (P=0.000).

Table – 17: Local examination findings in patients (n=287)

Findings	Group A (n=96) No.	(%)	Group B (n=93) No.	(%)	Group C (n=98) No.	(%)	Total (n=287) No.	(%)
Tender nail	90	(93.75)	82	(88.17)	94	(95.920)	266	(92.68)
Swollen soft tissue	0		6	(6.25)	1	(1.02)	7	(2.43)
Rough brittle nail	11	(11.45)	10	(10.75)	13	(13.26)	34	(11.84)
Ingrown nail Colour	0		3	(3.22)	0		3	(1.04)
Brownish	9	(9.37)	9	(9.67)	4	(4.08)	22	(7.66)
Brownish- yellow	18	(18.75)	5	(5.37)	25	(25.51)	48	(16.72)
Blackish	24	(25.00)	41	(44.08)	18	(18.36)	83	(28.92)
Blackish- yellow	25	(26.04)	36	(38.70)	38	(38.77)	99	(34.49)
Blackish- grey	12	(12.50)	3	(3.22)	9	(9.18)	24	(8.36)
Bright- white	12	(12.50)	6	(6.45)	6	(6.12)	24	(8.36)

Tender nails were found in 266 (92.68%), which were consistent with pain as the chief complaints of the patients. Surrounding soft tissue swelling was found in 7 (2.43%) cases. Rough and brittle nails were in 34 (11.84%) patients. Ingrown nails were found in 3 (1.04%) cases. However, 100% patients showed decolourisation of nails. Bright white decolourisation was found in 24 (8.36%) patients.

Onychomycosis associated findings and adjunctive therapies were described in table – 18.

Table – 18:Onychomycosis associated findings and adjunctive therapy provided (n=287)

Parameters	Group A (n=96) No.	(%)	Group B (n=93) No.	(%)	Group C (n=98) No.	(%)	Total (n=287) No.	(%)
Diagnosis								
Paronychea	47	(48.95)	70	(75.27)	51	(52.04)	168	(58.54)
Paronychea with koilonychea	1	(1.05)	0		0		1	(0.35)
None	48	(50.00)	23	(24.73)	47	(47.96)	118	(41.11)
Therapy Given	20	(41.66)	40	(57.14)	38	(74.51)	98	(57.99)
Not given	28	(58.34)	30	(42.86)	13	(25.49)	71	(42.01)

Out of 287 cases, paronychea was observed in 168 (58.54%) cases. Paronychea with koilonychea was found in 1 (0.35%) patient. No associated finding was found in 118 (41.11%) case. Out of 169 cases of paronychea 98 (57.99%) of the study population received adjunctive therapy while the rest 71 (42.01%) was free of therapy (P<0.01).

Table – 19: Pre treatment microscopic findings for detection of fungus (n=287)

Findings	Group A (n=96) No.	(%)	Group B (n=93) No.	(%)	Group C (n=98) No.	(%)	Total (n=287) No.	(%)
Positive	87	(90.62)	88	(94.62)	98	(100.0)	273	(95.12)
Negative	9	(9.38)	5	(5.38)	0		14	(4.88)

$X^2 = 8.842$, df = 2, P=0.012 (significant at P<0.05)

Pre-treatment microscopic findings in KOH preparation revealed that out of 287 cases, 273 (95.12%) were found to be positive and the rest 14 (4.88%) cases were negative (P<0.05)

Table – 20: Pre-treatment culture findings for detection of fungus (n=287)

Findings	Group A (n=96) No.	(%)	Group B (n=93) No.	(%)	Group C (n=98) No.	(%)	Total (n=287) No.	(%)
Positive	37	(38.54)	59	(63.44)	54	(55.10)	150	(52.26)
Negative	59	(61.46)	34	(36.56)	44	(44.90)	137	(47.74)

$X^2 = 12.841$, df = 2, P=0.002 (significant at P<0.01)

Pre treatment culture revealed that out of 287 cases, 150 (52.26%) were found to be positive and the rest 137 (47.74%) cases were negative (P<0.01).

Table – 21: Clinical diagnosis of the different groups of patients (n=287)

Diagnosis	Group A (n=96) No.	(%)	Group B (n=93) No.	(%)	Group C (n=98) No.	(%)	Total (n=287) No.	(%)
Onychomycosis	89	(92.71)	91	(97.85)	93	(94.90)	273	(95.12)
Superficial white onychomycosis	5	(5.21)	2	(2.15)	5	(5.10)	12	(4.18)
Onychomycosis with candida	2	(2.08)	0		0		2	(0.70)

$X^2 = 5.334$, df = 4, P=0.255 (not significant)

It was observed that out of 287 patients, 273 (95.12%) patients were found to be suffering from onychomycosis, 12 (4.18%) had superficial white onychomycosis while the rest 2 (0.70%) were carrying onychomycosis with candida (P<0.255).

Table – 22: Duration of treatment of the different groups of patients (n=287)

		P value[a]	
		Group B	Group C
	Duration of treatment (weeks) (Mean±SD)	3.00±0.00	12.16±4.03
Group A	14.52±4.90	0.000***	0.000***
Group B	3.00±0.00		0.000***

[a]Unpaired Student's 't' test, ***Significant at P<0.001

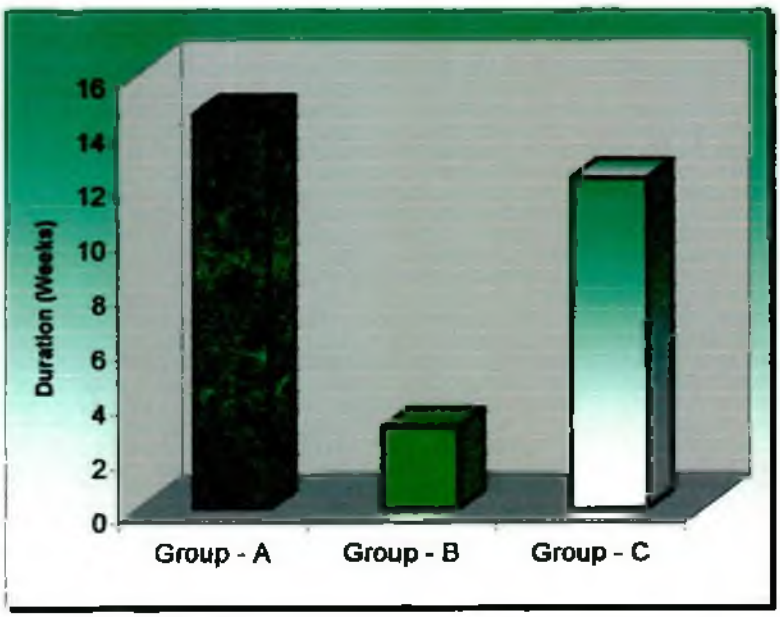


Fig.20: Duration of treatment in different groups of patients

Average duration of treatment required by individual patient in each Group (A, B and C) was 14.52 ± 4.9, 03.00 ± 0.00 and 12.16 ± 4.03 weeks respectively. The duration is significant between A&B, A&C and B&C as well (p<0.001).

The cost effectiveness amongst the three modalities was carefully evaluated and the observed treatment-costs were presented in Table – 23.

Table – 23: Cost incurred in the treatment of patients in different groups (n=287)

		P value[a]	
		Group B	Group C
	Cost incurred (Taka) (Mean±SD)	90.00±0.00	462.80±158.66
Group A	606.50±204.08	0.000***	0.000***
Group B	90.00±0.00		0.000***

[a]Unpaired Student's 't' test., ***Significant at P<0.001

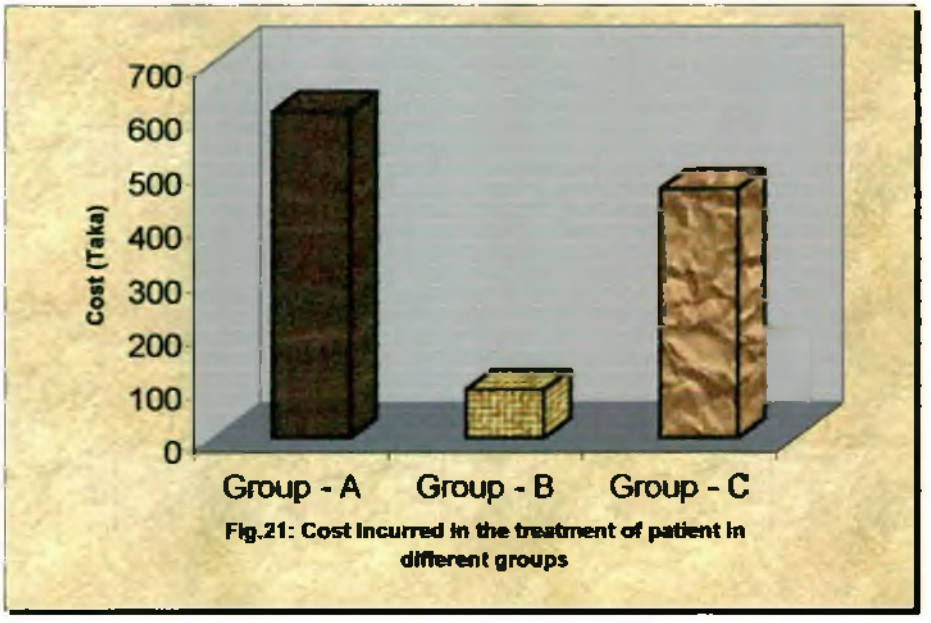


Fig.21: Cost incurred in the treatment of patient in different groups

The cost of treatment was found lowest in group –B (beta radiation), highest in group A (Griseofulvin) but in group C (beta radiation & Griseofulvin) it was in between A and B. Cost variation between A & B is highly significant (P=0.000). Similar variation has also been observed between B & C (P=0.000). Cost variation between A & C is very high and variation is highly significant (P=0.000).

Table – 24: Biochemical parameters in study population (n=287)

Group A	Group B	Group C	Normal Range
Random blood glucose (mmol/L) (Mean±SD)			**Up to 7.8mmol/L**
Baseline	6.19±1.32	6.32±1.48	6.65±1.82
1st follow-up	6.34±1.74	6.63±2.08	7.10±2.48
2nd follow-up	6.32±1.18	6.52±1.49	6.94±2.09
3rd follow-up	6.44±1.43	6.56±1.39	5.93±1.93
4th follow-up	6.48±1.39	6.48±1.23	6.98±1.98
Serum bilirubin (mg/dl) (Mean±SD)			**0.2 – 1.0 mg/dl**
Baseline	0.75±0.07	0.74±0.09	0.74±0.08
1st follow-up	0.81±0.17	0.78±0.14	0.85±0.63
2nd follow-up	0.71±0.19	0.71±0.18	0.70±0.17
3rd follow-up	0.67±0.17	0.68±0.17	0.66±0.18
4th follow-up	0.73±0.75	0.68±0.18	0.66±0.75
SGPT (U/L) (Mean±SD)			**Up to 12 U/L**
Baseline	9.70±1.62	8.40±1.61	10.00±1.58
1st follow-up	10.00±3.18	9.14±2.37	9.85±2.11
2nd follow-up	10.07±3.45	9.30±2.50	9.69±2.78
3rd follow-up	9.14±2.69	9.24±2.77	9.22±2.56
4th follow-up	8.90±3.40	9.07±2.56	9.14±2.93
Blood urea (mg/dl) (Mean±SD)			**15 – 50 mg/dl**
Baseline	30.67±3.29	30.74±2.25	32.88±2.33
1st follow-up	30.43±4.00	30.55±3.50	30.50±3.85
2nd follow-up	28.21±4.34	29.04±4.07	28.13±4.69
3rd follow-up	27.90±4.17	28.32±4.11	27.77±4.23
4th follow-up	27.14±3.70	28.02±3.92	27.40±4.03
Serum creatinine (mg/dl) (Mean±SD)			**0.6 – 1.5 mg/dl**
Baseline	1.02±0.04	1.03±0.05	2.03±10.00
1st follow-up	1.02±0.09	1.01±0.08	1.02±0.11
2nd follow-up	0.92±0.16	0.92±0.15	0.95±0.14
3rd follow-up	0.88±0.16	0.89±0.15	0.89±0.15
4th follow-up	0.86±0.16	0.86±0.16	0.86±0.16

Pre treatment baseline and post treatment follow up status for biochemical parameters were presented in Tables – 24 with mean ± 1SD values.

