BONEGRAFTING: KENYATTA NATIONAL HOSPITAL EXPERIENCE

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A DISSERTATION SUBMITTED IN PART FULFILLMENT FOR THE DEGREE OF MASTER OF MEDICINE IN SURGERY, UNIVERSITY OF NAIROBI



DECLARATION

I hereby certify that this dissertation is a result of my original work and has not been presented for a degree in any other university.

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DEDICATION

To my wife Lydia and my daughter Juliet, for their patience, understanding and support during this period of study.

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ABBREVIATIONS

K.N.H

- KENYATTA NATIONAL HOSPITAL

MB.CHB

- BACHELOR OF MEDICINE AND BACHELOR OF

SURGERY.

U.O.N

- UNIVERSITY OF NAIROBI

MMED.SURG

- MASTER OF MEDICINE IN SURGERY

HIV

- HUMAN IMMUNODEFICIENCY VIRUS

AIDS

- ACQUIRED IMMUNODEFICIENCY SYNDROME

USA

- UNITED STATES OF AMERICA

T.B

- TUBERCULOSIS - OSTEOARTHRITIS

O.A ORIF

- OPEN REDUCTION AND INTERNAL FIXATION.

EG

- FOR EXAMPLE

I.E

-THAT IS

DNA

-DEOXYRIBONUCLEIC ACID

SUMMARY

This is a retrospective study in which data was retrieved from the patients' files at KNH Medical records department from January 1991 to December 2000. This data was analysed and various indices established.

One eighty nine cases of bone grafting operations were considered. Males who underwent bone grafting were more than females in a ratio of 1.7:1. The most common indication for the operation was found to be non-union (33%) followed by arthrodesis (20%), most of these operations (71.4%) were done in young people in the age group (10-49) years, the youngest being 2 years and the oldest 95 years.

There were a total of 157 donor sites and the most utilized donor site was the iliac crest (47.8% or 75 instances) followed by the tibia (19.1% or 30 instances). There were a total of 200 recipient sites and the commonest recipient bone was the femur (69 instances or 34.5%).

Most of the cases developed no complication (142 or 75.1%) but among the once who did, donor site pain (19 patients) and recipient site infection(17 patients) were the most frequent. It is evident from this study that bone grafting using autografts is an established practice in KNH.

INTRODUCTION

Bone has been successfully transplanted since 1809 when Merrem obtained successful healing of bone plates in the skull of animals after trephining. The first systematic study of bone grafting was made by Ollier who published a monograph on bone transplantation in 1867. (1,2)

Different kinds of bone grafts (i.e. autografts, allografts, isografts and xenografts) have been used for different indications. As time has gone by the indications for bone grafting have increased, the success of these operations has also increased, while the complication rate has decreased. Use of bone substitutes and bone tissue engineering are the latest additions in this field.

There exists a lot of literature on bone grafting especially from the Western hemisphere in books and journals but no study has been done in K.N.H.

LITERATURE REVIEW

NOMENCLATURE

Autografts = autogenous grafts

A bone graft taken from one part of a patient's body to another part of the same patient. (1,2)

Isograft = isogenous graft (syngeneic)

Grafts exchanged between genetically identical individuals like identical twins, cloned animals or inbred strains. (1,2)

Allograft (old term is homograft)

Grafts exchanged between two individuals of the same species. (1,2)

Xenograft (old term is heterograft)

Grafts exchanged between subjects of different species like those obtained from animals and transplanted on to human subjects. (1,2,3)

Implant - for dead graft. (2)

Transplant - for a living graft. (2)

PHYSIOLOGY OF BONE FORMATION

Bone formation is a complex and closely regulated process. Sites of bone formation include:

- (i) Sites of previous osteoclastic bone resorption in adult humans.
- (ii) In the growing long bones during endochondral bone formation.
- (iii) Periosteal surfaces appositional bone formation.

The last two occur during growth and adolescence. (1)

The cellular events involved in bone formation include: -

- (i) Chemotaxis of osteoblast precussors.
- (ii) Proliferation of committed osteoblast precussors.
- (iii) Differentiation including expression of growth regulating factors and the structural proteins of bone.
- (iv) Mineralization.

These cellular events are under very tight regulatory control. The factors involved in this modulation include;

- A. Local factors or cytokines generated in bone cell microenvironment are probably the most important.
- B. Systemic hormones including parathyroid hormone, vitamin D3 and other systemic hormones e.g the pituitary and thyroid hormones and sex steriods (1).

An important part of the therapeutic approach to bone defects and the promotion of fusion is the implantation of materials that support new bone formation. Such implants may hasten healing by three mechanisms i.e. osteoconduction, osteogenesis and osteoinduction. (1)

In osteoconduction, the inert material serves as an inert scaffold for the ingrowth of host bone i.e. creeping substitution replaces the implant with new bone to form a functional skeletal element.

OSTEOGENESIS is the synthesis of new bone brought about by surviving preosteoblasts and osteoblasts within a bone autograft.

OSTEOINDUCTION - is the formation of new bone by the active recruitment of host pluripotent cells that differentiate into chondroblasts and osteoblasts. (5,6,7,8,9)

HISTORICAL BACKGROUND

Only a few historical events will be quoted here for it is not possible to quote all the milestones made in this field of bone grafting.

1809 - First documented successful bone transplant by Merrem in the skulls of animals. (1)

1867 - Ollier published a monograph of bone transplantation. (2)

Ollier was both a surgeon and an experimentalist and he made great contributions to tissue transplantation especially of periosteum and skin and to a lesser extent of bone and marrow. He clearly distinguished autograft, homograft (allograft) and heterograft.

- 1881 MacEwen was the first to transplant successfully fresh allogeneic bone that he used to replace a 10.8 cm defect of the humeral shaft lost through osteomyelitis. (10)
- 1915 Albee after experimental work with dogs initiated a mechanical approach to bone transplantation. The widespread use of bone grafts in surgery is due to Albee. (1)
- 1931 Phemister introduced a more biological approach to bone grafting. (12)
- 1941 Raisford Mowlem introduced iliac cancellous bone into maxillofacial surgery (13).
- 1944 Mowlem used iliac cancellous bone to treat continuity defects in long bones. (13)
- 1961-Burwell used autograft red marrow to impregnate dead allogeneic cancellous bone. (11)

INDICATIONS OF BONE GRAFTS

The uses of bone grafting have been expanding over time but the following can be listed as the principal ones.

- Non-union of fractures.
- Delayed union of fractures.
- Replacement or strengthening of bone weakened or destroyed by benign or malignant disease/growth.
- ❖ To replace bone destroyed by infection.
- To repair traumatic bone injuries.
- Filling of cavities in bone.
- Arthrodesis of joints.
- Fusion of growth plate cartilages.
- Bone block operations.
- ❖ Augmentation of acetabulum and cranium.
- ❖ To correct congenital or acquired deformities of extremities, trunk or face (1,2)

BONE GRAFTING OF INFECTED FRACTURES AND NONUNION

Bone grafting can be done successfully even in cases of osteomyelitis. Two kinds of protocols have been advocated. These are:-

- (a) one-stage protocol.
- (b) Staged (two-stage) protocol.

The one-stage protocol usually involves;

- thorough debridement of the septic focus
- stabilisation with an external fixator or cast
- pure or antibiotic-impregnated fresh autogenous cancellous bone grafting.

The wound is then left open for secondary closure or skin grafting if necessary. (2,14)

Note: there are several modifications of this protocol.

The two-stage protocol usually involves:-

- first stage thorough debridement of the septic focus and obliteration of the debrided osseous defect with polymethylmethacrylate (PMMA) bead chains and external skeletal fixation.
- Second stage- the beads are removed and the defect reconstructed with antibiotic-impregnated autogenous cancellous bone graft.

 The time between first and second stages of treatment is between two and six weeks. (15,16)

COMMON DONOR AND RECIPIENT SITES

DONOR SITES

Several bones may be used as donor sites for bone autografts. Among the common ones are; iliac crest, fibula, ribs, proximal tibia and the second metatarsal. (2)

Alt V. et al did a study on bone grafting from the proximal tibia. They noted that:

- The complication rates were low and that higher complication rates had been noted for iliac graft sites.
- The amount of bone that could be harvested was more than adequate.
- The patient could start immediate post-operative weight bearing (17).

RECIPIENT SITES

Almost any bone may be a recipient site for bone grafts. Common sites include tibial shaft, femur, hip, calcaneus, spine, radius and humerus. (1)

OUTCOMES AND COMPLICATIONS OF BONE GRAFTING

The outcome following bone grafting can be assessed both clinically and radiologically. The outcome is dependent on the indication for which bone grafting was performed.

Certain complications are associated with bone grafting procedures. This may involve the donor or recipient site and may vary according to the type of graft.

Listed below are some of commonly encountered complications: (1,2,17,18,19)

For autografts; donor site pain, donor site haematoma, infection, injury to vessels, lack of adequate graft material especially in children.

(See studies below)

For allografts; immunologic reactions from the host, risk of transmission of diseases like HIV/AIDS, lack of wide acceptance of cadaver grafts in certain religious and cultural groups.

For both allografts and autografts; nonunion, infection, graft failure and fracture of graft. (See studies below).

Below are some studies showing outcomes and complications following bone grafting for different indications:

NONUNION

Boyd H. B. et al. assessed the results of treatment of nonunion (diagnosed both clinically and radiologically) of 842 patients involving different bones and treated by different methods using autografts. Of the 842 patients, 790 (94%) eventually obtained union with different types of grafting methods but 64 required two or more operations to do so. (75)

The bones mainly involved in order, from the most to the least common were; tibia, femur, humerus, radius, ulna and clavicle. In a latter study where the researchers studied 122 patients, the bone with highest incidence of nonunion was found to be the femur ⁽⁷⁵⁾.

D'aubigue reported 814 consecutive nonunions. His rate of union after grafting was similar to the one of Boyd in the study above. D'aubigue emphasised the importance of infection as a cause of nonunion after acute fracture and as a cause of failure to obtain union after bone grafting.

In the tibia he recommended that a nonounion should never be approached through previously infected scars. (66)

Kim S. J. et al. performed endoscopic bone grafting for 8 patients of delayed union and nonunion which developed after humeral and femoral shaft fractures. Six of the patients healed at 4.1 months on average. Two of the patients had unsatisfactory healing and eventually underwent non-endoscopic revision surgery. No intraoperative or post-operative complications were recorded. (67)

TUMOUR SURGERY

Mankin H. J. et al. did 718 procedures over 24 years using allografts from bone banks. The authors implanted cadaveric allografts in defects created by resection of bone tumours. The complications that occurred are as listed below; (68)

Total number of procedures 718

| complication | Number of procedures | percentage |
|----------------|----------------------|------------|
| infection | 82 | 11% |
| fracture | 140 | 19% |
| nonunion | 122 | 17% |
| Unstable joint | 28 | 4% |

Infection accounted for most of the graft failures. Many of the patients in this series had more than one complication.

