for a possible case of Trisomy 21 or Down Syndrome, 85.3% were confirmed to have Trisomy 21, while 14.7% had other diagnoses. Likewise, for Trisomy 13, of the 69 requests, only 44 or 63.8% were confirmed to have Trisomy 13. In contrast to these, there were 186 requests for confirmation of Trisomy 18 but the Cytogenetics Laboratory was able to diagnose 228 cases. These were probably patients presenting with multiple congenital anomalies wherein Trisomy 18 was not the primary consideration. This data lends support to the importance of performing chromosomal studies to resolve diagnosis inasmuch as correct diagnosis is critical for management and prognostication of the patient. Our data from this review of cases show that Full Trisomy 21, Full Trisomy 18 and Full Trisomy 13 were still the most predominant sub-types ascertained, accounting for 88.3% (1640), 95.2% (217) and 86.4% (38) of the respective groups.

Among the different sex chromosome abnormalities, Turner Syndrome was the most commonly seen accounting for 80.1% of the cases with 38.9% of these were the classical Turner syndrome type.

A variety of different structural chromosome rearrangements were described. Rearrangements occurring within a single chromosome included deletions, duplications, isochromosome and ring formation. Rearrangements involving more than one chromosome included translocations, insertions, marker chromosomes and complex rearrangements. Deletions accounted for a third of the cases, followed closely by translocation cases (27.1%) and addition cases (17.5%).

Deletion of the long arm of the Y chromosome (Yq-), short arm of chromosome 5 (5p- or Cri-du-chat syndrome) and long arm of chromosome 18 (18q-) were the most common deletions ascertained accounting for 6.6%, 5.5% and 3.3% of structural chromosome abnormalities, respectively. Translocations involving chromosomes 9 and 22 (Philadelphia chromosome) were identified in 8.2% of cases and Robertsonian translocations [rob(13;14), rob(13;21), rob(14;21), rob(15;21)] were identified in 2.7% of structural chromosome abnormalities. Most of the chromosomal additions were in chromosomes 10, 22 and the Y chromosome. Fragile X and ring chromosome abnormalities involving chromosomes 4, 10, 13, 18 and 21 were identified.

The use of routine chromosomal analysis is limited to the gross structural appearance of the chromosomes. More recent techniques allow precise identification of chromosomes or parts of chromosomes that are beyond the resolution of routine cytogenetics. Fluoresence in situ hybridization (FISH) is one of these newer methods which utilize fluorescently labeled DNA probes to detect or confirm these different gene or structural chromosome abnormalities. Another technique is an array comparative genomic hybridization (CGH) which utilizes mapped DNA sequences in a microarray format as a platform for the detection of chromosomal deletions/duplications. Its advantage over conventional karyotyping includes a higher resolution and direct mapping of aberrations to the genome sequence.

Conclusion

In conclusion, visible changes in the number or structure of chromosomes form a major category of clinical conditions. They account for a large proportion of all reproductive wastage, congenital malformations, mental retardation and more than 100 identifiable syndromes. Thus, chromosomal analysis is an increasingly important diagnostic procedure in numerous areas of medicine.

This review presents the diverse types of chromosomal abnormalities detected on peripheral blood samples referred to the Institute of Human Genetics, National Institutes of Health, University of the Philippines Manila from government/private hospitals and from private health practitioners for the past sixteen years (1991-2007). Numerical chromosome abnormalities were more common than structural chromosome abnormalities. Full Trisomy 21 was the most common aneuploidy seen. Classic Turner Syndrome was the most frequent sex chromosome abnormality identified. Deletions, additions and translocations were the most common structural chromosome abnormalities ascertained.

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Cytogenetic Analysis of Primary Bone and Soft Tissue Tumors in Filipino Patients

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ABSTRACT

Primary bone and soft tissue tumors comprise about 1% of all malignancies. Cytogenetic studies of malignancies have been reported to play a significant role in their diagnosis, prognosis and management. However, there have been no similar studies reported in the Philippines. The study was conducted to identify the specific tumor karyotypes associated with primary bone and soft tissue tumors among Filipino patients. Subjects included patients seen at the UP-PGH Musculoskeletal Tumor Unit. This paper presents chromosomal studies on tumor specimens of 14 patients, 50% of which had abnormal karyotypes.

Key Words: cytogenetics, bone tumors, soft tissue tumors

Introduction

In the last three decades, cytogenetic analysis has been shown to be increasingly important in the study and understanding of oncogenesis and its management. The identification of more than 600 acquired, recurrent, balanced and highly consistent chromosome rearrangements has led to the characterization of tumors according to particular chromosomal attributes which have proven to be useful in the diagnosis, classification and management of myeloproliferative disorders and soft tissue tumors.[1] Results of cytogenetic investigations have been used in treatment, specifically to tailor therapeutic protocols to individual patients. Although cytogenetic investigations are more recent and less established in solid tumors, these have resulted in important applications in diagnosis and management.[2] Prognostic markers have been identified for neuroblastoma and rhabdomyosarcoma.[2,3] Likewise, specific chromosomal regions have been identified to be involved in the development of osteosarcomas.[4,5,6] Recurrent and specific chromosomal rearrangements in small round cell tumors have also been useful in establishing diagnosis and in distinguishing between those in which histopathologic distinctions are unclear or difficult, i.e. neuroblastoma, primitive neuroectodermal tumors, rhabdomyosarcoma and Ewing's sarcoma among others.[2, 3, 7, 8]

The objective of this study was to describe tumor karyotypes associated with primary bone and soft tissue tumors among a series of Filipino patients.