This biochemical tests comprised of random blood sugar, S. Bilirubin, SGPT, blood urea and S. Creatinine etc. From the tables we saw that the test values in each cases were within the normal limit in both pre and post treatment conditions.

Table – 25: Haematological parameters of study population (n=287)

	Group A	Group B	Group C	Normal Range
Haemoglobin % (Mean±SD)				**72% – 103%**
Baseline	71.12±12.33	71.05±9.40	70.57±10.73	
1st follow-up	70.73±10.83	69.70±9.24	69.80±9.98	
2nd follow-up	70.70±10.42	69.91±9.11	69.97±9.87	
3rd follow-up	70.68±10.22	69.81±8.56	70.13±9.62	
4th follow-up	71.26±10.26	70.40±9.00	70.57±9.48	
Total Count (cmm) (Mean±SD)				**4,000 – 10,500**
Baseline	7753.00±358.58	7710.00±482.73	8042.00±390.07	
1st follow-up	7748.00±1073.51	8304.00±6039.98	7986.50±796.86	
2nd follow-up	7570.50±800.48	7597.00±765.74	7665.00±621.72	
3rd follow-up	7547.00±664.61	7474.00±563.83	7570.00±547.45	
4th follow-up	7512.00±568.41	8116.00±6272.77	8089.50±5268.89	
Platelet count (cmm) (Mean±SD)				**1,50,000 – 4,50,000**
Baseline	235550.00±10072.84	234800.00±11654.11	234030.00±8803.64	
1st follow-up	230410.00±33635.63	227810.00±16084.18	227410.00±15738.45	
2nd follow-up	223800.00±20769.54	222250.00±18359.14	223200.00±17859.16	
3rd follow-up	225150.00±18097.03	227450.00±17858.80	227780.00±18093.80	
4th follow-up	230250.00±20155.64	230750.00±20540.36	228550.00±19257.62	
Polymorph (%) (Mean±SD)				**40% – 70%**
Baseline	60.51±5.16	64.66±4.55	58.92±4.31	
1st follow-up	62.34±5.38	64.23±4.44	61.89±4.90	
2nd follow-up	62.48±4.31	63.29±4.09	62.13±3.63	
3rd follow-up	62.31±3.63	63.20±3.15	62.23±3.31	
4th follow-up	62.11±3.58	62.91±3.30	62.30±3.34	
Lymphocytes (%) (Mean±SD)				**20% – 45%**
Baseline	33.69±3.78	29.85±4.31	35.28±4.51	
1st follow-up	32.78±5.05	30.87±4.30	33.57±4.74	
2nd follow-up	32.63±4.14	32.17±4.08	32.97±3.49	
3rd follow-up	32.81±3.72	31.94±3.44	32.76±3.18	
4th follow-up	32.90±3.38	32.42±3.54	32.95±3.60	

Cont.

	Group A	Group B	Group C
Monocytes (%) (Mean±SD)			**2% – 8%**
Baseline	2.70±1.06	2.68±1.12	2.18±0.46
1st follow-up	1.15±0.82	1.22±1.12	1.09±0.82
2nd follow-up	1.51±0.96	1.41±0.87	1.49±0.82
3rd follow-up	1.47±0.88	1.50±0.82	1.51±0.92
4th follow-up	1.63±0.81	1.65±0.85	1.68±0.89
Eosinophils (%) (Mean±SD)			**1% - 6%**
Baseline	2.77±1.81	2.83±1.25	3.63±2.29
1st follow-up	3.48±1.96	3.62±1.76	3.39±1.60
2nd follow-up	3.26±1.51	3.33±1.29	3.39±1.52
3rd follow-up	3.57±1.39	3.55±1.59	3.39±1.38
4th follow-up	3.33±1.55	3.12±1.42	3.29±1.47

These haematological tests included the followings: Haemoglobin estimation, WBC total counts, differential counts and platelets counts etc. From the tables it was evident that the entire test values in both pre and post treatment conditions were within normal limit.

The post therapy assessment, which consists of Clinical assessments, findings of microscopic examination in KOH preparation and Culture findings were presented in tables: 26, 27 and 28.

Table – 26: Microscopic findings at follow-up (n=287)

Findings	Group A (n=96) No.	(%)	Group B (n=93) No.	(%)	Group C (n=98) No.	(%)
1st follow-up						
Positive	93	(96.87)	87	(93.55)	87	(88.77)
Negative	3	(3.13)	6	(6.45)	11	(11.23)
Chi-square test: $X^2 = 5.984$, df = 2, P=0.050* (P<0.05)						
2nd follow-up						
Positive	88	(91.66)	83	(89.25)	83	(84.69)
Negative	8	(8.34)	10	(10.75)	15	(15.30)
Chi-square test: $X^2 = 1.600$, df = 2, P=0.449^{NS}						
3rd follow-up						
Positive	46	(47.92)	45	(48.38)	39	(39.80)
Negative	50	(52.08)	48	(51.62)	59	(60.20)
Chi-square test: $X^2 = 1.792$, df = 6, P=0.408^{NS}						
4th follow-up						
Positive	46	(47.92)	49	(52.69)	33	(33.67)
Negative	50	(52.08)	44	(47.31)	65	(66.33)
Chi-square test: $X^2 = 5.925$, df = 2, P=0.052^{NS}						

In Table – 26 microscopic findings were depicted. In post therapy first follow up 93(96.87) 87(93.55) and 87(88.77) were positive in Group – A, B & C respectively while 3(3.13%), 6(6.45%) and 11(11.23%) were negative (P<0.05).

In case of second follow up 88(91.66%), 83(89.25%) and 83(84.69%) were microscopically positive and 8(8.34%),10(10.75%) and 15(15.30%) were negative in Group – A, B and C (P<0.449) respectively. During the 3rd follow up 46(47.92%), 45(48.38%) and 39(39.80%) were microscopically positive and 50(52.08%), 48(51.62%) and 59(60.20%) were negative (P<0.408).

In 4th follow up – 46(47.92%), 49(52.69%) and 33(33.67%) were positive and 50(52.08%), 44(47.31%) and 65(66.33%) cases were negative (P<0.052).

Table – 27: Culture findings at follow-up (n=287)

Findings	Group A (n=96) No.	(%)	Group B (n=93) No.	(%)	Group C (n=98) No.	(%)
1st follow-up						
Positive	52	(54.16)	43	(46.24)	48	(48.98)
Negative	44	(45.84)	50	(53.76)	50	(51.02)
Chi-square test: $X^2 = 1.307$, df = 2, P=0.520^{NS}						
2nd follow-up						
Positive	38	(39.58)	37	(39.78)	27	(27.55)
Negative	58	(60.42)	56	(60.22)	71	(72.45)
Chi-square test: $X^2 = 8.000$, df = 2, P=0.018* (P<0.05)						
3rd follow-up						
Positive	19	(19.79)	17	(18.27)	13	(13.26)
Negative	77	(80.21)	76	(81.72)	85	(86.74)
Chi-square test: $X^2 = 4.722$, df = 6, P=0.094^{NS}						
4th follow-up						
Positive	10	(10.42)	9	(9.68)	4	(4.08)
Negative	86	(89.58)	84	(90.32)	94	(95.92)
Chi-square test: $X^2 = 0.499$, df = 2, P=0.779^{NS}						

Culture findings at follow up were described in Table – 27.

During the 1st follow up in Group – A, B & C 52 (54.16%), 43 (46.24%) and 48 (48.98%) cases were positive while 44(45.84%), 50 (53.76%) and 50 (51.02%) were negative respectively. (P=0.520)

In case of 2nd follow up 38 (39.58%), 37 (39.78%) and 27 (27.55%) were culture positive and 58 (60.42%), 56 (60.22%) and 71 (72.45%) were negative in 3 groups. (P=0.018)

In 3rd follow up culture finding was positive in 19 (19.79%), 17 (18.27%) and 13 (13.26%) cases and 77 (80.21%), 76 (81.72%) and 85 (86.74%) were negative in Group – A, B & C respectively (P=0.094).

In 4th follow up in Group – A, B and C positive findings are found in 10 (10.42%), 9 (9.68%) and 4 (4.08%) cases where negative culture findings were found in 86 (89.58%), 84 (90.32%) and 94 (95.92%) cases. (P=0.779)

Table – 28: Outcome of treatment after mycological evaluation.

	Group A (n=96)		Group B (n=93)		Group C (n=98)	
Outcome	No.	(%)	No.	(%)	No.	(%)
1st follow-up						
Cured	3	(3.13)	0		0	
Not cured	93	(96.87)	93	(100.0)	98	(100.0)
Chi-square test: $X^2 = 6.061$, df = 2, P=0.048* (P<0.05)						
2nd follow-up						
Cured	7	(7.29)	0		2	(2.04)
Not cured	89	(92.71)	93	(100.0)	96	(97.96)
Chi-square test: $X^2 = 11.045$, df = 2, P=0.004** (P<0.01)						
3rd follow-up						
Cured	33	(34.37)	33	(35.48)	34	(34.70)
Not cured	63	(65.63)	60	(64.52)	64	(65.30)
Chi-square test: $X^2 = 0.088$, df = 6, P=0.957NS						
4th follow-up						
Cured	41	(42.70)	36	(38.70)	65	(66.33)
Not cured	34	(35.42)	43	(46.24)	29	(29.59)
Recurred	21	(21.88)	14	(15.06)	4	(4.08)
Chi-square test: $X^2 = 20.738$, df = 4, P=0.000*** (P<0.001)						

In Table – 28 Outcome of treatment at follow up was described.

During the 1st follow up in Group – A, B & C 3(3.13%), 0(0%) and 0(0%) were cured, 93 (96.87%), 93(100%) and 98(100%) were not cured (P<0.05)

In case of 2nd follow up 7(7.29%), 0(0%) and 2(2.04%) were cured in Group A, B & C and 89(92.71%), 93(100%) and 96(97.96%) were not cured (P<0.01) while in 3rd follow up percentages of cured patients were 33(34.37%), 33(35.48%) and 34(34.70%) and not cured were 63(65.63%), 60(64.52%) and 64(65.30%) (P=0.957).