- 21 had both nonunion and infection
- ☐ 16 had graft failure and infection
- 6 had all the three conditions, that is nonunion, infection and graft failure.

The graft failure rate was highest in the first year then declined rapidly.

In twenty five patients primary union was achieved within twelve months and in two in twenty months, while twelve patients required a second supplementary cancellous graft at the site of nonunion to obtain stability.

One patient required removal of an infected graft. Fractures occurred in eighteen of forty patients after union had occurred. The stress fractures healed in fifteen of these patients, in six with no treatment, in seven with external immobilization and in two after bone grafting of the ununited

fracture. There were three persistent nonunions of stress fractures despite bone grafting, internal fixation and electrical stimulation. There was little morbidity (three of forty patients) associated with graft procurement. (69)

SOLITARY BONE CYST

Solitary bone cysts treated with curretage and filling the cavity with autogenous bone chips have had different outcomes reported. Henry L.quotes a recurrence rate of 40-50% after the first operation. (64) The ones that recurred were almost always cured by a second intervention. Brashear J. R. quotes a recurrence rate of 25% after operation. (65)

OTHER STUDIES

Alt V. et al. did a study where 54 patients underwent bone grafting harvested from the proximal tibia with a mean follow up time of 26.4 weeks. The indications for bone grafting were;

- fresh fractures with primary grafting
- nonunions.

The overall complication rate was 1.9% with one patient suffering a local haematoma. No major complications such as fractures, sensory deficits or wound infection were observed. (17)

Lim E. V. et al gives a case report of injury to superior gluteal artery during bone graft harvesting from posterior iliac crest. This case was successfully managed by arterial embolization. (70)

CONTRAINDICATIONS

The only contraindications to the surgical use of bone grafts are a markedly septic field of operation and excessive scar tissue over the site being grafted.

Syphilis should be cured before operation (1).

AUTOGRAFTS:

FATE OF FREE AUTOGRAFTS

The fate of a bone graft is controlled by many factors that mainly relate;

- to the graft
- The bed in which it lies and
- Whether or not it becomes infected.

The cellular changes that occur in fresh bone autografts are of three broad types:

- Degenerative
- Proliferative
- Differentiative (2,20)

Autografts are by far the most trusted forms of bone grafts. It is known that an autogenous bone graft always "takes" and becomes permanent if it is put under aseptic conditions, and if it has function to perform it stays there and adapts itself in structure, size, contour and in strength to the new environment. (21)

NATURAL HISTORY OF FREE BONE AUTOGRAFTS

- Necrosis
- Mitosis, cell migration and differentiation
- Revascularization
- Osteogenesis
- Remodelling
- Growth/hypertrophy. (2)

Cortical bone largely dies after transplantation whereas cancellous bone grafts actively contribute to osteogenesis.

The internal remodelling of a bone graft is similar to the internal remodelling of normal bone in the intact skeleton but occurs in a much more exaggerated manner in a bone graft.

Autogenous live bone is the only material that can be implanted with safety in a bed free of periosteum ^(1,22). The best transplant is a live (not boiled) piece of autogenous bone with all its elements namely; periosteum, compact bone, endosteum and marrow substance.

Cortical bone ultimately becomes spongy if implanted in or connected with bone of that character and vice versa. (1)

LAW OF FUNCTIONAL IRRITATION

The law of functional irritation as laid down by Roux states that; if a graft is placed in a location where there is no mechanical function for it to perform its cells retain their vitality but nearly always there will be few or no proliferative changes in the transplant. On the other hand if it is transplanted into a defect where there is demand

for it to perform mechanical function, proliferative changes are usually marked and it rapidly becomes united and similar in structure to the part in which it is grafted.

The more perfect the technique of transplantation the greater will be the effect of this law of irritation

THE FATE OF VASCULARISED FREE SKELETAL AUTOGRAFTS

Haw et al in 1978 using tibial segments of dogs with and without microvascular anastomosis stated that, with microvascular anastomosis:

- (i) Two thirds of the grafts were successful.
- (ii) The rate of infection was reduced.
- (iii) Bone union was guaranteed and.
- (iv) The rate of union accelerated. (23)

The vascularity of a bone graft has a significant effect on long-term thickness and histomorphometric parameters of bone remodelling and deposition are accelerated during the initial period following graft placement. Continued bone deposition renders vascularized grafts better suited for the long-term maintenance of thickness and contour relative to non-vascularized grafts. (23)

Bergrran et al (1982) using vascularized rib grafts in adult dogs found osteoclasts, osteoblasts and marrow survived 25 hours of ischaemia after storage in tissue culture at 5°C. (27)

Vascularised bone grafts are standardized procedures in reconstructive surgery but there are some disadvantages; donor site morbidity, limited number of natural donor sites and complex technique. (26)

PEDICLE BONE GRAFTS

Chacha et al 1981 using monkeys showed that fibular grafts raised on pedicle remained viable as opposed to free fibular grafts. (28)

PERIOSTEAL GRAFTS

King (1976) using puppies raised periosteal flaps from proximal and distal ends of the tibia and sutured them to form periosteal tubes along the length of the bone. He found that ossification developed in the periosteal tubes. This result has been reproduced many times after that and it is an established fact that periosteal grafts do work. (59)

FREE MARROW AUTOGRAFTS

The formation of woven bone and then an ossicle from marrow autografts transplanted heterotopically can occur. The origin of bone is considered to be from:

- a) Endosteal osteoblasts
- b) Stromal cells in marrow and
- c) Possibly host cells at the site of grafting.

It has been shown that adult bone marrow contains a stem cell that can form bone in vitro. (29)

ALLOGRAFTS

Bone allografts both fresh frozen and freeze dried play an important role in the treatment of orthopaedic conditions. They have been used most commonly for benign and malignant bone lesions and in spinal fusion procedure ⁽²⁾. (See previously quoted studies).

An advantage of allografts is that donor-site discomfort and morbidity associated with autografts harvesting can be avoided (1).

The most important issues concerning allograft implantation are the standards, quality and size of the bone bank from which the graft is obtained. No other single factor is more critical than the maintenance of a safe and competent bank with an adequate inventory so as to offer optimal sizing for the replacement part. (2)

Allografts are mainly used when massive amounts of bone are required and especially when it is difficult to source this from the same patient like in children. (2)

CELLULAR AND HUMORAL IMMUNE RESPONSE OF ALLOGRAFTS

Fresh allograft bone excites an actively acquired immune response in the recepient. Both humoral and cell mediated immunity are elicited ^(20,31,32). Musculo D. L. ⁽¹⁸⁾ in 1976 studied long-term cellular and humoral immune response in rats in which bone was allografted. Grafts of complete bone and bone free of marrow elicited both types of immune response and he proposed that transplantation antigens for both types of reactions exists in the bone tissue itself. A gene dose effect was found. In this study, the authors suggested that the graft tissue should be matched to the host for major transplantation antigens to help prevent unexpected failures.

CONCLUSIONS OF THIS STUDY (18)

- 1. Allogeneic bone grafts elicited the specific cellular and humoral responses when transplanted across major histocompatibility barriers.
- 2. Although complete bone gave stronger reactions, marrow free bone grafts also showed specific and high reactivity suggesting that major histocompatibility antigens are present in the bone itself
- 3. There was a gene dose-effect in the response.

4. A hypersensitivity type of humoral immune response was found in rats regrafted with allogeneic bone.

Other researchers who made similar findings were; Bos et al in 1983 (32) and Friendlender in 1983. (35)

PREPARATION TECHNIQUES FOR BONE ALLOGRAFTS (Processing and storage)

Several preparation techniques have been proposed but fresh frozen, freeze dried and gamma irradiation are most common (4,33).

The challenge is to prepare the allografts that are well cleaned, sterile and free of viruses while still preserving the natural biologic and biochemical properties of the tissue. (34)

Other methods of sterilization include boiling and autoclaving, chemicals e.g. Merthiolate solvinor, Benzalkonium chloride, ethylene oxide gas and beta propriolactone antibiotic.

While boiling is inefficient, both boiling and autoclaving cause denaturation of bone proteins ⁽³⁵⁾. High-energy ionizing radiation alters the colours of bone, chemistry, physics and its structure. ⁽³⁶⁾ McAnulty J.F. evaluated the effect of various short-term storage methods on the viability of cancellous bone fragment ⁽⁶³⁾. Among the preservations solutions used were; 0.9% Nacl, phosphate buffered sucrose, eurocollins, V.W. Lactobeonate, hyperionic citrate and blood soaked sponges. The preservation was done at 22°C and also at hyporthermic conditions and storage was for 3 hours.

After warm reperfusion, viability was significantly better for fragments stored in cold phosphate buffered sucrose solution. It was concluded that hypothermic storage in solutions designed to prevent temperature dependent cell injury were best for maintaining cancellous bone fragments viability.

Clinical relevance; hypothermia may be advantageous for use in storing cancellous bone fragments during procedures that dictate prolonged period between harvesting and placement of graft fragments. (37)

Gamma irradiation is one of the common methods used for preparation of bone allografts but gamma irradiation of human bone allografts alters medullary lipids and releases toxic compounds for oesteoblast like cells and hence strongly influences the biocompatibility of the bone graft so defatting procedures should be added when preparing bone allografts in human bone banks. Despite the foregoing irradiation remains the most convenient and acceptable method of bone sterilization. (33)

BIOMECHANICAL PROPERTIES OF BONE ALLOGRAFTS

Pelker R. R. et al in 1983 showed that fleeze drying of bone diminishes its torsional and bending strength without affecting compressive or tensile strength. Irradiation combined with freeze-drying appears to cause a significant reduction in breaking strength ⁽⁴⁰⁾.

EFFECTS OF GAMMA IRRADIATION ON HIV

Fideler B.M. et al ⁽³⁹⁾ conducted a study on frozen bone patellar ligament with bone grafts obtained from infected cadaver.

Several different doses of gamma irradiation were studied ranging from 20,000 to 40,000 grays with respect to the inactivation of the HIV virus in fresh frozen whole bone patellar ligament bone grafts.