Finally in 4th follow up cured case were 41 (42.70%), 36 (38.70%) and 65 (66.33%) respectively, while not cured cases were 34 (35.42%), 43 (46.24%) and 29 (29.59%) and percentage of recurrence was 21 (21.88%), 14 (15.06%) and 4 (4.08%) in Group – A, B & C respectively ($p<0.001$).

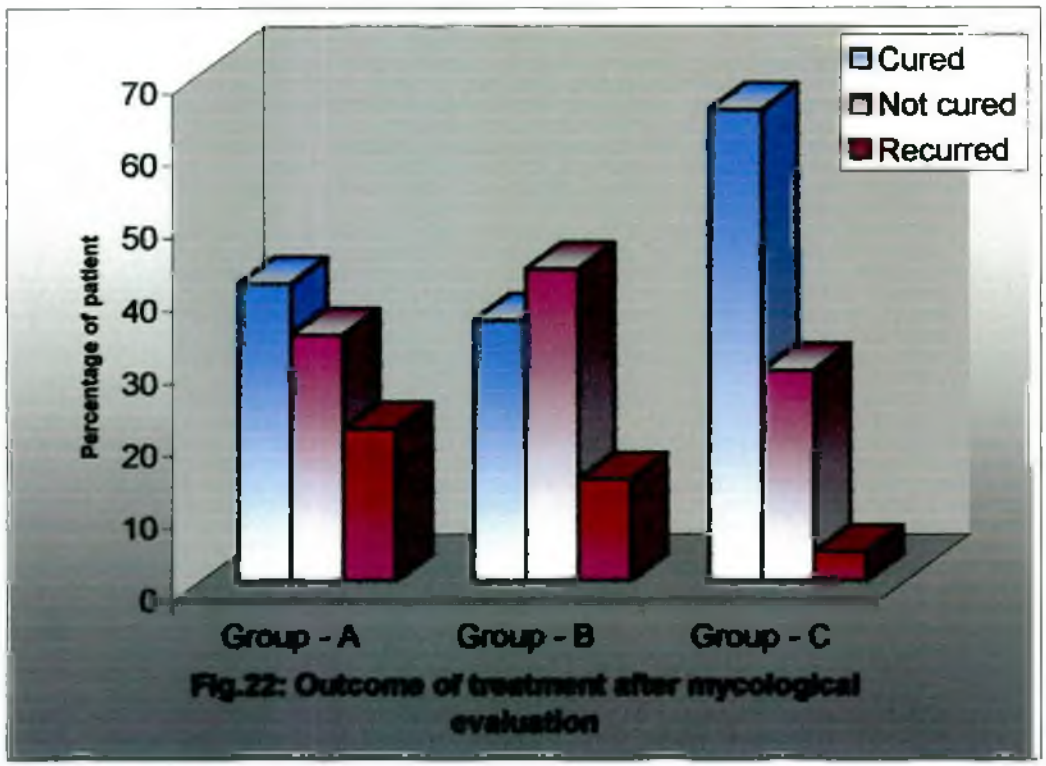
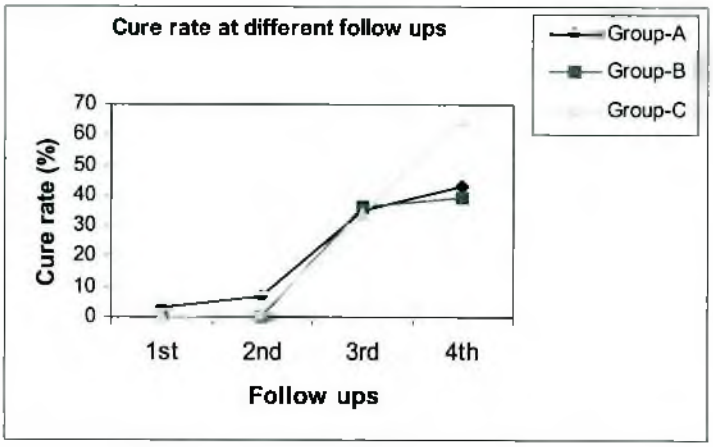
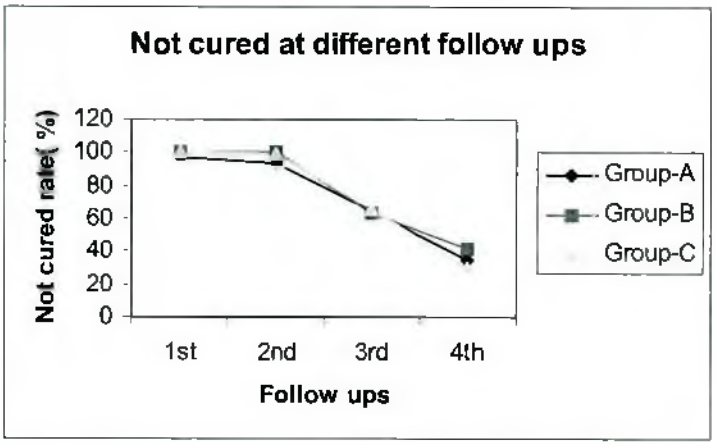


Fig.22: Outcome of treatment after mycological evaluation





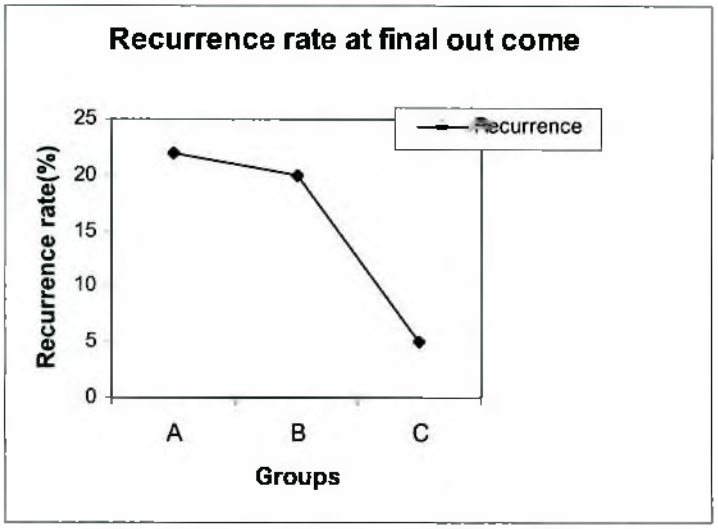


Fig. 23: Outcomes of the therapy at different follow-ups.

In cases of cured group highest number of patients belonged to Group - C (Combined therapy), less than that was occupied by group – A (Griseofulvin) and in the lowest position was Group – B (beta radiation alone).

In case of non-cured group, Group – B showed the highest position than Group – A and in lowest position is Group – C.

In case of recurrence, highest rate of recurrence was in Group – A, followed by Group – B and lowest rate of recurrence was observed in Group – C.

Table – 29: Clinical Response of patients in different treatment groups (n=287)

Improvements	Group A (n=96) No.	(%)	Group B (n=93) No.	(%)	Group C (n=98) No.	(%)
1st follow-up						
None	83	(86.46)	86	(92.47)	87	(88.77)
Mild	13	(13.54)	7	(7.53)	11	(11.23)
Chi-square test: $X^2 = 2.015$, df = 2, P=0.365^{NS}						
2nd follow-up						
None	72	(75.00)	75	(80.64)	75	(76.53)
Mild	21	(21.87)	18	(19.36)	23	(23.47)
Marked	3	(3.13)	0		0	
Chi-square test: $X^2 = 6.647$, df = 4, P=0.156^{NS}						
3rd follow-up						
None	49	(51.04)	42	(45.16)	33	(33.68)
Mild	30	(31.25)	46	(49.46)	56	(57.14)
Moderate	10	(10.42)	3	(3.23)	6	(6.12)
Marked	7	(7.29)	2	(2.15)	3	(3.06)
Chi-square test: $X^2 = 17.978$, df = 6, P=0.006** (P<0.01)						
4th follow-up						
None	44	(45.84)	27	(29.03)	21	(21.43)
Mild	21	(21.87)	45	(48.39)	50	(51.02)
Moderate	16	(16.67)	17	(18.28)	17	(17.35)
Marked	15	(15.62)	4	(4.30)	10	(10.20)
Chi-square test: $X^2 = 32.326$, df = 6, P=0.000*** (P<0.001)						

Table – 29: In consideration of patients follow-up clinical assessment showed mild improvement in 13 (13.54%), 7 (7.53%) and 11 (11.23%) in group A, B and C respectively, while no improvement was observed in 83 (86.46%), 86 (92.47%) and 87 (88.77%) cases during the first follow-up (p=0.365). In case of second follow up marked improvement was seen in 3 (3.13%), 0 (0%) and 0 (0%) cases in-group A only. Mild improvement was seen in 21 (21.87%), 18 (19.36%) and 23 (23.47%) cases in group A, B and C respectively, while no improvement was observed in 72 (75.0%), 75 (80.64%) and 75 (76.53%) patients (P=0.156).

In third follow-up, marked improvement in 7 (7.29%), 2 (2.15%) and 3 (3.06%) cases, moderately improved in 10 (10.42%), 3 (3.23%) and 6 (6.12%) cases and mild improvement was evidenced in 30 (31.25%), 46 (49.46%) and 56 (57.14%) cases, while no improvement was seen in 49 (51.04%), 42 (45.16%) and 33 (33.68%) cases in group A, group B and group C respectively (P< 0.01).

In fourth follow-up – marked improvement was found in 15 (15.62%), 4 (4.30%) and 10(10.20%) cases, moderately improved cases were 16 (16.67%), 17 (18.28%) and 17 (17.35%), mild improvement was observed in 21 (21.87%), 45 48.39%) and 50 (51.02%)cases and no improvement was seen in 44 45.84%), 27 29.03%) and 21(21.43%) cases in group A, B and C respectively (P<0.001).

This response was independent of the site of the lesion or the type of pathogenic organism.

Table - 30: Overall results obtained after different types of treatment applied in the cases of Onychomycosis based on clinical and mycological evaluation (n=287)

Treatment applied	Clinical Response	No. of Patients	Mycological Result		Overall Assessment	No. of Patients
			Positive	Negative		
Group – A (Griseofulvin)	Responsive (including mild, moderate & marked improvement)	52	10	42	Cured*	42
	Un-responsive	44	44	0	Not cured**	54
Group – B (beta radiation)	Responsive (including mild, moderate & marked improvement)	66	30	36	Cured*	36
	Un-responsive	27	27	0	Not Cured**	57
Group – C (Combination of Griseofulvin and beta radiation)	Responsive (including mild, moderate & marked improvement)	77	12	65	Cured*	65
	Un-responsive	21	21	0	Not Cured**	33

Chi-square test: $X^2 = 20.738$, df = 4, P=0.000 (P<0.001)

*Total cured value excludes mycological positive values

**Total not cured value includes mycological positive values including recurrence

In table – 33 the overall results, based on clinical and mycological response were described. Responsive group included mild, moderate and marked clinical improvement. Out of 52 clinically responsive cases, 42 were mycologically cured. In overall assessment 42(43.75%) were considered to be clinically cured in Group – A. In the same way 36 (38.70%) and 65 (66.33%) were clinically cured in Group – B & C respectively. 54, 57 and 33 cases were found to be not cured in Group – A, B and C respectively. The cure rate between A, B & C is statistically highly significant (P=0.000) Not cured group includes clinically unresponsive and

recurrent cases. In clinically cured cases A, B & C groups the difference was statistically significant (P=0.000), while clinical cure and mycological cure in three different groups showed no significant difference (P=0.255). The response was independent of the site of lesions or type of pathogenic organism.