Although the international atomic energy agency had recommended the use of 25,000 gray of gamma irradiation for the sterilization of medical products; using polymerase chain reaction (PCR) test this dose was found not to destroy the genes of HIV virus effectively. DNA of the virus was detectable in the DNA of bone marrow tissue obtained from grafts treated with this dose but not in grafts treated with 30,000 to 40,000 gray of gamma irradiation. They concluded that a dose of 30,000 gray of gamma irradiation or more is necessary for the sterilization of fresh frozen patellar ligament - bone allografts so that it can be used for reconstructive procedures without the risk of transmission of the virus to the recipient.

XENOGRAFTS

Though several types of animal bone have been evaluated as an alternative to using allografts, only those commercially prepared from calves have had clinical application.

Calf bone as supplied by Unilab inc (New Jersey USA) is termed Surgibone. Calf bone as supplied by B. Brown Melsung (Germany) is termed Kiel bone.

Successful results (figure not quoted) have been obtained using kiel bone for the calcanean osteotomy of Dwyer. Successful results have also been reported for the use of kiel bone in pelvic osteotomy for hip dysplasia. (40,41) (No figures quoted). Clinical experience using surgibone has been reported for cervical interbody fusion. Surgibone has also been used in the lumbar spine operations. (42,43) (No figures quoted)

Salama R. (1983) has reported experience in the UK using kiel bone-marrow composite (patients own red marrow) grafts in 98 patients. The results were said to be excellent particularly for the treatment of non-union. (45) (No figures quoted)

Prior work done in 1978 by Salama and Weissman showed that the shortcoming of xenogeneic bone grafting is the intense immune rejection that makes its clinical practice limited. (46)

Luo Z. et al ⁽³⁾ did experimental studies of immune response of antigen extracted bovine cancellous bone for grafting. They performed lymphocyte proliferation assay, enzyme linked immunosorbent assay (ELISA) and histological observation to evaluate the levels of humoral and cellular immunity tissue reaction to the grafting of Balbic mice receiving fresh bovine cancellous bone (FCB),antigen extracted bovine granular cancellous bone (GCB) and antigen extracted bovine massive cancellous bone (MCB).

The results suggested that antigen extracted bovine cancellous bone whether granular (GCB) or massive (MCB) showed less antigenicity and can be used as osteoconductive material or as a carrier of bone growth factors.

BONE SUBSTITUTES AND BONE TISSUE ENGINEERING

Due to the shortcomings of;

- autografts (donor site morbidity and limited amounts)
- allografts (infection transmission, difficult processing and sterilization) and
- xenografts (intense immune rejection),

research has shifted to other ways of filling bone defects and promoting union. In the 1970s and 1980s a lot of work was done on bone substitutes and in the 90s and the year 2000 a lot of work has been done and is continuing to be done on bone tissue engineering as shown below.

BONE SUBSTITUTES

Materials that provoke bone repair can be categorised broadly as bone substitutes. The concept of repair may be viewed as the restoration of form and function to deficient osseous tissue.

CLASSIFICATION OF BONE SUBSTITUTES

Bone substitute may be classified broadly into polymers and ceramics. These two broad categories may be sub classified into biodegradable or non-biodegradable.

Bone substitutes may also be classified according to their interaction with living bone at the implant bed (Bio-dynamics) as suggested by Osborne and Newesley (1980). (47)

| Biodynamics | Material | Reaction of implant |
|----------------|---|------------------------|
| bed | | |
| | | |
| 1. Biotolerant | - bone cement | -Distance osteogenesis |
| | - stainless steel | separated by fibrous |
| | | layers |
| | | |
| 2. Bio inert | - Alumina (A ¹ 2 ⁰ 3) | - Contact osteogenesis |
| | - carbon materials | |
| | | |
| 3. Bio active | - Glass ceramics | |
| | - calcium phosphate | -Bonding osteogenesis |
| | ceramics | |
| | - hydroxyapatite ceramics | |

POROUS VERSUS COMPACT MATERIALS

Bone substitutes may be compact or porous. The porous ceramic structures allow in-growth of bone. To create pores in a material the replamine form process has been used, i.e. using coral (from coral reef) to obtain these pores.

Porous materials have been found to be superior to the compact ones since the in-growth of bony tissue in the implant allows good binding of the material to the bone.

POROUS BIODEGRADABLE AND BIOACTIVE CERAMICS

For a long time hard tissue implants (eg of calcium sulphate) were being used with the hope that local release of calcium ions would stimulate osteogenesis.

Later two principle groups of ceramics were studied; these were hydroxyapatite (HA) and tricalcium phosphate (TCP) both from calcium phosphate. Jarcho in 1981⁽⁵¹⁾ concluded that in addition to being unusually well tolerated both porous and dense forms of these materials have demonstrated the ability to become chemically bonded to bone via natural appearing bone cementing mechanisms.

Knaack D. et al ⁽⁵⁸⁾ studied the in Vitro and in Vivo properties of a novel fully resorbable apatitic calcium phosphate (ABS) bone substitute using rabbits. In this study they showed the new bone formation in ABS filled bone defects followed a time course comparable to autologous bone graft filled defects.

The ABS material was greater than 99% resorbed within 26 weeks. Quantitatively and qualitatively the autografts and ABS were associated with similar new bone growth and defect filling characteristics.

Hydroxyapetite ceramics (HA) are widely used in clinical applications as bone substitutes or dental implants because they have been shown to be biocompatible and exhibit excellent oesteoconductivity when grafted into the bone tissue. (49)

Whereas there have been conflicting evidence in the literature whether autologous bone marrow transplantation alone is as effective as the combination of hydroxyapatite ceramics and bone marrow combined, Wipperman et al showed that hydroxyapatite ceramics do improve healing of segmental defects filled with autologous bone marrow (50).

SPECIFIC CERAMICS

Calcium Phosphates

Though calcium phosphates have an enviable record of biocompatibility, the primary troubling issue of tricalcium phosphate (TCP) is an unpredictable biodegradation profile. Moreover TCP biodegradation within bone defects is not routinely accompanied by bone formation.

Tricalcium phosphate (TCP) is biodegradable while hydroxyapatite may be biodegradable or non-biodegradable.

Tricalcium phosphates have been formulated as pastes, particles and discs for bone repair. Tetracalcium phosphates and dicalcium phosphates dehydrate cement is a relatively new composition with clinical appeal.

The two calcium salts are mixed with water to a dense paste and shaped intraoperatively. Working time is approximately 10-15 minutes and as the cement hardens it is converted to microporous hydroxyapatite. (51) Another hydroxyapatite is trorian SRS which is a powder made up of monocalcium phosphate monohydrate, alpha - TCP and calcium carbonate combined with a solution of sodium phosphate. In situ setting time is approximately 10 minutes. (52)

Some products may be combined with autogenous bone marrow for optimal effects. Some hydroxyapatite products e.g. 'tru bone' can be injected directly into osseous defects and then harden in situ.

BIOGLASSES

Bioglasses are silicophosphatic chains that may bond ionically to compounds such as calcium monoxide, copper monoxide and sodium monoxide.

Biomedical devices composed of bioglass can exchange ions or molecular groups with contiguous physiologic milieu. This property may enable bioglass devices to osseointergrate

(i.e. chemically bond to bone). (63)

BONE TISSUE ENGINEERING

Over the years research has moved from using bone grafts to bone substitutes and now to bone tissue engineering.

There are three leading strategies for using bioactive factors for bone tissue engineering (53).

- (i) Extraction and partial purification of growth factors
- (ii) Recombinant protein synthesis and
- (iii) Gene therapy.

It is now accepted that osteoinduction is controlled at least in part by osteogenic bone matrix proteins [which are often referred to as bone morphogenetic proteins (BMPS) or oesteogenic proteins (OP).

These proteins are low molecular weight polypeptides that have been isolated from the bones of a variety of mammalian species including rat,

bovine, monkey and human. (54,55,56,57,) They are also produced by osteogenic sarcoma cell lines (60,61). In recent years oesteogenic proteins have been produced by recombinant DNA method. (55,62)

The recombinantly produced human oesteogenic protein I (OPI) when combined with a bioabsorbable carrier matrix and when introduced at the bony site initiates the recruitment, attachment, proliferation and differentiation of mesenchymal cells leading to new bone formation containing fully functional marrow components. Similar results have been described for bone morphogenetic protein -2 (BMP-2). (71)

These devices potentially can replace conventional autologous bone grafts in repair of nonunion of bone fractures craniofacial defects and spinal fusions. These oesteogenic proteins may also be useful in promoting the osteointegration of metallic implant devices. (71) The oesteogenic implants that have been evaluated have no initial structural properties and will require the combination of allograft bone or synthetic materials in their application. (71)

JUSTIFICATION FOR THE STUDY

Bone grafting plays an important role in management of certain fractures and their complications and also of various orthopaedic conditions. Despite this no local study has been done on this important topic. The need for a study (ies) is increased by the ever increasing road traffic accidents and assaults that are causing serious fractures. The future of orthopaedic practice seems to be in bone substitutes and bone tissue engineering. Therefore we need to evaluate our current practice on bone grafting before we go to bone substitutes and bone tissue engineering.

This study looks at the various aspects of bone grafting (i.e. indications, outcomes, complications, age and sex distribution and involved sites.) as has been practiced in KNH in a period of ten years (1991-2000). It is intended to form a basis that other workers may use to study specific aspects of bone grafting. The study also gives recommendations on the future practice of bone grafting in KNH.

STUDY OBJECTIVES

BROAD OBJECTIVE

To review the practice of bone grafting at KNH over a period of 10 years (1991-2000).

SPECIFIC OBJECTIVES

- 1. To assess the indications for which bone grafting was performed.
- 2. To determine the commonest donor and recipient sites for bone grafts.
- 3. To determine the age and sex distribution in bone grafting.
- 4. To determine outcomes and complications following bone grafting.
- 5. To give recommendations on future practice of bone grafting in KNH.

MATERIALS AND METHODS

STUDY DESIGN

This was a retrospective study from January 1991 to December 2000.

STUDY POPULATION

The study was conducted on patients for whom bone grafting was done in KNH between January 1991 and December 2000.

SAMPLE SIZE/INCLUSION CRITERIA

All patients for whom bone grafting was performed in the study period and whose records were available, legible and complete.

EXCLUSION CRITERIA

- 1. Patients for whom bone grafting was performed but whose records were unavailable.
- 2. Those whose records were illegible or mutilated.

STUDY METHODOLOGY

The study was conducted by the principal investigator under the guidance of the supervisor from the department of orthopaedic surgery University of Nairobi. The instruments comprised of the medical records and radiographs of patients who had undergone bone grafting during the study period.

The investigator studied the records in order to determine the indications of bone grafting, the donor and recipient sites and the complications encountered. The questionnaire had all the demographic data including name, age, sex, district of residence and occupation.

The investigator used the theatre register and KNH records department coding books to get the details of the files to be retrieved. For files retrieved, the investigator went through the details of:- Patient's demographic data, diagnosis, preoperative care, operation notes, postoperative care and complications. Available radiographs were also examined.

LIMITATIONS OF THE STUDY

- 1. Incomplete data due to loss of patients to follow up.
- 2. Failure by surgeons to write detailed surgical notes indicating donor and recipient sites of grafts and also types of grafts (i.e. chip grafts, segmental grafts etc).
- 3. Where bone grafting was done as an adjunct to an implant or in combination with another operation, the coding was for the other operation (Examples ORIF, plating, K-nail) and bone grafting was just given a mention in the details of the operation notes. This necessitated the investigator to go through many files before getting the desired ones.

ETHICAL CONSIDERATIONS

Permission to carry out the study was sought from the Kenyatta National Hospital Ethical and Research Committee and approval was given.

Necessary confidentiality was maintained when conducting the study and the information obtained was only used for the intended purpose.

DATA MANAGEMENT AND ANALYSIS

The data was recovered from patients' records using a data sheet. It was then carefully monitored and entered into IBM compatible computer.

Analysis was carried out using SPSS (version 9.0) computer software programme and results presented in form of tables and figures.

DATA PRESENTATION

Data is presented in tabular and geographical forms, Bar charts and pie charts are used as necessary

RESULTS

This was a retrospective study between January 1991 and December 2000. One hundred and eighty nine patients files were retrieved from the medical records department, Kenyatta National Hospital and the following results obtained.

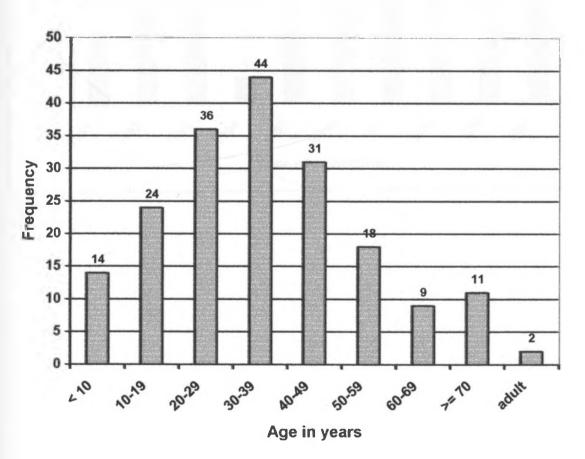
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AGE DISTRIBUTION

The age range was 2 years to 95 years with a median age of 33 years.

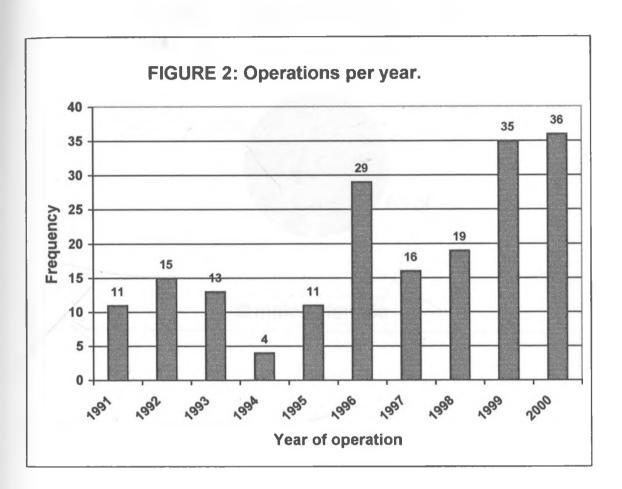
Most patients (135 out of 189 or 71.4%) were between 10 and 49 years.

FIGURE 1: Age distribution



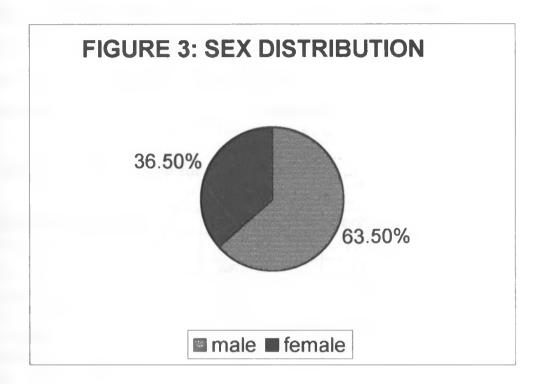
NUMBER OF PATIENTS OPERATED PER YEAR

The average number of patients operated per year was 18.9 (Approximately 19 operations). The fewest patients (4) were in 1994 while the most (36) were in the year 2000.



SEX DISTRIBUTION

Males (120 or 63.5%) were more than females (69 or 36.5%). The ratio being male: female 1.7:1



INDICATION FOR OPERATION

The commonest indication for operation was non-union with 66 cases (33%) followed by joint arthrodesis with 40 cases (or 20%). The category labelled 'others' included; congenital pseudoarthrosis (2 cases or 1%), bone tumours without fractures (3 cases or 1.5%), broken plate (1 case or 0.5%), kienbock's disease (1 case or 0.5%), delayed union (3 cases or 1.5%) and brodies abcess (1 case or 0.5%).

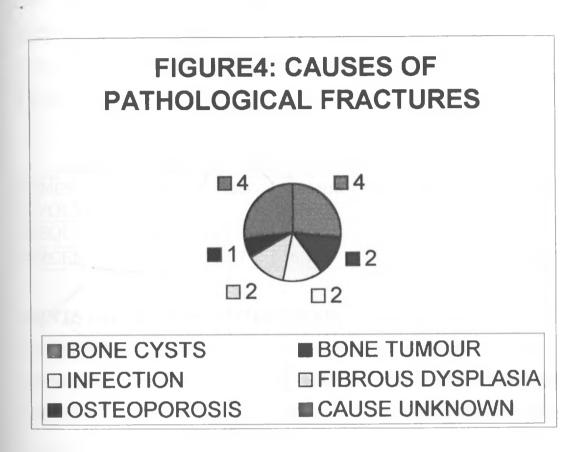
TABLE 1: - Indication for operation

| Reason for operation | Frequency | Percent |
|------------------------|-----------|---------|
| Fresh fractures | 31 | 15.5 |
| Non-union | 66 | 33 |
| Joint arthrodesis | 40 | 20 |
| Mal union | 20 | 10 |
| Pathological fractures | 15 | 7.5 |
| Bone cysts without | 7 | 3.5 |
| fractures | | |
| Bone defect/loss | 5 | 2.5 |
| Arthroplasty | 5 | 2.5 |
| Others | 11 | 5.5 |
| Total | 200 | 100 |

^{*} The total adds up to 200 (> 189) since some patients had more than 1 indication.

CAUSES OF PATHOLOGICAL FRACTURES

There were 15 cases of pathological fractures for which bone grafting was performed. The causes of these pathological fractures are as shown in the figure below.



BONES INVOLVED IN NONUNION

The femur was the bone with the most cases of nonunion with 25 cases (or 37.9%), followed by the tibia with 15 cases (or 22.7%).

There were 11 cases (or 16.7%) of nonunion of forearm bones. In 6 of these, both radius and ulna were involved, in 3 only the radius and in 2 only the ulna was involved.

In the category of 'others' were; clavicle 2 cases, fibula 2 cases, phalanx 1 case.

TABLE 2: Nonunion - bones involved

| BONES | Femur | Tibia | Forearm | Humerus | others | Total |
|-----------|-------|-------|---------|---------|--------|-------|
| INVOLVED | | | bones | | | |
| FREQUENCY | 25 | 15 | 11 | 10 | 5 | 66 |
| PERCENT | 37.9 | 22.7 | 16.7 | 15.2 | 7.5 | 100 |

JOINTS INVOVED IN ARTHRODESIS

In cases where bone grafting was performed during arthrodesis, the ankle joint was the most commonly involved joint (22 cases or 55%). This was followed by knee joint (4 cases or 10%), and metartarsal phalangeal joint (3 cases or 7.5%). Other joints are as shown below.

TABLE 3: Arthrodesis- joints involved

| ITEDLE | TABLE 5. THE MODE SISTEMAN AND THE SISTE | | | | | | | |
|-----------|--|------|------|----------|-------|-------|--------|-------|
| JOINT | Ankle | Knee | MTP. | Subtalar | Elbow | Spine | Others | Total |
| FREQUENCY | 22 | 4 | 3 | 2 | 2 | 2 | 5 | 40 |
| PERCENT | 55 | 10 | 7.5 | 5 | 5 | 5 | 12.5 | 100 |

^{*}MTP - Metatarsalphalangeal

BONE CYST SITES (BONES INVOLVED)

Eleven patients who were diagnosed to have bone cysts underwent bone grafting procedures. four of these (or 36.4%) were in the neck of femur followed by 3 cases (or 27.3%) in the humerus. The other involved bones were the tibia, radius and ulna as shown below.

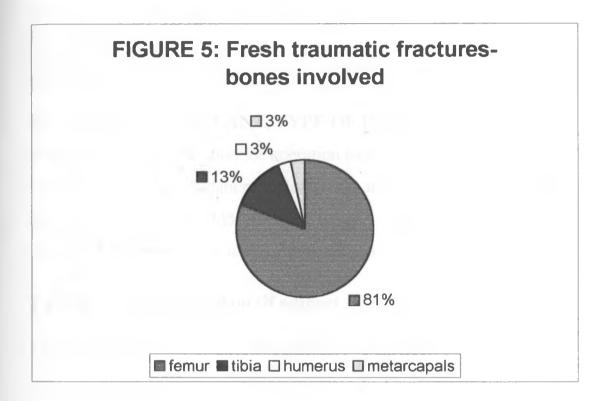
TABLE 4: Bone cysts- bones involved

| BONES | femur | humerus | tibia | ulna | radius | total |
|-----------|-------|---------|-------|------|--------|-------|
| INVOLVED | | | | | | |
| FREQUENCY | 4 - | 3 | 2 | 1 | 1 | 11 |
| PERCENT | 36.4 | 27.3 | 18.1 | 9.1 | 9.1 | 100 |

^{*} Four of these patients had sustained pathological fractures while 7 had not.

BONES GRAFTED FOR FRESH TRAUMATIC FRACTURES

Thirty one cases of fresh traumatic fractures underwent bone grafting operations as adjuncts to other implants. The femur contributed 25 cases (or 81%) while all the other bones contributed only 6 cases (or 19%).