Table – 31: Adverse reactions of Griseofulvin, beta radiation & their combination treatments on Onychomycosis (n=287)

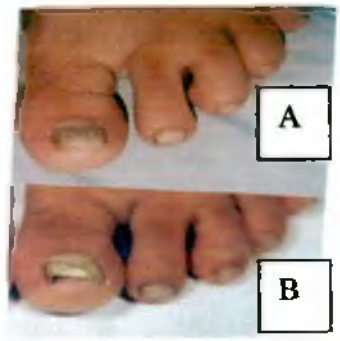
Effects	Group – A (Griseofulvin)	Group – B (Radiation)	Group – C (Combination of Griseofulvin & Beta Radiation)
Reaction	Number	Number	Number
Headache	04	-	02
Pruritus	01	-	-
Nausea	02	-	-
Diarrhoea	02	-	-
Blackening of surrounding soft tissue of nail	-	93*	98*
Total	08	93	98

*Transient effect

All 287 patients who entered the study and took therapy were included in the safety evaluation. Eight of ninety-six, griseofulvin treated patients (8.34%) reported systemic adverse reactions. In rest of the groups i-e. B & C, all 191 patients showed soft tissue blackening in surrounding of the nail (Table – 31). This was transient and local reaction and mostly faded off at the time of first follow-up. In addition to this, only 2 patients in-group – C showed headache in mild degree. Otherwise no systemic involvement was observed in those patients.

EVIDENCE OF SUCCESS & FAILURE

GROUP – A

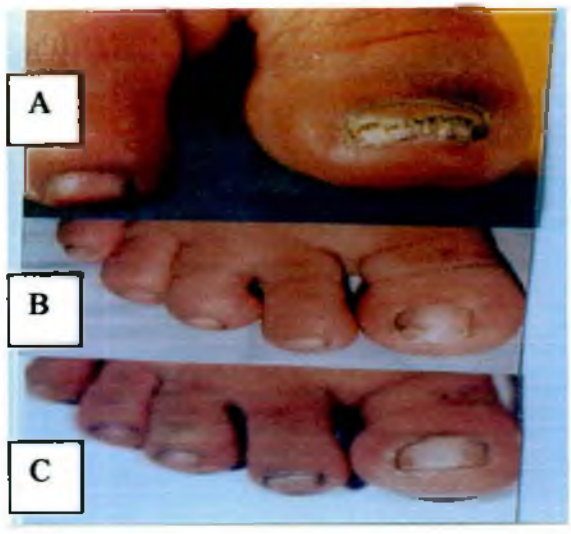


SEQUENTIAL IMAGES: A - POST THERAPY 2ND FOLLOW UP (IMPROVED)
B - POST THERAPY FINAL FOLOW UP (RECURRENCE)

GROUP – B

PRE & POST TREATMENT OF TOE NAIL ONYCHOMYCOSIS

GROUP - C



SEQUENTIAL IMAGES: A – BEFORE TREATMENT; B – POST THERAPY 2ND FOLLOW UP
AND C – POST THERAPY FINAL FOLOW UP

Fig.24: Pictorial evidence of success and failure in treatment Group – A, B & C.

CHAPTER 5: DISCUSSION

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The incidence of nail fungal infections are increasing day by day, because of several factors:

i. Improved methods for detection.

ii. Growing population of immunocompromised patients who have a greater susceptibility to such infections.

iii. Increased use of systemic antibiotics and immunosuppressive drugs.

In addition, it appears to have a heightened public awareness, specially in younger patients with regard to fungal infections of the nails. Onychomycosis is not only a cosmetic problem; rather severe nail dystrophy may produce functional impairment. Hence, the demand for treatment is not unexpected. (Cohen PR, Scher RK, 1994)

Onychomycosis, particularly of the toenails, is a therapeutic challenge. Experience with antifungal therapy alone has been uniformly disappointing. (Assaf RR, Elewski BE & Cleveland MD 1996) There is debate regarding the optimum treatment of onychomycosis with variations from countries to institutions.

Toenail onychomycosis has been a vexing problem. Topical preparations are generally ineffective and Griseofulvin has had a dismal record in treating onychomycosis. (Korting HC, Schaler – Koring M 1992) Other study concludes that topical agents have limited efficacy in the treatment of onychomycosis. Oral Griseofulvin or Ketoconazole requires long-term treatment, cure rates are

disappointing and high relapse rates are equally discouraging. (Richard B. Odom et al 1997; Robert DT 1994) Surgical removal of nails is invasive while chemical removal has its inherent limitations. (Daniel CR, 1996)

Under the above circumstances, it was felt necessary to wander a modality that would overcome the aforesaid limitations and consequently the present study was undertaken with a view to have the procedure in existence.

Application of nuclear medicine therapy has been employed for more than 40 years in CNM&U, DMCH. The prime type of nuclear medicine radiation used in this field is beta ray. Present study confirms the efficacy of beta radiation alone and in combination with antifungal drug in the treatment of dermatophytic onychomycosis.

Firstly our objective was to establish beta radiation as a curative and innovative modality of treatment in patients with onychomycosis and the optimisation of its doses.

Secondly we wish to verify the efficacy of beta radiation and to establish it as a replacement or supplement to antifungal drug.

Our third hypothesis was the most important one for this study, which was to reduce the duration of drug exposure and to make a cost effective treatment protocol.

Our present study was a clinical trial., so prevalence of the disease was not looked for. Despite, a good number of onychomycosis cases were observed when

mycological confirmation was done for detection of fungus. Moreover, the study was performed irrespective of etiological species. It was found in a study in Bangladesh that isolated dermatophytes were T. rubrum 84.9% and T.mantagrophytes 11.3%. (Islam S, 1993) In a similar study in Bangladesh T.rubrum was found 88.2%, T.mantagrophytes was the second common type of species (Alam, 1971). Same type of findings was observed by others (Wong KO and Chan YF, 1968; Blank et al., 1974 and Mc Lean et al., 1987) where T. rubrum was found to be 100% responsible for Tinea unguium (onychomycosis). (Alam, 1971) Others also got comparable reflections in USA, England, Hong Kong and so on. (Neils R, 1966; Zaias N et al 1969; Ealshe and English 1966; Wong KO and Chan YF 1968). All these findings are in good agreement with high prevalence of onychomycosis in Bangladesh.

The results of the current study show that highest frequency of onychomycosis occurred in 31 – 40 years age group, which is not consistent with the findings in literature for developed countries. In a recent study in North Ohio in United States, the frequency of onychomycosis found that may approach to approximately 50% in individual over 70 years of age. (Elewski BE and Charif MA 1997) In another study in USA it is reported that fungal infections of nails are more common among older adults because after age 40, nails grow more slowly and begin to be thickened, making them more susceptible to infection. (First Aid and self-cured guide, Mayo Foundation, Aug 2000). In a study for fungus in Bangladesh, which included all varieties of superficial fungi showed majority of

the patients (60.4%) were in 21– 40 years age group which is in good agreement with the present study population.

However, the mean age among these groups of treatment in study population did not show significant variation, which indicates well randomisation of patients.

Distribution of height and weight of our study population showed similar observation as in other study of Bangladesh. (Islam S, 1993) In the present study height and weigh were considered to determine the obesity of patients, which is an important predisposing factor for the occurrence of onychomycosis. (Gupta A K 1997, Daniel SR, Lowsom LA, 1987) However, in the present study no unusual obese patient was found.

In the present study economic status of patients were low and middle-income group. The possible explanation may be that, patients from low and middle-income group of our country usually attend out patient department to seek medical advice and treatment from where our study subjects were collected. It also reflects the total economic status of the country in miniature form. However, many studies found similarly higher incidence of onychomycosis among low socio-economic groups, where malnutrition, poor personal hygiene, overcrowding and ignorance were encountered as important contributing factors for occurrences and persistence of the disease. (Islam S, 1993; English & Lewis, 1974; Sanyal M, 1969; Khan and Anwar, 1969; Blank et al., 1974; Al-Dorry, 1975; Chowdhury & Huq, 1979; Mooney, 1986; Babel et al., 1990)

Past history, history of antifungal drug intake and duration of signs and symptoms of present illness seem not to imply any reasonable impact in the present treatment strategy and not statistically significant, although nail symptoms with prolonged duration showed delayed mycological and clinical improvement in the present study as evidenced in the literature. (Hanke E, Tausch I, Brautigam M, et al., 1995)

To optimise the dose of beta radiation, invitro method was used. Although a total dose of 2500 rads of beta radiation was considered as effective dose for nail fungus destruction, it was given in fractionated dose of 500 rads per exposure. The idea of fractionation was- (i) it helps repair mechanism of normal tissue, (ii) it increases tolerance to radiation by surrounding normal tissue, (iii) it successively helps inhibiting repairable capacity of the diseased tissue (i-e fungi). (Johns HE, 1978)

In our study population there was preponderance of female patients over male (56.45% versus 43.55%), although this result was not statistically significant. The reason might be due to higher attendance of female patients to out patient department of Skin & VD in hospital as they assumed to be highly health conscious and more cosmetic cared. Further to this our research is a city-based study, females are less shy to attend hospital in comparison to rural women. Our results were in contrary to the findings of Rao A, 1969; Sanyal M, 1969; Roberts SOB et al 1984; Mc Lean et al 1987 etc. and also Alam, 1971 in Bangladesh where male preponderance were evidenced. However, it was not statistically significant.

In the present study, in demographic record it was observed that frequency of the disease is comparatively higher in literate people (p=0.009), influence of occupation was not statistically significant although service holders were found to have maximum occurrence (p=0.197) and building resident showed higher occurrence of the disease than slum people (p=0.987). Although the variations in the above stated findings were statistically not significant they are all in good agreement with the literature as said that onychomycosis is characteristically an infection of urbanised areas of the world. (Philpot CM, 1977)

In consideration of family history, our study showed higher positive incidence of family history, which is concomitant with the disease although statistically it is not significant. This is well matched with the findings in literature. The importance of cross-infection in the family has been investigated by many workers with somewhat convincing results that family history is a vulnerable factor for onychomycosis. (English MD, 1957; Gupta AK et al., in press; Zains N et al 1996, Philpot CM, 1977).

In our study population, the highest site involved was single fingernail, which was followed by multiple fingernails and the finding was statistically significant. In case of multiple nails involvement, all the nails do not response to therapy uniformly. In that case, to determine the "cure" a target nail is selected and usually the clinically bad nail is considered as the target one. Cause for involvement of more single nail instead of multiple nails is not very clear and remain unanswered. It may depend on type of species responsible for the infection, regarding which this study is not concerned. Still it may be mentioned

that C.albicans are responsible for multiple nail infection and mostly fingernail (Harry LA 1998), while T.rubrum causes infections in both finger and toenails similarly.

It is worthful to mentioning the fact of fingernail preponderance, which has to be brought into consideration for discussion. The possible explanation is that in the present study relatively high incidence of the disease occurred among the female as washing of clothes and utensils is everyday job of women in our society, which causes wet hands and excessive use of detergents as no protection (like gloves etc.) are worn. It pre disposes fungal infection of fingernails in female patients. (First Aid and Self Cured Guide, Mayo Foundation, Aug. 2000)

Females mostly wear open slippers rather than closed shoes that assume to reduce the prevalence of fungi in toe nail as suggested by literature (English MP et al., 1959) and same observation is also evidenced amongst Bangladeshi population and thus proportionately fingernail infection increases.