^{*}Percentages are rounded off to whole numbers.

BONES INVOLVED IN MALUNION

Twenty cases of malunion underwent osteotomy and bone grafting. All these cases involved the long bones as shown in table below.

TABLE 5: MALUNION – BONES INVOLVED

| BONES | Femur | tibia | ulna | radius | humerus | fibula | total |
|-----------|-------|-------|------|--------|---------|--------|-------|
| INVOLVED | | | | | | | |
| FREQUENCY | 5 | 4 | 4 | 3 | 3 | 1 | 20 |
| PERCENT | 25 | 20 | 20 | 15 | 15 | 5 | 100 |

IF ADJUNCT TO IMPLANT, TYPE OF IMPLANT

Irrespective of the indication for operation most bone graft operations involved one type of implant or the other, the most common being plates and screws (88 cases or 44%). Only 36 cases or 18% of the operations involved bone grafts alone without an additional implant.

TABLE 6: Type of implant (if adjunct to implant)

| Type of implant | Frequency | Percent |
|--------------------------|-----------|---------|
| None | 36 | 18 |
| Plates and screws | 88 | 44 |
| Nails | 17 | 8.5 |
| Wires | 15 | 7.5 |
| Screws | 21 | 10.5 |
| Rush pins | 6 | 3 |
| External fixators | 3 | 1.5 |
| Chanley clamps | 3 | 1.5 |
| Hip prosthesis | 3 | 1.5 |
| Plates, screws and wires | 7 | 3.5 |
| Wires and screws | 1 | 0.5 |
| Total | 200 | 100 |

^{*} The total adds to $200 \ (> 189)$ since some patients had more than one indication.

RECIPIENT SITE

The most commonly grafted site was the femur (69 cases or 34.5%) followed by the tibia (34 cases or 17%). The other sites are as shown below.

TABLE 7: Recipient sites

| RECIPIENT SITE | FREQUENCY | PERCENT |
|----------------|-----------|---------|
| Femur | 69 | 34.5 |
| Tibia | 34 | 17 |
| Ankle joint | 22 | 11 |
| Forearm bones | 22 | 11 |
| Humerus | 19 | 9.5 |
| Acetabulum | 8 | 4 |
| Knee joint | 4 | 2 |
| Other joints | 14 | 7 |
| Other bones | 8 | 4 |
| total | 200 | 100 |

^{*} Total (200) exceeds patients operated (189) since some patients had more than one recipient site.

In the category of "other joints" were; spine, metatarsalphalangeal, subtalar, elbow, interphalangeal, hip and wrist joint.

In the category of "other bones" were; clavicle, lunate bone metatarsals and phalanges.

DONOR SITE

Bone was harvested from the iliac crest in 75 cases or 47.8% and from the tibia in 30 cases or 19.1%. Donor site was indicated in the operation notes in 154 cases only and was not indicated in 35 cases.

TABLE 8: Donor site

| Donor bone | Frequency | Percent |
|----------------------------|-----------|---------|
| Iliac crest | 75 | 47.8 |
| Tibia | 30 | 19.1 |
| Callous/ osteotomized bone | 27 | 17.2 |
| Femur | 8 | 5.1 |
| Fibular | 8 | 5.1 |
| Radius | 4 | 2.6 |
| Patella | 2 | 1.3 |
| Phalanx | 1 | 0.6 |
| Metetarsal | 1 | 0.6 |
| Rib | 1 | 0.6 |
| Total | 157 | 100 |

^{*} Total number of donor sites (157) for autografts exceeded total number of patients (154) since some patients had multiple donor sites.

COMPLICATIONS

Of the 189 cases reviewed 142 patients (or 75.1%) had no recorded complications. The ones who developed complications (47 patients or 24.9%) were divided into those involving the donor sites and those involving the recipient sites.

TABLE 9: Donor site complications

| 1718 EE 27 B OHOT Site Comprise to the | | | | | | | |
|--|------|-----------|----------|-----------|-------|-------|--|
| COMPLICATIONS | Pain | Infection | Bleeding | Haematoma | Nerve | Total | |
| 1 | | | | | palsy | | |
| FREQUENCY | 19 | 3 | 3 | 2 | 1 | 28 | |
| PERCENT | 67.9 | 10.7 | 10.7 | 7.1 | 3.6 | 100 | |

TABLE 10: Recipient site complications

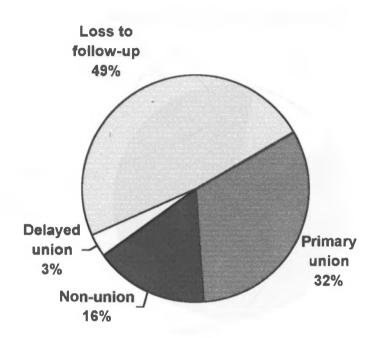
| CO | MPLICATIOS | Infection | Pain | Bleeding | Haematoma | Total |
|-----|------------|-----------|------|----------|-----------|-------|
| FRI | EQUENCY | 17 | 11 | 1 | 1 | 30 |
| PEI | RCENT | 56.7 | 36.7 | 13.3 | 13.3 | 100 |

^{* 11} patients had both donor and recipient site complications.

OUTCOME OF BONE GRAFTING FOR FRESH TRAUMATIC FRACTURES

There were 31 cases of fresh traumatic fractures. Ten cases or 32% achieved primary union, 5 cases or 16% developed non-union, 1 case or 3% developed delayed union while 15 or 49% were lost to follow up.



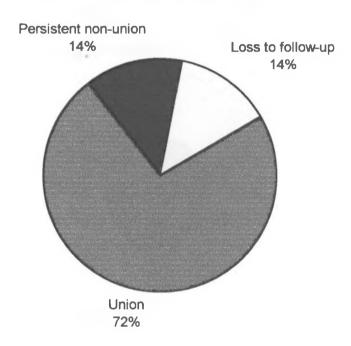


^{*}Percentages are rounded off to whole numbers.

OUTCOME OF BONE GFAFTING FOR NONUNION

Out of the 66 cases of non-union which had bone grafting operation, 48 cases or 72% union was achieved, 9 cases or 14% continued to have persistent non-union despite the grafting and 9 cases or 14% were lost to follow up.

FIGURE 7: Outcome of Non-union

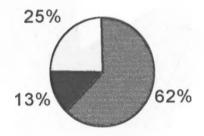


^{*}Percentages are rounded off to whole numbers.

OUTCOME OF BONE GRAFTING FOR JOINT ARTHRODESIS

There were 40 cases of joint arthrodesis, in 25 cases or 62 % joint fusion was achieved, in 5 cases or 13% there was failure of joint fusion and 10 cases or 25% were lost to follow up.

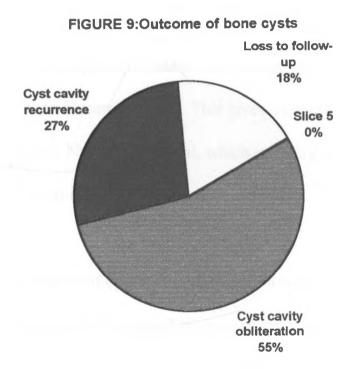




■ joint fusion ■ failure of joint fusion □ loss to follow up

OUTCOME OF BONE GRAFTING FOR BONE CYST

Eleven cases of bone cysts had bone grafting operation, 6 cases or 55% had obliteration of the cyst, 3 cases or 27% cyst cavity persisted or recurred and 2 cases or 18% were lost to follow up.



^{*}Percentages are rounded off to whole numbers.

DISCUSSION

This is the first local study on bone grafting and it is meant to form a basis which other researchers may use to study specific aspects of bone grafting. Due to the foregoing, there are no other local studies to compare with.

There were 189 patients who had bone-grafting operations between

January 1991 to December 2000. This gives an average of 19 patients per
year in Kenyatta National Hospital, which is a national referral and
teaching hospital in Kenya.

The lowest number of operations was in 1994 with only 4 cases and the highest in 2000 with 36 cases. This may be explained by the fact that in 1994 there was a doctor's strike reducing the number of elective operations done. In the year 2000 the records department in K.N.H computerized the coding system improving retrieval system. The year 2000 had 36 retrieved files, the most in the series.

From the many varied indications shown in this study for which bone grafting was done, it is clear that bone grafting is not a disease or a

condition- specific operation rather it may be used in many different bone and joint pathologies /conditions. This great value of the bone graft in the field of orthopaedic surgery was shown by Fred Albee when he enumerated the different general and specific indications of bone grafts.⁽¹⁾

Those indications compare well with the ones identified in this study.

AGE

In this study, the age of patients who underwent bone grafting operations ranged from 2 years to 95 years with a median age of 33 years. One hundred and thirty five patients (or 71.4%) were between 10 and 49 years.

The youngest patient was 2 years old who developed pathological fracture secondary to chronic osteomyelitis of the right femur while the oldest was 95 years old who developed traumatic fracture of upper third of right femur. In 2 cases the age of the patient was simply recorded as adult.

Eleven patients (or 5 .8%) were 70 years old and above while 14 patients (or 7.4%) were under 10 years. These are problematic ages; in the young due to inadequate donor sites and in the old due to osteoporosis. (1, 2)

No study was found showing the age distribution for overall bone grafting operations, but in a study by Buckley P. D. et al on bone grafting for atraumatic avascular necrosis of the femoral head the patients ranged from 31 to 55 years. ⁽⁷³⁾ In another study by Kumta S. M. on vascularized bone grafting for fibrous dysplasia of the upper limb the age range was 17 to 36 years. ⁽⁷⁴⁾ Several studies have also been published dealing with bone grafting in children with congenital scoliosis. ⁽⁴⁾

SEX

Most of the patients requiring bone grafting were male (120 cases or 63.5%) compared to females (69 cases or 36.5%) giving a ratio of male: female 1.7:1.

This can be explained by the fact that the main indications for grafting were nonunion, arthrodesis and fresh traumatic fractures all of which mainly started as trauma cases which are more in males than in females. (66)

INDICATION FOR OPERATION

In this study 189 patients underwent bone grafting operations for more than ten different indications (TABLE 1), some were general and others were specific indications. Fred Albee having done over 400 bone grafting operations listed 10 general and 13 specific indications. The indications in this study compare well with Albee's list. (1)

There were 66 cases (or 33%) of nonunion, this was the commonest indication for bone grafting. Sixty two of these cases followed traumatic fractures while 4 followed pathological fractures.

The bone, which had the highest incidence of nonunion in this study, was the femur (37.9%) followed by tibia (22.7%). In one series Boyd and others studied 842 patients with a total of 1013 bone grafting operations. In that series the bone with the highest incidence of nonunion was the tibia (35%). In a later series, they studied 122 patients with 146 bone grafting operations. In that series the bone with highest incidence of nonunion was the femur (31%) followed by the tibia, humerus, radius and clavicle in that order. (75) This compares well with the current study.

In the current study there were only two cases of bone grafting for spinal fusion as compared to study done by Glenn et al in 1996 in California where there were 63 cases of spinal fusion procedures. ⁽⁴⁾ The two spinal fusion cases in the current study were due to TB spine whereas in the California study all the cases were due to spinal deformities, mainly scoliosis. Many other studies in the USA show that the indication for bone grafting during spinal fusion is mainly spinal deformities. ⁽⁴⁾

In the current study 31 cases of fresh traumatic fractures had bone grafting during primary fixation. Twenty five of the 31 cases (or 80.7%) involved the femur. Ruedi and Luscher studied 131 communited fractures of the femur which they fixed with AO plate technique. In 9 patients, the plate bent or broke and in 2 there was a refracture after plate removal. These researchers recommended the routine use of a bone graft medially in all severely communited fractures fixed with AO plates. (78) Sisk T. D. et al recommended the addition of cancellous bone grafts to severely communited fractures when the communition involved greater than one third of the circumfrence of bone. (72) In the current study all the 25 femoral fractures had communition.

There were in total 11 cases of bone cysts that underwent bone grafting operations. Out of these, 4 patients had presented with pathological fractures due to bone cysts while the other 7 had presented with other symptoms and the diagnosis of bone cysts made radiologically then confirmed histologically after operation. The operation done for all these cases was currettage and then the cavities were packed with bone grafts. This was what Henry L. and Brashear H. R. recommended in their different studies. (64, 65)

There were a total of 5 patients who had bone loss or defect. These were difficult to manage because the grafts harvested were inadequate to bridge the gaps. These are the kind of cases which may have benefited from segmental allografts or vascularised segmental autografts since the amount of bone required to bridge large gaps may be difficult to get as free autografts. (2)

There were 5 cases of bone tumours, 2 of which resulted in pathological fractures while 3 did not. The later 3 were histologically confirmed as giant cell tumour of bone. The histology results of the former 2 were not available. Bone grafting for bone tumour surgery is much commoner in

the USA as evidenced by a study done by Enneking W. F. et al on 40 patients. ⁽⁶⁹⁾

There were 20 cases of malunion. In most of these the osteotomized bone was used as bone graft with or without an added implant. The recommended method is to use the osteotomized wedge as a whole or to cut it into small fragments. (76)

The other indications included;

Arthroplasty- 5 cases (all 5 cases were of hip joint), broken plate-1 case, Congenital pseudoarthrosis of tibia- 2 cases, protrusio acetabulurale- 1 case, brodies abcess- 1 case, delayed union- 3 cases, kienbock's disease-1 case. The two cases of pseudoarthrosis of the tibia were difficult to manage. One had undergone 3 operations and the other, 2 operations and in both patients union was not achieved. It has been shown that an established pseudoarthrosis usually involves several operations and prolonged splintage before success results. (77)

The patient who had kienbock's disease had bone grafting and one year later he still had pain. In this patient, the donor bone for the graft was the distal radius and the patient developed fracture of the radius later at the donor site.

IF ADJUNT TO IMPLANT, TYPE OF IMPLANT

Of the 200 bone grafting operations in 189 patients, 36 (or 18%) had grafting alone without any added implant.

Plates and screws were the implants, which were most commonly used together with bone grafts (88cases or 44%), this was followed by screws without plates (21 cases or 10.5%) and intramedullary nails (17 cases or 8.5%). In the previously quoted study Ruedi and Luscher recommended routine use of bone grafts on severely communited femoral fractures fixed with AO plates. (75)

RECIPIENT SITE

The researcher came across no other study comparing the different recipient sites but in this study the femur with 69 cases (or 34.5%) was the most frequent recipient site followed by the tibia in 34 cases (or 17%) and the ankle with 22 cases (or 11%) respectively.

It may be concluded that the femur is the most frequent recipient site because it commonly gets involved in both traumatic and pathological fractures and its nonunion rates are some of the highest. (66,72,75)

DONOR BONE / DONOR SITE

In the current study only in 154 of the 189 patients operated did the operation notes indicate the donor bone/site. These 154 patients had 157 donor sites (some patients had more than one donor site) and these were all autografts. Only in one case of a 10 year old boy who had pathological fracture of the femur secondary to bone cyst was the graft indicated to be an allograft. The notes indicated the source to have been the head of femur from a different donor but no other details were given.

These results differ significantly from the practice in many centers in USA and Europe where allografts are regularly used. (31,40,48)

In the current study, the iliac crest acted as the donor site in 75 cases (or 47.8%) making it the most used donor site. This compares well with studies done elsewhere which showed the iliac crest to be one of the most commonly used donor site for autografts. (4,66,72) The second most commonly used donor site was the tibia in the current study, with 30 cases (or 19.1%). In 1999 Alt V. and others did a study on bone grafting where bone grafts were harvested from the proximal tibia. 54 patients were enrolled in this study. The researchers noted the following;

- The amount of bone that could be harvested was more than adequate.

- The patients could start immediate post- operative weight bearing.
- The complication rates were low(only 1.9% in this study) and that higher complications rates had been noted for iliac graft sites. (17)

In this study, the iliac crest and the tibia together contributed 66.9% of all donor sites for bone grafts. Unfortunately some surgeons in their operation notes did not indicate which part of the tibia was used as the donor site making it difficult to know whether the graft was cortical, cancellous or corticocancellous.

In 27 patients (or 17.2%), the source of the bone grafts was callous or locally osteotomized bone. This was mostly in patients with hypertrophic nonunion and those with malunion. This reduced the donor site morbidity since the donor and the recipient sites were in the same area. Furguson, Thomas F. B. described a method of using locally osteotomized bone as grafts in malunion. ⁽⁷⁶⁾

In 3 cases the head of femur was crushed and used as bone graft. This was done in patients undergoing hip replacement who also required bone

grafting. This has the advantage of being a readily available graft, which would have otherwise been thrown away. It also avoids the problem of donor site morbidity.

The other donor bones /sites included; distal femur- 8 cases, fibular 9-cases, distal radius- 4 cases, patella 2- cases and then ribs, metatarsal and phalanx 1 case each. The patella was crushed and used as bone graft in 2 patients who were undergoing knee arthrodesis. One was a 16 year old girl who had severe knee injury following a road traffic accident. This patient was lost to follow up. The other was a 60 year old man with oesteoathritis, here joint fusion was achieved. In 35 cases (or 18.5%) the surgeons in the current study did not indicate in the operation notes the source (donor site) of the graft.

There was no case found on record in the current study where bone substitutes or bone tissue engineering were used. Bone substitutes are now increasingly being used in some centres instead of or in conjunction with autogenous bone grafts. (49,50,51) Preclinical and early clinical trial results using bone morphogenetic protein-2 (i.e. bone tissue engineering)have already been obtained. (48,53)

OUTCOME OF BONE GRAFTING FOR NONUNION

Out of the 66 cases of nonunion which were managed by bone grafting, union was finally achieved in 48 cases (or 72%) and in 9 cases (or 14%) there was persistent nonunion. Only 43 (or 65%) of the 66 nonunion cases achieved union after first operation while 5 cases required 2 or more operations to do so. Nine cases (or 14%) were lost to follow up. The union rate in this study was slightly lower than in a study done by Boyd et al.

In this later study Boyd et al assessed the results of treatment of nonunion of 842 patients involving different bones and treated by different methods using autografts. Of the 842 patients, 790 (or 94%) eventually obtained union. In that study 64 patients required 2 or more operations to do so and in some up to 4 operations were done before union was finally achieved. The union rate following the first operation was 88% (or 739 out of 842). (75)

In the current study there were 2 cases of infected nonunion both of femur, one case was lost to follow up and in the other nonunion persisted.

Generally the Management of infected non-union is a challenge to the surgeon. Chan Y.S. et al advocated the use of antibiotic impregnated

autogenic cancellous bone graft ⁽¹⁵⁾ but different people have advocated different methods. ^(2,14,15,16)

OUTCOME OF BONE GRAFTING FOR ARTHRODESIS

Twenty five (or 62%) of the 40 cases of joint arthrodesis resulted in fusion while in 5 (or 13%) fusion failed and 10 cases (25%) were lost to follow up. There were no comparable studies in literature on the outcome of bone grafting for joint fusion.

In 2 cases, arthrodesis of the ankle was done using onlay bone graft without the addition of an implant. In both these cases fusion failed and repeat operations were done with implants and bone chips. Fusion was finally achieved in both.

For the 2 cases of spinal fusion there was no comment on the files during follow up on whether or not fusion was achieved and the X-rays could not be traced. In this study it was noted that the use of bone graft for ankle joint fusion has increased as the use of chanley clumps has decreased.

OUTCOME OF BONE GRAFTING FOR BONE CYSTS

Only 6(or55%) of the 11 operated cases of bone cysts had obliteration of the cyst cavity, 3 cases (or 27%) had recurrence or persistence of the cyst cavity and 2 cases (18%) were lost to follow up.

These results were in keeping with studies done elsewhere. Henry L. quotes a recurrence rate of (40 - 50) % while Brashear JR quotes a recurrence rate of 25%. (64, 65)

OUTCOME OF BONE GRAFTING FOR FRESH TRAUMATIC FRACTURES

There were 31 cases of fresh traumatic fractures that underwent bone grafting which were retrieved from the records department of KNH.

Among the 31 cases, 15 patients(or49%) got lost to followup, 10 (or 32%) achieved primary union, 5 (or16%) developed non union, 1 case (or 3 %) had delayed union and no case of refracture was recorded. The reason for the high rate of nonunion could be because the cases that had primary bone grafting done were the difficult ones with severe communition and/ or bone loss.

This apparently high rate of non-union may also be because of the many patients (15 out of 31) who got lost to follow up. If one looked only at the patients who got followed up (16 patients) the union rate would have been 63% (10 out of 16), non-union 31% (5 out of 16) and delayed union 6% (I out of 16).