Our findings were in contrast to the findings of literature as toenail infection is several times, even four times more common than fingernail infection. (Elewski BE, Charif MA 1997, Robert DT 1994).

In case of chief complain and local examination, painful nail was the commonest finding (92.68%) in our study population, which was highly significant. This

finding supports that; this was the vital cause of patients with onychomycosis reporting to Dermatologist's office, otherwise patients in our society are very reluctant to see the doctors. In our study 58.54% cases needed adjunctive therapy for paronychea, which may be the cause of painful nail. As the present study was not species specific, the causative species was not looked for. Signs and symptoms suggest that, paronychea may be due to candida albicans in addition to the major species, dermatophytes. Candia albicans are common causes of paronychea (Harry LA, 1998). Literature suggests that fingernails are more commonly infected by C.albicans than toenails, which is consistent with our present findings and thus justifying the high incidence of fingernail fungus. (Harry LA, 1998, Galimberti RL et al., 1991, Clayton YM, Hay RJ, 2000, Scher RK, 1994 and Finlay AY, 1994)

As a pre-treatment investigative work up procedure, direct microscopic detection of nail fungus showed significant positive findings in our study (95.12% $P<0.05$). It is consistent with the expected findings of literature (Islam S, 1993). Detection efficiency of direct microscopy seems to be higher in our research work. Culture analysis for fungal detection was 52.26% positive and 47.74% negative ($P<0.01$), which is in good agreement with other study in Bangladesh (Islam S, 1993).

Initially there was hesitation regarding culture negative cases for inclusion in the present study population. As literature suggested that microscope positive but culture negative does not exclude possibility of fungus (Jolly HW et al., 1983), the clinically suspected cases with microscope and / or culture positive cases were

included for the study. In addition, as this is a time limited study and for better accumulation of patients the above-mentioned criteria was set.

The duration of treatment is very important to be discussed. In case of conventional antifungal drug alone in Group – A the treatment period was 14.52 weeks ± 4.90. In case of beta radiation alone in Group – B it was 3 weeks ± 0.00 and in Group–C that is combination of antifungal and beta radiation the duration was 12.16 weeks ± 4.03. In comparison to conventional medical therapy i.e Group – A, Group –B and Group – C showed less duration of treatment which is statiscally significant ($P< 0.001$).

Another vital point of this treatment regimen is cost perspective. Conventional anti-fungal therapy showed highest cost involvement, which was followed by combination of antifungal and beta radiation (Group – C). Lowest cost involvement was observed with beta radiation alone i-e. Group – B.

Duration of treatment and cost perspective, these two factors are vital to be taken into account. Reduction of the duration of drug exposure and cost was the very important hypothesis of the present study in context of the country like Bangladesh. This hypothesis very consistently fulfils the desire of literature where high cost and long duration of therapy were considered to be the major limitation for treatment of onychomycosis. (Roberts DT, 1994; Pierard G et al 1993; Scher RK, 1990)

Biochemical and haematological test values before and after treatment were compared to each other in all three groups of A, B & C. The only reported

laboratory abnormalities were moderately elevated blood glucose level in 4 cases during the inclusion of the patient and slight elevation of liver function test in 3 cases after therapy. At the post therapy visits after discontinuation of treatment all the laboratory values had returned to normal. No other laboratory abnormalities were seen in any of the groups, which is in agreement with the findings of literature (Jolly HW et al., 1983).

In post therapy state of onychomycosis, mycological cure and overall cure rate were separately evaluated, where mycological cure stands for negative values both in direct microscopy and culture analysis while associated clinically normal nails were considered as clinical cure. In our present study, a cure means mycological cure following the definition of cure in literature where it was shown that from a scientific stand point, it is imperative to note that the definition of "cure" varies between studies, for example some studies define cure as mycological negative (determined from KOH test and culture analysis) and clinically normal nails; this is considered the strict definition of clinical cure (Tosti A, 1996; Branfigam OJ, 1995; DeDoncker P, 1996). We are in favour of mycological cure, as the patients in our country usually appear to the doctors in late with somewhat worse nail condition where we usually see that the nails are thickened, disfigured and more over, long time follow up is quite impossible to see clinical cure. It is in good agreement with literatures "support" a full "cure" for onychomycosis is not always possible (Tosti A, 1996; Branfigam OJ 1995; DeDoncker P 1996). In the present study the observed mycological cure in Group

– C, was the highest in percentage, while Group – A & B showed similar results, which was significantly less than Group – C. ($P<0.001$)

However, achievement towards clinical cure was observed to some extent, which was seemed to be quite encouraging in our present study.

In the present study, group – C (Combination therapy) can explicitly be accepted as a choice of treatment for onychomycosis due to highest cure rate and lowest recurrence rate, as combination therapy is popular in nuclear medicine practice now a days, less treatment duration in comparison to the prevailing treatment procedures, duration may even be reduced further, if radiation and antifungal drugs are provided simultaneously, cost is obviously less than the conventional antimycotic drugs and overall patients demand for direct evidence of cure which was better evidenced in Group – C.

Most researchers acknowledge that analysing the adverse reactions associated with the prescribed drug is difficult because the drug is used primarily in patients with severe underlying disease. (Scher RK, 1999) In a study of 4,000 patients receiving griseofulvin, the incidence of adverse reaction was 16% (Elewski BE, 1996). Adverse effects included gastrointestinal disturbance, headache, rash and mild abnormal liver function.

In the literature regarding drug safety of griseofulvin it has been found to be relatively free of serious side affects over many years of use. However, it produces upper gastrointestinal upset and headache in about 20% of patients (Davies RR, 1980).

In our study, out of 96 patients, who had griseofulvin, 8.34% cases had adverse reactions including headache, pruritis and gastrointestinal disorders. The findings were in good agreement with literature. One hundred and ninety one post-irradiated cases of our study population, although all (100%) cases had blackening of irradiated nails surroundings, this was a local and transient reaction and mostly cleared up during the first follow up. No systemic effect was observed in radiation group of patient.

Finally to justify beta radiation as an innovative and acceptable modality over the newer antifungal agents, which are considered to be the highest efficient antimycotic drugs. It can be mentioned that cost effectiveness is one of the prime objectives of the present study.

Newer drugs are beyond the reach of more than 90% population in Bangladesh in context of cost perspective. On the other hand beta radiation applicator being a portable device, it can easily be reached to mass population at negligible cost.

In addition to this, several side effects causing systemic involvement of oral drugs even in the case of newer one are already being experienced, while beta radiation, as a local agent is free from all of those side effects.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATION

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6.1 CONCLUSIONS

From the present study it is worthy to mentioned that Group – C (Combination of antifungal and beta radiation) can be considered as an acceptable therapeutic procedure for onychomycosis in a local population. Group – B (beta radiation) can also be accepted in special occasions to replace Group – A (Antifungal).

The proposed new treatment modality exhibited a low risk to benefit ratio. It is a well-tolerated and efficacious method to treat onychomycosis. It may be possible to combine with other newer oral therapies with radiation, there by further increasing efficacy rates and the cost effectiveness while decreasing adverse effects and duration of oral therapy. From the observations, the following recommendations can be drawn which are expected to be helpful to the treatment policy adapters for proper and efficient management of onychomycosis either alone or in combination.

6.2 RECOMMENDATIONS

- The advent of this innovative therapy of beta radiation for management of onychomycosis in the present study has been significant and could be a welcome addition to the armamentarium of therapies at the disposal of the physician.

- From observations of the present study it can reasonably be recommended that Group–C (Combination of antifungal and beta radiation) can be adopted as a routine treatment modality for efficient management of onychomycosis.

- Since the beta applicator is portable, patients in remote areas could be brought under this cost effective treatment protocol.

- In certain special cases, Group B alone can be employed for achieving sequels of the treatment by group –A.

- It is possible to combine the radiation therapy with newer oral antifungal drugs, topical and surgical treatments, which would further increase efficacy rates and the cost-effectiveness while decreasing adverse effects and duration of treatment.

- This innovative treatment procedure could be introduced in other Nuclear Medicine Centres of the country with a view to expand the procedure, so that the large number of patients could be beneficiary as end user.

CHAPTER 7: REFERENCE

REFERENCES

Ajello L. A taxonomic review of the dermatophytes and related species. Sabourandia,1968; 6:147 – 59.

Al Sogar SM, Moawad MK, Al-Humaidan YM. Fungal infection as a cause of skin disease in the eastern province of Saudi Arabia: Prevailing fungi and pattern of infection. Mycoses 1991; 34:333-7.

Alam SA. Dermatophytes in East Pakistan. M.Phil, Thesis. University of Dhaka; 1971 : 48–52.

Albom MJ. Avulsion of nail plate. J. Derm Surg Oncol 1977; 3:34–35.

Alcantara R, Garibay JM. Itraconazole therapy in Dermatomycosis and vaginal candidiasis: effects and adverse effect profile in a large multi-centre study. Adv Ther 1988, 5:326 – 34.

Al-doory Y. The Epidemiology of Human Mycotic Disease, 1st ed. USA: Charles C. Thomas Publisher 1975 : 290.

Andre J, Achten G. Onychomycosis. Int J Dermatol. 1987; 26: 481-490. MEDLINE.

Assaf RR, Elewski BE & Cleveland MD. Intermittent fluconazole dosing in patients with onychomycosis: Results of a pilot study. J Am Acad Dermatol 1996; 35:216-9.

Babel DE, Rogers AL., Beneke ES. Dermatophytosis of the scalp: Incidence, immune response and epidemiology. Mycopathologia 1990; 109:69-73.

Banasree C. Deka et al. Treatment of keloids with strontium-90 Beta rays. Indian Journal of Cancer, March 24 (1987) 15–21.

Banerjee U, Sethi M, Pasricha JS. Study of onychomycosis in India. Mycoses 1989; 33:411–5.

Baran R. Haneke E. Surgery of the nail, skin surgery (Epstein E, Epstein E Jr eds) 6th edn. Philadelphia : WB Saunders Co. 1987; 534 – 547.

Baran R. More on a avulsion of nail plates. J Derm Surg. Oncol 1981; 7: 854.

Barham, D and Trinder, P. Analyst 97 (1972).

Bartels, H. Bohmar, M.; Heirli, C., Clin.Chim. Acta 37, 193(1972).

Baucham V. Dr. Head, Department of Nuclear Medicine, Chulalongkorn Hospital., Bangkok, Thailand, personal communication.

Beneke ES, Rogers A Al. Medical Mycology Manual., 3rd ed USA: Burgerss Publishing Company, 1970 : 44, 45, 51, 55, 56.

Benham RW. Nutritional studies of the dermatophytes – effect on growth and morphology. Trans NY Acad Sci, 1953: 15:100-6.

Bernard C, Miarcello L M, David R, Maria P, Vincent F, Jean-Marie Lang, Edonard. Nail changes in patients infected with Immuno Deficiency Virus. Arch. Dermatol, 1998; accepted for publication.