Other studies done on fresh traumatic fractures include; a study by Ruedi and Luscher on 103 closed communited femoral fractures (study quoted previously), study by Magerl et al on 67 femoral shaft fractures and a study by Pearlman et al on 85 femoral shaft fractures. (78, 79, 80) In these three studies the researchers used plates and screws without bone grafts and reported excessive complication rates. Their complications included; bent or broken plates, refractures, nonunions and infections. This led Ruedi and Luscher to recommend routine application of bone grafts medially in all communited femoral fractures fixed with AO plates. The researchers do not show their results after they used bone grafts. (78) McBirne and others described a technique of open reduction, bone grafting and fixation with a single kitchner wire of fresh unstable fractures of the distal radius. (30)

COMPLICATIONS

In 142 (or 75.1%) of the 189 patients studied in the current study, there were no recorded complications. Twenty eight (or 14.8%) of the patients developed donor site complications while 30 patients (or 15.9%) developed recipient site complications. Eleven patients had both donor and recipient site complications. In a study done by Enneking et al where they used autogenous cortical bone grafts in the reconstruction of segmental skeletal defects, 3 out the 40 patients (or 7.5%) developed donor site morbidity associated with graft procurement. The recipient site complications were not mentioned. (69)

Among the donor site complications in the current study, donor site pain was the most frequent with 19 out of 28 patients (or 67.9%). This is in keeping with studies done elsewhere which showed donor site pain to be one of the most common complications. (4) Infection was the most frequent recipient site complication with 17 patients out of 30 (or 56.7%) patients developing recipient site infection. This could be attributed to longer operation time associated with bone grafting procedures together with other unrelated factors like theatre conditions, aseptic technique and others.

Lim E.V et al gives a case report of superior gluteal artery injury during iliac bone graft harvesting. (19) No such complication was encountered in the current study. Complications encountered in the current study included; donor site- infection, haematoma, bleeding and nerve palsy recipient site- infection, pain bleeding and haematoma. These complications are similar to those quoted in different studies. (1,2,17,18,19)

In one study by Kim SJ et al on 8 patients of delayed union and non-union no complications were recorded. These researchers did these bone grafting procedures endoscopically. (67) No case of endoscopic bone grafting was found on record in the current study.

CONCLUSIONS

- 1. There were many varied indications of bone grafting but the commonest was non-union (33%).
- 2. Most patients requiring bone grafting were in the productive age group (10-49) years.
- 3. There were more males than females undergoing bone grafting procedures Ratio Male: Female 1.7:1.
- 4. In most cases (82%) bone grafting was done as an adjunct to another implant .
- 5.Plates and screws were the most common implants (44%) used in conjunction with bone grafts.
- 6. The most commonly grafted bone/site was the femur (34.5%) followed by the tibia (17%).
- 7. The iliac crest was the most utilized donor site (47.8%) followed by the tibia (19.1%).
- 8. Free autografts are almost exclusively the types of bone grafts used at K.N.H.
- 9. The most commonly encountered complication are donor site pain and recipient site infection.
- 10. In most of the patients undergoing bone grafting for nonunion, union was achieved.
- 11.In about a quarter of the patients undergoing bone grafting for bone cysts, there was recurrence.
- 12. Following bone grafting arthrodesis was achieved in about two thirds of the patients.
- 13. There were many patients who were lost to follow up ranging from 14% to 49% for different indications.

14. For fresh traumatic fractures the assessment of outcome is heavily biased by the many patients who were lost to follow up (49%).

RECOMMEDATIONS

- There is need for surgeons to make clear notes concerning the
 indication for grafting, the donor and the recipient sites of grafts
 and the complications encountered: This will help in research and
 hence improve future practice.
- 2. There is need for other researchers to do studies on specific indications of bone grafting. These will clearly bring out the issues, which may not have been resolved by this study and will help set specific guidelines for the specific indications
- 3. Mechanisms need to be devised to improve patients' follow up since there are too many patients getting lost to follow up making it difficult to assess outcome well.
- 4. Pain should be specifically and adequately managed in patients undergoing bone grafting procedures since donor site pain was the commonest complication encountered in this study.

APPENDIX

DATA COLLECTION FORMAT

| Study | y code no | |
|-------|---|-------|
| 1 | Name | |
| 2 | I.P. No | |
| 3 | Age | |
| 4 | Sex | |
| 5 | District of residence | |
| 6 | Occupation | |
| 7 | Date of admission | |
| 8 | Date of operation | |
| 9 | Date of discharge/death | , |
| 10 | 0 Reason for operation . Yes = 1 | No =2 |
| | Fresh traumatic Fracture Non union Bone tumour Bone cysts Infection Joint arthrodesis Other (specify) | |
| 11 | 1. If Arthrodesis, joints involved. Yes=1 | No=2 |
| | Ankle joint Knee joint Hip joint Others –specify | |

| Hip joint Knee joint Shoulder Others - specify | | |
|---|-------|------|
| 13.If adjunct to implant, type of implant | Yes=1 | No=2 |
| Plates and screws | | |
| 14.Bones grafted (=recipient site) | Yes=1 | No=2 |
| Tibia Femur Humerus Forearm bones Spine Others (Specify) | | |
| 15. Type of bone graft | Yes=1 | No=2 |
| Autograft | | |
| 16.If autograft, donor bone | Yes=1 | No=2 |
| Radius Iliac crest Tibia Fibular Others (Specify) | | |

12. If arthroplasty, joints involved Yes= No=2

| 17.Complications | Yes=1 No=2 | |
|--|--------------------|---------------|
| Donor site pain Donor site haematoma Donor site Bleeding | | |
| Infection of donor site | | |
| Infection of recipient site | | |
| Graft fracture Others(Specify) | | |
| 18.Outcome of bone grafting for nonunion Union Persistent nonunion Lost to follow up | | No=2 |
| 19.Outcome of bone grafting for bone cys | sts. Yes=1 N | Ion=2 |
| Obliteration of cyst cavity | | |
| 20. Outcome of bone grafting for fresh fra | actures. Yes=1 | No=2 |
| Union Delayed union Nonunion Lost to follow up | | |
| 21. Outcome of bone grafting for joint arth | nrodesis. Yes=1 | No=2 |
| Joint fusion achieved | | |
| Lost to follow up | to arouted by reco | otion of hone |
| 22.Outcome of bone grafting of defect tumours. | is created by rese | ction of bone |
| Yes=1 No=2 | | |
| Primary union achieved | • | |

REFERENCE

- 1. Fred Houdlette Albee . Bone graft surgery . Clinical Orthopaedics And related research 1996; 324:5-12.
- 2. Hughes, S.P.F., Benson, M. K., D'A., Colton, C.L. Bone grafting. The principles and practice of musculoskeletal surgery. Churchill Livingstone. First edition. 1987: 500 535.
- 3. Luo, Z., Hu, Y., Wang, Q. The experimental studies of immune response of antigen extracted bovine cancelous bone grafting. Chung-hua Waikotsa Chi. 1997; 35: 11.
- 4. Glenn, R., Buttermann, M. D., Paul, A. et al. The use of bone allografts in the spine. Clinical orthopaedics and related research. 1996; 324: 75 –85.
- 5. Friedlaender G. E. Editorial comment. Clinical orthopaedics and related research. 1983; 174:2 4.
- Reddi, A. H., Weintroub, S., Muthukuruman, W. Biological principles of bone induction. Ortho. Clinic worth. (AM). 1987; 18: 207 212.
- 7. Urist, M.R. Practice application of basic research on bone graft physiology. Instruction course Lact 1976. 25: 1-26
- 8. Urist, M. R., and Iwata, H. Preservation and biodegradation of morphogenetic property of bone matrix. J. Theory Bioi 1923; 38: 155-167.
- 9. Vukicevic, S., Legin, V., Che, P. et al. Localization of osteogenic proteins (bone morphogenetic protein 7) during human embryonic development; High affinity binding to basement membranes. Biochem. Biophys. Pes common. 1996; 18: 693 700.
- 10.MacEwen, W. Observation concerning transplantation of bone illustrated by a case of interhuman osseum transplantation whereby

- over two thirds of the shafts of human are stored. Proceedings of royal society London. 1881; 32: 232.
- 11.Burwell R.G. A study of homologous red marrow after transplantation to a muscular site. Journal of anatomy. (London). 1961; 95: 613.
- 12. Phemister, D. B. Split grafts in the treatment of delayed and non-union of fracture, surgery, gynaecology and obstetrics. 1931; 52: 376 381.
- 13. Mowlem, R. Bone and cartilage transplants, their use and behaviour. British Journal of surgery. 1941: 29: 182 193.
- 14.Lei, H. and Yi, L. One stage often cancellous bone grafting of infected fracture and nonunion. J. Orthop. Sci (Japan). 1998; 3 (6): 318 23.
- 15.Chan Y. S., Ueng, S.W., Lee, S. S., ET AL. management of small infected tibial defects with antibiotic-impregnated autogenenic cancellous bone grafting. J. trauma (United States). 1998; 45 (4): 758-64.
- 16.Ueng, S.W., Wei, F. C., shih, C. H. Management of femoral diaphyseal infected nonunion with antibiotic beads local therapy, external skeletal fixation and staged bone grafting. J. Trauma (United states). 1999; 46 (1): 96 103.
- 17.Alt, V., Nawab, A., Seligson, D. Bone grafting from the proximal tibia. J. trauma (United States). 1999; 47(3): 555 7.
- 18. Musculo, D. L., Kawai, S., Ray, R. D. Cellular and humoral response analysis of bone allografted rats. J. Bone Joint Surg. (AM). 1976; 58(6): 826 32.

- 19.Lim, E. V., Lavadia, W.T., Roberts, J.M. Superior gluteal artery injury during iliac bone grafting for spinal fusion: a case report and literature review. Spine (United States). 1996; 21(20): 2376 8.
- 20. Mowlem, R. Cancellous Chip bone grafts report on 75 cases. Lancet ii. 1994: 746 748.
- 21. Mors, W., Kaminiski, E. Oesteogenic replacement of tricalcium phosphate ceramic implants in the dog plate. Arch Oval trot. 1975; 20: 265 376.
- 22. Stenovic, M., Gutow, A. P., Sharpe, F. The management of bone defects of the Forearm after trauma. Hand clinics. 1999; 15(2): 299 318.
- 23. Haw, C. S., O' Brien, B. MCC., Kurata, T. The Microsurgical revascularization of resected segments of tibia in the dog J. Bone Joint Surg. 1978; 60B: 266 269.
- 24. Gosain, A.K. Song, L., Santoro, T. D. et al. Long term remodelling of vascularized and non-vascularized onlay bone grafts; a macroscopic analysis. Plastic and reconstructive surgery. 1999; 103(5): 1443 50.
- 25. Macgueen, A. K., Pianta, R.M., Lucas, J. Bridging of a large defect with a microvascular autogenous bone graft an experimental study in dogs. J. Bone Joint Surg. 1978; 60B: 287.
- 26.Busa, R., Adani, R., Castagenetti, C., et al. Neovascularized bone grafts. Experimental investigation. Microsurgery. 1999; 19(6): 289 95.
- 27. Bergran, A., Welland, A. J., Dorfman, H. The effect of prolonged ischaemic time on osteocytes and osteoclast survival in composite bone grafts vascularized by microvascular anastomosis. Plastic and reconstructive surgery. 1983; 69: 290 8.