Bert RS et al. X-ray therapy of skin cancer. Evaluation of a "Standardized" method for treating basal cell epitheliomas. Proceeding of the 6th National Cancer Conference. Philadelphia, JB Lippincott, 1968.

Bickers DR. Antifungal therapy: potential interactions with other classes of drugs. J Am Acad Dermatol 1994; 31:S87 – 90.

Blank F, Mann SJ, Reale RA. Distribution of Dermatophytosis according to age, ethnic group and sex. Sabouraudia 1974; 12:352-61.

Bonasree C. Deka., A C Deka, J S Avadhani, VK Sathiyanarayam. B.Sc, Dip RP RR Kalghatgi, R.B. Batil, S J Supe. Treatment of Keloids with Strontium-90 Beta Rays. Indian Journal of Cancer, March 24(1987), 15-21.

Brodell RT, Elewski BE. Clinical pearl: Systemic antifungal drugs and drug interactions. J Am Acad Dermatol 1995; 33:259-60.

Chaney, AL and Marbach, AL., Clin. Chem., 1962; 8:130.

Cheesbrough M. Medical Laboratory Manual for tropical countries, 1st ed., Vol.II Cambrigeshire, England: Tropical Health Technology, 1984 : 372, 373, 375, 376, 382.

Chhabra, AS: 90Sr- beta-ray (and Bremsstrahlung depth-dose measurements in Lucite. Radiology, 79:1001, 1962.

Chiritescu MM, Chiritescu ME, Scher RK. Newer systemic antifungal drugs for the treatment of onychomycosis. Clin Podiatr Med Surg 1996; 13 : 741 – 58.

Chowdhury M R, Haq M N. Mycotic disease in Bangladesh with special reference to types causative fungi. Bangladesh Medical Journal 1979; 8: 39–44.

Chowdhury M Z, Rahman M A K, Islam K M S, Muhammad F and Islam A K M S. Pattern of superficial fungi infection at Barisal. Bangladesh Journal of Dermatology Venereology and Leprosy 1994; 5-7.

Chowdhury S. J. Head, Nuclear Section, Al-Sabah Hospital., Kuwait Cancer Control Centre, 1996.

Clark RE. Tope WD: Nail Surgery. Cutaneous Surgery (Wheeland RG ed) 1st edition. Philadelphia: WB Sanaders Co. 1994; 375–402.

Clayton Y M. Clinical and mycological diagnostic aspects of Onychomycosis. Clin Exp Dermatol 1992; 17 (Suppl 1) 37 – 40.

Clayton YM, Hay RJ. Epidemiology of fungal skin and nail disease : roundtable discussion held at Dermatology 2000, Vienna, 17 May 1993. Br J Dermatol 1994; 130;9-11.

Clayton YM, Hay RJ. Epidemiology of fungal skin and nail disease: roundtable discussion held at Dermatology 2000, Vienna, 17 May, 1993. Br J Dermatol 1994; 130:9–11.

Clayton YM, Midgley G. Pocket Picture Guide to Medical Mycology. London: Gower Medical Publishers, 1985: 5 – 81.

Clayton YM, Midgley G. Pocket Picture. Anide to Medical Mycology. London : Bultger worths, 1967 : 25–6.

Cockrell CJ. Cataneous manifestation of HIV injection other than kaposi's surcome : J Ars Acad Dermatol 1990; 22 : 1260-1290.

Cohen PR, Scher RK. Topical and surgical treatment of onychomycosis. J Am Acad Dermatol 1994; 31: S74-7

Collins GH, Taylor CED. Microbiological Methods, 2nd ed, London: Butterworths, 1967 : 25 – 6.

Crislip MA, et al: Candidiasis. Infect Dis Clin North America, 1989; 3:103.

Daniel CM, Daniel MP, Daniel CR. Onychomycosis update. J Miss State Med Assoc 1995; 36 : 37 – 9.

Daniel CR, Jackson MD. Traditional management of onychomycosis. J Am. Acad Dermatol 1996; 35 : S21 – S25.

Daniel CR, Lawson LA. Tinea unguium. Cutis 1987; 40 : 326 – 7.

Daniel CR, Norton LA, Scher RK. The spectrum of nail disease in patients with human immunodeficiency virus infection. J Am Acad Dermatol 1992; 27: 93-7.

Davies RR, Everall JD, Hamilton E. Mycological and clinical evaluation of Griseofulvin for chronic onychomycosis. Br Med J 1967; 3:464-8.

Davies RR. Griseofulvin. In: Speller DCE, editor. Antifungal chemotherapy. New York: John Wiley, 1980; 149 – 82.

Diflucan® (fluconazole tablet) package insert. NY: Pfizer Inc.

Dollery C. Griseofulvin. In: Therapeutic drugs. Edinburgh: Churchill Livingstone, 1991: G66 – 9.

Domonkos AN : Treatment of eyelid carcinoma. Arch Dermatol 91: 364, 1965.

Domparastin D. Domparartin A. Deluol Am, Grosshan SE, Couland JP. Onychomycosis and AIDS : Clinical and laboratory findings in 62 patients. Int J Dermatol 1990; 29 : 337-339.

Dover JS, Hohnson RA. Cataneous manifestation of Human Immunodeficiency Virus injection syndrome. Arch Dermatol. 1992; 127 : 1383-1391.

Drake L A, Dinehart S M, Farmer E R, Goltz R W, Graham G F et al. Guidelines of care for superficial mycotic infections of the skin: Onychomycosis. J Am Acad Dermatol, 1996; 34: 116 -21.

Drake LA, Scher RK, Smith EB, et al. Effect of onychomycosis on quality of life. J Am Acad Dermatol, 1998; 38: 702 – 4.

Early P.J, Sodee D.B.: Principles and practice of Nuclear Medicine, 2nd ed. Mosby – year book, Inc, St. Louis, 1995 : 23-49.

Edsmyr. F. Larson L. G., Onyango J., Wangarnu's and Wood. M : Radiation therapy in the treatment of Keloids in East Africa. Acta Radiol 13 : 102-106. 1974.

Elewski BE : Diagnostic techniques for confirming onychomycosis. J Am Acad Dermatol 1996, 35 : 86.

Elewski BE and Roderick J Hay. Novel treatment strategies for superficial mycosis : introduction. J Am Acad Dermatol 1999; 40 : 21 – 22.

Elewski BE, Charif MA. Prevalence of Onychomycosis in patients attending a dermatology clinic in north-eastern Ohio for other conditions. Arch Dermatol. 1997; 133 : 1172 – 1173.

Elewski BE, Hay RJ. Update on the management of Onychomycosis: Highlights on the Third Annual International summit in Cutaneous Antifungal Therapy. Clin Infect Dis 1996; 23 : 305 – 13.

Elewski BE. Once – weekly fluconazole in the treatment of Onychomycosis. J Am Acad Dermatol 1998; 38 : S73 – 6.

Elewski BE: Onychomycosis. Fitzpatrick's Journal of Clinical Dermatology, 1994; 1:48 – 54.

English M.P.M.D., Gibson EHL, Duncan RP, Warin & RR Wethred. Studies in Epidemiology of Tinea pedis. I. Tinea pedis from school children; BMJ. 1959; i : 1442 – 1448.

English MD: Trichophyton rubrum in infection in families. BMJ 1957; 1:744-746.

English MP, Lewis L, Ringworm in the South-West England, 1960-1970. with special reference to onychomycosis. Br J Dermatol 1974; 90:67-75.

Epstein E: How often does oral treatment of toenail onychomycosis produce a disease-free nail? Arch Dermatol. 1998; 134:1551- 1554.

Farrer JJ, Toft Ad, Iodine-131 treatment of hyperthyroidism: Current issues. Clin Endocrinol 1991; 35 : 207 – 212.

Fawcett, JK and Scott, JE, J.Clin.Path. 1960; 13:156.

FDA: A review of the use of ionising radiation for the treatment of benign disease . Washington, DC, US Dept HEW, Publication 78-8043, 1977.

Feulard H: Teignes et Teigneux, Paris, Georges Steinheil, Editeur, 1886.

Finlay AY. Global overview of lamisil. Br. J Dermatol, 1994; 130:1-3.

Forslind B. 1970. Biophysical studies of the normal nail. Acta Dermatol Venereol (Stockh) 50; 160-168

Frain-Bell W, Riddell RW, Stevension CJ, et al. Chronic ringworm infection of the skin and nails treated with Griseofulvin. Lancet 1960; 1:1141-7.

Franklyn JA, Patric M Michael S, Joan B, Peter B, Cancer incidence and majority after radioiodine treatment for hyperthyroidism : a population based cohort study. Lancet 1999 : 355 : 2111-2115.

Friedell, H.L.; Tomas, C.I., and Krohmer, J.S.: An evaluation of the clinical use of a strontium 90 beta-ray applicator with a review of the underlying principles. Amer J Roentgen, 71:25, 1954.

Friedman, M., and Lewis, L.G.: A new technique for the radium treatment of carcinoma of the bladder. Radiology, 53:342, 1949.

Galimberti R, et al: Onychomycosis treated with a short course of oral terbinafine. Int J dermatol 1996. 35:374.

Galimberti RL, Kowalczuk AM, Flores V, et al. Onychomycosis: Therapeutical alternativa mediante tratamiento local. Med Cut ILA, 199; 19:209–214.

Galimberti RL, Negroni R, Iglesias de Elias MR, Casala AM . The activity of ketoconazole in the treatment of onychomycosis . Rev Infect Dis 1980; 2: 596- 598.

Gates, W: The De La Cruz-Badiano Aztec Herbal of 1952, Baltimore, The Maya Society, 1939.

Ghaunoum MA, Hajjeh R, Scher R, et al., A large Scale North American study of fungal isolates from nails: the frequency of Onychomycosis, organism distribution, and antifungal susceptibility patients. J Am Acad Dermatol. In press .

Gold Schmidt H, Sherwin WK : Reactions to ionising radiation. J Am Acad Dermatol 3 : 551 – 579, 1980.

Goldschmidt H,et al: Dermatological radiation therapy . In dermatology , 2nd ed. Moschella SL,et al.(eds) . Philadelphia . WB Saunders, 1985,pp 2048-2081.

Goldschmildt H : Dermatologic radiation therapy. Current use of ionizing radiation in the United States and Canada. Arch Dermatol 111: 1511, 1975.

Goldschmildt H : Dermatological radiation therapy. Current use of ionising radiation in the United States and Canada. Arch Dermatol III : 1511, 1975.

Goldschmildt H : X-ray therapy. Andrews' Disease of the skin – clinical dermatology (Harry LL, Richard B.O, Willium DJ eds). 9th ed. Philadelphia : WB Sannders Co. 1990 Page 1016 – 1025.

Gray H: Appendages of skin . Gray's Anatomy (Williams PL, Warnick R, Dyson M, Bannister LH eds) 37th ed. Churchill Livingstone, London, NY. 1989; 89-94.

Greer J L and Viekers B. : Combined surgical and X-ray therapy of Keloid. J La Med Soc 112 : 107, 1970.

Gugnani HC. Foot infection due to Hendersonula toruloidea and Scytalidium hyalinum in coal miners. J. Med Vet Mycol 1989; 27 : 169 – 79.

Gupta AK, et al. A higher prevalence of onychomycosis in psoriatic compared with non-psoriatic: a multicentre study. Br. J. Dermatol. 1997; 136:786-9.

Gupta AK, et al: Current management of onychomycosis. Dermatol Clin, 1997, 15:121.

Gupta AK, et al: Itraconazole for the treatment of tinea pedis: a dosage of 400mg/day given for 1 week is similar in efficacy to 100 or 200 mg/day give for 2 to 4 weeks. J Am Acad Dermatol 1997, 36:789.

Gupta AK, Sauder DN, Shear NH: Antifungal agents: An overview. Part I. J Am Acad Dermatol, 1994; 677 – 698.

Gupta AK, Sauder DN, Shear NH: Antifungal agents: An overview. Part II. J Am Acad Dermatol, 1994; 30:911 – 933.

Gupta AK, Scher RK, Doncker PD: Current management of onychomycosis – an overview. Dermatologic clinics, 1997; Vol.15: No.1. P121-135.

Gupta AK, Shear NH, Sauder DN: New antifungal agents. Curr Opin Dermatol, 1993; 2:200 – 206.

Gupta AK, Shear NH: Onychomycosis – going for cure. Can Fam Physician, in press, 1966.

Gupta AK, Shear NH: Terminafine in the treatment of superficial dermatomycoses. Can J Derm, 1994; 6:561 – 565.

Gupta AK, Sibbald RG, Lynde CW, et al: A survey and treatment of onychomycosis in children. J Am Acad Dermatol, in press.

H.E.Johns, 1978. The physics of radiology. Pub. Library of Congress cataloguing in Publication Data. 3rd Ed. 1978; p524-27.

Hamilton J. G. and Lawrence J. H.: Recent clinical development in the therapeutic applications of radio phosphorous and radio iodine. Jc.l 21, 624, 1942.

Haneke E. Baran R: Nail Surgery and traumatic abnormalities. Diseases of the nails and their management (Baran R. Danber RPR eds) 2nd ed. Oxford: Blackwell Scientific Publications 1994; 345-416.

Haneke H. Fungal infections of the nail. Semin Dermatol 1991;10:41–53.

Hanke E, Tausch I, Brautigam M, et al. Short-duration treatment of fingernail dermatophytosis: a randomised, double-blind study with terbinafine and griseofulvin. J AM ACAD DERMATOL 1995; 32:72-7.

Haq E. A Study on Onychomycosis in Bangladesh. M.Phil. Thesis. University of Dhaka, 1994.

Haria M, Bryson HM. Amorolfine. A review of its pharmacological properties and therapeutic potential in the treatment of onychomycosis and other superficial fungal infections. Drugs 1995; 49: 103-20.

Harry L. A, Richard B.O., Willium D. J. Andrews' Diseases of the skin, Clinical dermatology 8th ed. W. B. Saunders company, 1990:318-374.

Harry L. A, Richard B.O., Willium D. J. Andrews' Diseases of the skin, Clinical dermatology 9th ed. W. B. Saunders company, 1998: 318-374.

Hashimoto K. 1971. Ultrastructure of the human toenail. II. Keratinization and formation of marginal band. J Ultrstruct Res. 36:391 –410.

Hay RJ. Risk/benefit ratio of modern antifungal therapy: Focus on hepatic reactions. J Am Acad Dermatol 1993; 29:S50 – 4.

Hay RJ: Onychomycosis: Agents of choice. Dermatol Clin, 1993; 11:161 – 169.

Hay RJ: Treatment of dermatomycoses and onychomycoses – state of the art. Clin Exp Dermatol, 1992; 17(Suppl): 2 – 5.

Hekkila H, Stubb S. The prevalence of Onychomycosis in Finland. Br. Dermatol 1995; 133 : 699 – 703.

Henry, RJ; Cannon, DC; Winkelman, JW. Clinical Chemistry – Principles and Technics. Harper & Row 2nd ED (1974).

Hertz and Roberts A. Application of radioiodine in the therapy of Graves Disease. J.C.1.21.31.1942.

Hoefnagel, C.A, Radionuclide therapy revisited, Eur. J. Nucl Med.18, 408-431. 1991.

Hollander MB: Ultra soft x-rays. Baltimore, Williams & Wilkins, 1968.

Human Gaselischaft for Biochemical und Diagnostically mbH Max-Planck-Ring 21 – D-65205 Wiesbaden – Germany

Human, Cat. No 12012 Complete test kit . Clin. Chim. Acta 105, 147 – 172, 1980.

Hunter MS, Weitzman I, Rrosenthal SA. Cutaneous mycosis. In : Balous A, Hausler WJ, editors. Diagnostic Procedure for Bacterial., Mycotic and

Parasitic Infection, 6th ed. Washington: American Public Health Association, 1981: 852, 863, 869, 978, 881 – 86.

International Atomic Energy Agency CRP Project # 7876: Standardization and optimisation of Iodine – 131 therapy for hyperthyroidism, 1995.

International Committee for Standardization in Haematology (ICSH), Brit. J. of Haematology 13 Suppl., 71(1967)

Islam S. A. study on Dermatophytosis in Bangladesh. M. Pill. Thesis. University of Dhaka. 1993 : 8.

James E Turner. Atoms, Radiation, and Radiation Protection. Pub. Pargamon Press inc. Ed. 1st. 1986; p77-95)

Janssen PAJ, Symoens JE. Hepatic reactions during ketoconazole treatment. Am J Med 1983; 74:80 – 5.

Jendrassik, L., Grof, P, Biochem. Z. 81, 297(1983)

John William C. Eckeloman & Domid D. Newmann, C. Herbert Nuclear Medicine Diagnosis and therapy. 1st ed. Thieme Medical Publishers, the New York 1996 : 951-1141.

Jolly H W, Arthur D, Ira H R, Iris K, Theodore A , Samuel J and Richard G, A Multicenter Double – Blind Evaluation of Ketoconazole in the treatment of Dermatomycoses. CUTIS Vol- 31, Feb 1983.

Kaleque KA and Mamun KZ, 1995. Practical Pathology and Microbiology 8th ed. Dhaka. Mamun KT, 136, 137.

Khan KA, Anwar AA. The etiology of tinea cruris in Karachi. Br. J Dermatol 1969; 81:858-60.

Kisch B: Forgotten Leaders in Modern Medicine, Trans. Amer. Phil. Soc. 44:139-317, 1954.

Klein I , Becker DV and Levely GS : Treatment of thyroid diseases . Ann. Intern . Med. 121, 281-8.

Kombila M , Martz M, Gomez de Diaz M, et al. Handersonula toruloidea as an agent of mycotic foot infection in Gabon. J Med Vet Mycol 1990; 28: 215-23.

Korting H C, Schaler-Korting M. Is tinea unguium still widely incurable? A review three decades after the introduction of griseofulvin. Arch Dermatol. 1992; 128:243- 48

Kotrajaras R, Chongsathien S. Rojanavanich V, et al. Hendersonula toruloidea infection in Thailand. Int J Dermatol 1988; 27 : 391 – 5.

Kovacs MJ, Alshammari S, Guenther L, et al. Neutopenia and pancitopenia associated with oral terbinafine. J Am Acad Dermatol 1994; 31:806.

Kyung BP, et al: Study on the preparation of Holmium-166 patch for skin cancer treatment presented in 1999. International conference on future nuclear system, 28th August – 3rd September, Wyoming, USA (1999).

Lamisil® (terbinafine tablet) Package insert. East Handover (NJ): Novartis Pharmaceutical Corp.

Laskey and Sunthalaringam: Beta radiation treatment can prevent repeat blockages in blood vessels after angioplasty according to a new study, released April 11, 2000. Source – Internet, bcrawfor@umm.edu.

Lawrence JH. Nuclear Physics and therapy: Preliminary report on a new method for the treatment of leukaemia and polycythemia. Radiology 35, 81-90, 1940.

Lederman M: Some applications of radioactive isotopes in opthalmology, Br. J. Radiology, 1956; 337,1.

Lele RD. Principles and Practice of Nuclear Medicine, 1st ed. Arnold Heinemann publishers (India) Pvt. Ltd. 1984 :364-374.

Lesher JL: New antifungal agents. Dermatol Clin, 1992; 10:799 – 805.

Lindelof B, et al : Incidence of malignant skin tumours in 14,140 patients after Grenz- ray treatment for benign skin disorders . Arch Dermatol 122:1391-1395, 1986.

Lubeck DP, Patrick DL, McNulty P, Fifer SK, Birnbaum J. Quality of life of persons with onychomycosis. Qual Life Res, 1993; 2:341- 48.

M. N. Maisey and I. Gosplman. Thyroid disease. Clinical Nuclear Medicine. 2nd ed. M. N. Maisey, K.E. Britton and D.L. Gilday. Chapman & Hall Medical., 1991:210.

Mayo foundation for medical education and research – First Aid and Self cured guide, Aug. 2000.

Mc Lean T, Levy H, Lue YA. Echology of dermatophyte infections in South Bronx, NY 1969 to 1981. J Am Acad Dermatol 1987; 16:336-40.

Meinhof-W. Investigations of the effect of beta rays on dermatophytes. Influence on growth and germinations, determinations of lethal ray dosage- Arch-Klin-Exp-Dermatol. 1965 Sep 1:223(2): 185-98.

Midgley G, Moree MK, COOK JC and Phan QC. Mycology of nail disorders. J Am Acad Dermatol1994; 31: S68-S74.

Mitchell TG. Medical mycology. In : Joklik WK, Willet HP, Amos DB, Wilfert CM, editors. Zinsser Microbiology, 20th ed. USA: Prentice-Hall International Ltd., 1992: 1123, 1126, 1128, 1129, 1130.

Mooney E. Dermatophytes in Iceland. Intern J Dermatol 1986; 25:305-6.

Myrvik QN, Weiser RS. Fundamentals of Medical Bacteriology and Mycology, 2nd ed. Philadelphia: Lea and Febiger, 1988:538 – 40.

National Academy of Sciences (NAS). Health affects of exposure to low level of ionising radiation [(Biological Effects of Ionising Radiation (BEIR V)] Washington DC: National Academy Press 1990.

Neils R. Infections with T.rubrum. Br J Dermatol 1966; 78:209-12.

Odom RB: New Therapies for Onychomycosis. J Am Acad Dermatol 1996. 35 : S26.

Osterloh IH. Safety In : Powderly WB, Van Wout JW, editors. Fluconazole. Camforth, UK: Marias press, 1992:40.

Park KB, Kim YM, Kim SJR et al. Therapeutic application of new Holmium – 166. Chitosan complex in malignant and begin disease. Presented in symposium held in Lisbon, Portugal., 30th March to 3rd April, 1998.

Philip R, Cohen and Richard K. Scher. Topical and surgical treatment of onychomycosis. J Am Acad Dermatol 1994; 31 : S74 – S77.

Philpot CM. Some spects of the epidemiology of Tinea. Mycopathologia, 1977; 62,1:3-13.

Physician's Desk Reference. 49th ed. Montvale, NJ: Medical Economics Publishers, 1995: 2255-6.

Pierard G, Arrese-Estrada J, Pierard-Franchimont C. Treatment of onychomycosis: traditional approaches. J Am Acad Dermatol 1993; 29:S41–5.

Ramani R, Srinivas CR, Ramani A, et al Molds in onychomycosis .Int J Dermatol ; 1993;32:877-878.

Rao A. Fungus disease in India. Bull Calcutta Sch Trop Med 1969; 5:76-9.