- 28. Chacha, P. B., Ahmed, M., Dawuwale, S. Vascular pedicle graft and the ipsilateral fibular for non-union of the tibia with a large defect. An experiment and clinical study. J. Bone Joint Surg. 1981; 63B: 244 253.
- 29. Hing, I. L. F. Periosteal pedicle grafting in dogs. J. Bone Joint Surg. 1976; 58B: 117 121.
- 30.MCBirne, J., Court Brown, C.M., MCQueen, M. M. Early open reduction and bone grafting for unstable fractures of the distal radius. J. Bone Joint Surg. Br. 1995; 77(4): 571 5.
- 31. Miller, F., Sussman, M.D., Stemp, W.G. current practice. J. Paediat. Orthopaedics. 1984; 4: 353 355.
- 32.Bos, G. D., Goldberg, V.M., Zika, J.M., et al. Immune responses to frozen bone allografts. J. Bone Joint Surg. 1983; 63A: 239 246.
- 33. Morean, M.F., Gallois, Y., Basle, M.F. et al. Gamma irradiation of human allografts alters medullary lipids and releases toxic compounds for osteoblast like cells. Biomaterials. 2000; 21(4): 369 76.
- 34. Boyce, T., Edwards., Scar Borongh, N. Allograft bone. The influence of processing in safety and performance. Orthopaedic clinics of North America. 1999; 30(4): 571 81.
- 35. Friend laender, G. E. Immune responses to osteochodral allografts. Current knowledge and future directions. Clinical orthopaedics and related research. 1983; 74: 58 68.
- 36.Burwell, R. G., the fate of freeze-dried allograft. Transplantation proceedings supplement 1976; 1: 95 111.

- 37. Hollinger, J. O., Schrimitz, J. P., Mizgala, J. W. et al. An evaluation of two configurations of tricalcium phosphate for treating craniotomies. J. Biomed meter Res. 1989; 23: 17 29.
- 38. Tomford, W. W., Mankin, H. J. One hundred and eighty three investigational preservation. Clinical orthopaedics and related research. 1983; 174: 22 27.
- 39. Fideler, B. M., Vangsness, C. T. Jr., Moore, T. et al. Effects of gamma irradiation on the human immunodeficiency virus. A study in the frozen human bone patella ligament bone grafts obtained from infected Cadavars. J. Bone Joint Surg. A. M. 1994; 76(7): 32 –5.
- 40.Pelker, R. R., Friendlaender, G. E., Malkhem, T. C. Biomechanical properties of bone allografts. Clinical orthopaedics and related research. 1983; 174: 54 68.
- 41.Lemperg, R. K., Smith, T. W. D. The calcaneal osteotomy of Dwyen. An indication for Iliac bone. Acts. Orthopidice scandinavics. 1976; 47: 125 128.
- 42. Goram, A., Muthy, K. K. Fracture dislocation of the cervical spine. Value of anterior approach with bovine interbody fusion spine. 1978; 3: 95 102.
- 43. Siguera, E. B., Kranzler, L.I.I. Cervical interbody fusion using calve bone. Surgical neurology. 1981; 37 39.
- 44. Tacheri, Z. E., Gueramy, M. Experience with calf bone in cervical interbody spinal fusion. Journal of neurosurgery. 1972; 36: 67 71.
- 45. Salama, R. Xenogeneic bone grafting in human. Clinical arthopaedics and related research. 1983; 174: 113 121.

- 46. Salama, R., Weissmann, L. the clinical use of combined Xenografts of bone autologous red marrow. Preliminary report. J. Bone Joint Surg. 1978; 60B: 111 115.
- 47.Osborn, J. F., Newesley, H. Dynamic aspects of the implant bone interface. In: Henke G(ed). Dental implants can herges verlag. Munice. 1980 a: 108 111
- 48.Zelein, G. Linde, A. Bone neogenesis in domes made of expanded polytetrafluoroethylene: efficiency of rhBmp 2 to enhance the amount of achievable bone in rats. Plastic and reconstructive surgery. 1999; 103(4): 1229 37.
- 49. Kurioka, K., Imeda, M. Teranobu, O. et al. Effects of various properties of hydroxyapatite ceramics on osteoconduction and stability. Kobe journal of medical sciences. 1999; 45 (3-4): 149 63.
- 50. Wipperman, B., Donow, C., Schratt, H. E. et al. The influence of hyoroxyapatite granules on the autologous bone marrow. Annales Chirurgiae et gynaecologiae. 1999; 88(3): 194 7.
- 51. Jarcho, M. Calcium phosphate ceramics as hard tissue prosthesis. Cinical orthopaedics and related research. 1981; 157: 259 278.
- 52. Costeoino, P. D., Friendman, C.D., Cane, A. Biomaterials in facial plastic and reconstructure surgery. Facial plastic surgery. 1993; 9: 1 15.
- 53. Boden, S. D. Bioactive Factors for bone tissue engineering. Clinical orthopaedics and related research. 1999; 367: 84 94.
- 54. Sampath, T. K., Desimone, D.P., Reddi, A. H. Extracellular bone matrix derived growth factor. Exper. Cell Resp. 1982; 142: 460 464.

- 55. Sampath, T. K., Muthukuruman, N., Reddi, A. H., Isolation of osteogenin and extracellular matrix associated bone inductive protein by heparin affinity chromatography. Proc. Nalt acad Sci 1987; 84: 7109 7113.
- 56. Constant, Z.B.R., Ison, I. C., Fulmer, M. T. et al. Skeletal repair by in situ formation of the minerals phase of bone. Size 1995; 267: 1796 1799.
- 57. Sampath, T. K., Maliakal, J. C., Hanseka, P. V. et al. Recombinant human osteogenic protein (HPI) Induces new bone formation in vivo with specific activity comparable with natural bovine osteogenic protein proliferation and differentiation in vitro. J. Biol Chem. 1992; 267: 20352 20362.
- 58.Knaack, D. Goad, M. E., Aiolova, M. et al. Resorbable calcium phosphate bone substitute: Journal of biomedical materials research. 1998; 43(4): 399 409.
- 59.King K. F. Periosteal Pedicle grafting in dogs. J. Bone joint Surgery. 1976; 58B: 117 121.
- 60. Sampeth, T. K., Reddi, A. H. Homology of bone inductive proteins from human monkey, bovine and rat extracellular matrix. Proc natt acad sci. 1983; 60: 6591 6595.
- 61. Takgoke, K., Yoshikawa, H., Masuhara, K. et al. Establishment of cell line producing bone morphogenetic proteins from human osteosarcoma. Clin orthop. 1998; 244: 258 264.
- 62. Tsunda, T., Mesuchera, K., Yoshikawa, H. et al. Establishment of an osteoinductive murine osteosarcoma cloned cell line showing osteoblast phenotypic traits. Bone. 1989; 10: 195 200.

- 63.MCAnulty, J. F. Effects of various short term storage methods on viability of cancellous bone fragments. American journal of veterinary research. 1999; 60(1): 63 –7.
- 64. Henry, L. Jaffe, M.D. Solitary bone Tumours and tumorous conditions of bones and joints. Lea Febiger. Fifth edition. 1974: 74.
- 65.Brashear, H. R., Raney, R. B. Solitary bone Cyst. Shand's handbook of orthopaedic surgery. C. V. Mosby. ninth edition 1978; 236.
- 66.Crenshaw, A. H. Delayed union and nonunion of fractures.

 Campbell's operative orthopaedics. C. V. Mosby. Seventh edition.

 1987: 2053 2118.
- 67.Kim, S. J., Serin, S. J., Yang, K. H. et al. Endoscopic bone graft for delayed union and nonunion. Yonsei med. J. 2000; 41(1): 107 11.
- 68. Mankin, H. J., Tomford, W. W. Long term results of allograft. replacement of bone tumour. Clinical orthopaedics and related research. 1996; 324; 86 97.
- 69. Enneking, W. F., Eady, J. L., Burchadt, H. Autogenous cortical bone grafts in the reconstruction of segmental defects. J. Bone Joint Surg. (AM). 1980; 62(7): 1039 58.
- 70.Lim, E. V., Lavadia, W. T., Roberts, J. M. superior gluteal artery injury during Iliac bone grafting for spinal fusion. A case report and literature review. Spine (United States). 1996; 21(20): 2376 8.
- 71. Stephen D. Cook, David C. Rueges. Osteogenic protein 1, bilogy and applications. Clinical orthopaedics and related research. 1996; 324: 29 38.

- 72. Sisk, T. D. General principles and techniques of external skeletal fixation. Clin Orthorp. 1983; 180: 96.
- 73. Buckley, P. D., Geeran, P. F., Petty, R. W. Structural bone grafting for early atraumatic avascular necrosis of the femoral head. J. Bone Joint Surgery (AM). 1991; 73(9): 1357 64.
- 74. Kumta, S. M., Leung, P. C., Griffith, J. F. et al. vascularized bone grafting for fibrous dysplasia of the upper limb. J. Bone Joint Surgery. (Br) 2000; 82(3): 409 12.
- 75. Boyd, H. D., Anderson, L. D., Johnston, D. S. Changing concepts in the treatment of non-union. Clin. Orthop. 1965; 43: 37.
- 76. Thomas, F. B. Arthrodesis of the subtaler joint. J. Bone Joint Surg. 1967; 49B: 93.
- 77.Nigel H. Harris. Congenital pseudoarthrosis of the tibia and congenital deficiency of the tibia and fibular. Postgraduate textbook of clinical orthopaedics. Wright P. S. G. 1983: 194 219.
- 78.Ruedi, T. P., Luscher, J. N. Results after internal fixation of communited fractures of the femoral shaft with DC plates. Clin. Orthop. 1979; 138: 74.
- 79. Margel, F., Wyss, A., Brunnes, C. et al. Plate osteosynthesis of femoral shaft fractures in adults: follow up study. Clin. Orthop. 1979; 138: 62.
- 80.Pearlman, H.S., Patel, M. Sclafani, S.J. et al. Fractures of the femoral shaft. Treatment with solitary plate compression. Clin. Orthop. 1981; 10: 109.