Rashid A: Onychomycosis : A dilema –to treat or not to treat , abstract published in South Asian Regional Conference of Dermatologists, Dhaka, Bangladesh. Feb 7-9, 2002.

Richard B. Odom, Raza Aly, Richard K. Scher Ralph Daniel, Bone E. Elewski. A multi center, placebo – controlled, double – blind study of intermittent therapy with itraconazole for the treatment of Onychomycosis of the fingernail. J Am Acad Dermatol. 1997; 36:231 – 5.

Richard B.Odom. New therapies for onychomycosis. J Am. Acad Dermatol 1996; 35 : S26 – S30.

Richard K Scher. Onychomycosis : Therapeutic update. J Am Acad Dermatol 1999; 40 : S21 – 6.

Rippon JW , Medical Mycology , 3rd ed. Philadelphia : W.B.Saunders, 1988 : 170 –74, 182,183.

Rippon JW. Medical Mycology, 3rd Ed. Philadelphia : W.B. Sander, 1988; 170 – 74, 182, 183.

Rivkees S A, Sklar C, Freemark M : The Management of Graves' disease in children with special emphasis on radioiodine treatment. T Clin Endocervical Metal 1998; 83 : 3767-3776.

Robert SOB, Mackenzie DWR. Mycology. In: Rook A, Wikinson DS, Ebling FJC, Champion RH Burton JL, editors. Textbook of Dermatology, 9th ed, vol.II. London: Blackwell Scientific Publications, 1986: 893, 894, 910 –15.

Roberts DT. Oral therapeutic agents in fungal nail disease. J Am Acad Dermatol 1994; 31 (suppl) : S78-81

Roberts DT. Prevalence of Dermatophytes Onychomycosis in the United Kingdom : results of an omnibus survey. Br J Dermatol 1992; 126 (Suppl 39) : 23 – 7.

Roberts SOB, Hay RJ, Mackenzie DWR. A clinician's guide to fungal disease, Ist ed. NY, Basel : Marcel Denker, Inc., 1984: 67-70.

Rocha A.F.G., and Herbert J.C.: Textbook of Nuclear Medicine: basic science, Philadelphia, 1978. Lea & Febiger.

Rosenthal T: Aulus Cornelius Celsus, A.M.A. Arch. Derm. 84:613-618, 1961.

Sanyal M. Dermatophytosis in India Bulletin Calcutta Sch Trop Med 1969; 17 : 54 – 8.

Scher RK : The nail. Dermatological Surgery – Principles and Practice (Roenigk RK, Roenigk H eds) 1st ed., New York : Marcel Decker Inc. 1989; 509 – 526.

Scher RK. Diseases of the nails. In: Conn H, editor. Current therapy. Philadelphia: W.B. Saunders, 1990:736.

Scher RK. Onychomycosis : therapeutic update, J Am Acad Dermatol, 1999; 40: S21 - 6.

Scher RK. Onychomycosis is more than a cosmetic problem, Br. J dermatol 1994; 130: 431- 435.

Scher RR : Onychomycosis. J Am Acad Dermatol 1999, 40 : S21.

Shahidullah Dr. Md., Professor & Head, SSMC & Mitford Hospital. 1995, Personal Communication.

Shelly WB, Wood MG: The white spot target for microscopic examination of the nails for fungi. J Am Acad Dermatol 1982, 6:92.

Silverstein EB and Taylor A, Jr. Procedure guideline for bone pain treatment : 1.0,J.Nucl. Med., 37(5),881-4.

Sinclair, W.K, and Trott, N.G.: The construction and measurement of beta-ray applicators for use in Ophthalmology. Brit J Radiol, 29:15, 1958.

Singer PA. Treatment guidelines with Hyperthyroidism an Hypothyroidism. JAMA 1995; 273:808-812.

Smith KJ, Skelton HG, Yenger J. et al. Cataneous findings in HIV-1-positive patient : a 42 months' prospective study. J Am Acad Dermatol. 1994; 31 : 746-754 Medline.

Sporanx^{R}, (itraconazole) 100 mg capsules : package insert. Titusville (NJ) :Janssen Pharmaceutical Inc.

Stokes EJ, Ridgeway GL. Clinical Microbiology. 6th ed. Edward Arnold (Publishers) Ltd., 1987: 158, 160, 169.

Stricker BH, De-Jong PA, Schreuder F, et al. Loss of test sensation in terbinafine administration. Ned Tijdschr Ge – neeskd 1992; 136:2438 – 400.

Summberbell RC, Kane J, Krajden S. Onychomycoses, tinea pedis, and tinea manuumcaused by nondermatophytic filamentous fungi. Mycoses 1989: 32: 609-19.

Synopsis der Laberkrankheiten: H. Wallnofer, E. Schmidt and F. W. Schmidt, Georg Thieme Veriag, Stuttgart 1974.

Taplin D, Zaias N, Rebel G and Blank H : Isolation and Recognition of Dermatophytes on a New Medium (DTM) Arch Derm – 1969. 99 : 203 – 206.

Taylor RL, Kotrajaras R, Jotisanicasa V. Occurrence of dermatophytes in Bangkok, Thailand. Sabouraudia 1967-1968; 6:307-11.

Teuscher, A and Richterich P. Schweiz Med. Wscher, 101 (1971) 345 and 390.

Thefeld, W. et al., Dtsch. med. Wschr. 99, 343 (1974)

Tosti, A, Piraccini BM and Stinchi C et al. J Am Acad Dermatol. 1996; 34: 595-600

Turner J E. Atoms, Radiation and Radiation Protection. 1st Ed. Pergamon Press Inc.(Pub.) 1986; p225-227.

Van der Bergh, A. A. Muller, P., Biochem. Z.77,90(1916)

Van Kampen, Kampen, E. J., Zijlstra W. G., Clin. Chem. Acta 6, 538 (1961)

Ven Den Break, H A S and Minty CCJ : Radiation in the management of Keloids and hypertrophic scars. Brit J Surg. 47 : 595-605, 1960.

Villars VV, Johns DC. Special features of the clinical use of oral terbinafine in the treatment of fungal diseases. Br J Dermatol 1992; 126 (Suppl 39): 61 – 9.

Williams, Warkwick, Dyson and Bannister. Appendages of the skin. Gray's Anatomy. 37th Ed. Pub. Churchill Livingston 1989. p89-94.

Wintrobe, M. M., Clin. Haematology, Lea & Febringer, Philadelphia, Pa. 4th edit. 1956.

Wong KO, Chan YF: Dermatophytosis in Hong Kong. Br J Dermatol 1968; 80:287-92.

Zaias N, Dertel I, Elliott D. Fungi in toenails. J Invest Dermatol 1969; 53:140-42.

Zaias N. Tosti A, Rebbel G, et al: Autosomal dominant pattern of distal subungual onychomycosis caused by Trichophyton rubrum. J Am Acad Dermatol 34:302-304, 1996.

Zaias, N: The nail in Health and Disease. Jamaica, NY, Spectrum publications, 1980.

Zaug M, Befgstraesse M. Amorolfine in Onychomycoses and dermatomycoses (an overview). Clin Exp Dermatol 1992; 19 & Suppl 1) 60 – 70.

APPENDIX – I

Check Sheet & Treatment Plan

STUDIES OF THERAPEUTIC EFFECT OF BETA RADIATION ON ONYCHOMYCOSES FOR AN INNOVATIVE MODALITY OF TREATMENT

Code No:	**Registration No:**	**Date:**

Personal Data:

1. Name:			
2. Address:			
3. Age:	4. Sex:	5. Height:	6. Weight:
Socio-Economic status:			
1. Education (0, 0-5, 5-10, >10):		2. Occupation:	
3. Family Income:		4. Housing:	

History of Illness:

1. Past History: (If yes, Duration, Medication, How long):
2. Family History:
3. Chief Complaints:
4. Duration of signs and symptoms of the present illness:
5. Site involved:
6. History of recent medication:

Diagnostic Criteria

1. Local examination findings: (a) Clubbing/Koilonychea/Paronychea/None (If yes, any adjunctive therapy)	
(b) Colour and palpation findings of lesion:	
2. Microscopic Findings:	
3. Culture of Fungus:	

Biochemical Investigation:	**Blood Picture:**	
(a) Random Blood sugar:	(a) Hb% = (b) Total Count:	
(b) S. Bilirubin:	(c) Platelet Count:	
(c) SGPT:	(d) D.Count:	**P:**
(d) Blood Urea:		**L:**
(e) S. Creatinine:		**M:**
9. Clinical diagnosis:		**E:**

Treatment received [Group-A(Anti-fungal)/Group-B(Radiation)/Group-C(Combined)]:

Signature of the Physician

FOLLOW-UP

Code No.		Regn.No.		
Name:		Treatment received:		
Parameters	**1st Follow-up**	**2nd Follow-up**	**3rd Follow-up**	**4th Follow-up**
1. Physical appearance of the lesion ***(No /Mild / Moderate / Marked improvement)***				
2. Laboratory Findings **(a) Microscopic** *(Positive / Negative)*				
(b) Culture *(Positive/Negative)*				
(c) Biochemical values *(Within normal limit / Raised/ Reduced)*				
(d) **Hb%, Platelet count & WBC***(Within normal limit / Raised / Reduced)*				
3. Complication *(Yes/No)*				
4. Outcome *(Cured / Non-cured / Recurrence)*				

Note: Every follow-up was done on an average 6 weeks apart.

APPENDIX – II

CONSENT FORM

I, ..., was being very clearly briefed that for management of my nail disease I would be receiving radiation from ^{90}Sr applicator and /or antifungal drugs. About the hazard of radiation and side effects of antifungal drugs have been unambiguously explained to me.

With conscious and good knowledge, I do agree to receive the treatment and given the consent to commence the treatment.

Date_____ _____

Signature

APPENDIX – III

Fungobiotic agar media (selective medium for dermatophytes)

Dehydrated fungobiotic agar (Hi-Media)		35.1 g
Distilled water	....	1000.0 ml

pH 6.5±0.2

The ingredients were dissolved completely by heating in water-bath. pH was adjusted and distributed in 7 –10 ml amounts in test tubes. The tubes were autoclaved at 15 lbs pressure (121°C) for 10 minutes. The test tubes were kept in slanting position till medium was solidified.

APPENDIX – IV

Sabouraud dextrose agar with thiamine (Beneke and Rogers, 1970).

Dehydrated SDA (Difco)	...	65.0 g
Thiamine hydrochloride	....	10.0 mg
Distilled water	...	1000.0 ml

pH 6.5±0.2

Autoclaved at 121°C for 10 minutes under 15 lbs pressure and dispensed aseptically in 5 – 7 ml amounts in sterile test tubes. The media in the tubes were allowed to cool in a sloped position.

APPENDIX -V

Sabouraud dextrose agar (SDA) media

Dehydrated SDA (Difco) ... 65.0 g

Distilled water ... 1000.0 ml

pH 6.5±0.2

The ingredients were dissolved by heating in a boiling water-bath. pH was adjusted and autoclaved at 121°C for 15 minutes under 15 lbs pressure. The medium was allowed to cool at 50° – 55°C and dispensed aseptically in sterile petri dishes, sterile test tubes and sterile small sized screw-capped tubes. The medium was allowed to solidify in sloped position in the test tubes.

APPENDIX – VI

FLOW CHART