Materials and Methods

All primary bone and soft tissue tumor cases with sufficient soft tissue component, not previously treated (i.e. chemotherapy and radiotherapy) seen at the UP-PGH Musculoskeletal Tumor Unit from February 2002 to December 2003 were included in the study. Excluded from the study were other solid tumors (i.e. retinoblastoma and Wilm's tumor), cases with exposed or infected tumor sites, tumors that had been subjected to adjuvant treatment such as radiotherapy or chemotherapy, metastatic tumors and other 'tumor-like' lesions wherein infection was a differential diagnosis.

Tumor specimens were obtained aseptically by core (Tru-cut®) or open biopsy following previously determined specifications by the Tumor Service of the Department of Orthopedics (Appendix A). Submitted specimens were processed at the Institute of Human Genetics-National Institutes of Health-University of the Philippines Manila according to established protocols for Solid Tumor Culture.[9] Karyotyping was done for specimens that were successfully cultured and harvested. Reporting used the International System for Human Cytogenetic Nomenclature (2005).[10] All abnormal findings were reported including those found in only 1 cell.

Results and Discussion

Thirty nine patients (26 males and 13 females) were included in the study. Twenty seven patients had tumors in the lower extremity, 8 in the upper extremity and 4 in other sites, i.e sacrum, pelvis and scapula. Table 1 shows the histopathologic results and tissue sources.

Table 1. Histopathology and tissue source of tumors

Histopathology	Tissue Source	Number (N=39)	Per cent (%)
Benign	Bone/Osseous	4	10.3
	Soft Tissue	6	15.4
Malignant	Bone/Osseous	20	51.3
	Soft Tissue	9	23
Total		**39**	

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Only 14 patients had successful karyotyping. Fifteen samples were classified to be contaminated and 10 had absence of dividing cells. Of the 14, seven had normal results and seven had abnormal karyotypes (Table 2).

Table 2. Cytogenetic results of specimens with karyotypes

Histologic findings	Age/Sex	Location of tumor	Karyotype
Benign			
Osteofibrous dysplasia	2/F	Tibia	46 XX [14] Hypotetraploidy [1]
Aneurysmal bone cyst	23/M	Distal tibia	46 XY [9] 90 XXYY, -2, +6, -7, -7, +12, +13, -14, -15, -19, +20 [1] 46 XY, add (10p) [1] 46 XY, add (14p) [1] 46 Y, -X, +mar [1]
Giant cell tumor	20/F	Femur	46 XX [12]
Lipoma	28/F	Proximal thigh	46 XX [15] 47 XX, +mar [1] 46 XX, --15 +mar [1] 46 XX, --14 +mar [1] 46 XX, --19 +mar [1] 46 XX, add(10)(pter)[1]
Malignant			
Osteoblastic osteosarcoma	17/F	Femur	46 XX [16] 45 X [1] 46 XX, del(10)(q24→qter) [1] 47 XX +mar -1 [2] 46 XX, del(2)(p11→pter), add(14p), +mar --3 [1] 46 XX, add(15p) [1] 46 XX, del(1)(q32→qter) [1]
	14/M	Femur	46 XY [14] Polyploidy [1]
	7/F	Distal Femur	46 XX [6]
Fibroblastic osteosarcoma	12/M	Distal Femur	46 XY [5]
Parosteal osteosarcoma	33/M	Thigh	46 XY [11] 46 XY, t(11,14) [1] 46 XY, del(9q) [1] 46 XY, --12, +18 [1] 47 XY, +mar [1]
Chondrosarcoma	33/M	Pelvic	46 XY [18]
	32/M	Arm	46 XY [9]
Malignant round cell tumor	58/M	Arm	46 XY [12] 47 XY, del(3)(p22 → pter) –9, +mar [1] 47 XY, +mar [1] Polyploidy [1]
Well-differentiated liposarcoma	23/F	Rectus femoris	46 XX [15]
Low grade soft tissue sarcoma	30/M	Infraspinatus	46 XY [6]

Osteofibrous dysplasia. Osteofibrous dysplasia is a rare bone tumor which most commonly arises in the tibia of young individuals. Fluorescence in situ hybridization analysis revealed trisomies 7, 8, and/or 12.[11] In another study, multiple copies of chromosomes 8, 12, and/or 21 were identified but other cases exhibited either a completely normal karyotype or single cell aberrations.[12] In our study, a 2 year old female with a mass on her tibia showed a karyotype of hypotetraploidy.

Aneurysmal bone cyst. Aneurysmal bone cysts are benign, but often rapidly expanding osteolytic multicystic lesions. They usually occur in young patients and exhibit a slight female preponderance. The metaphyseal region of long bones and vertebrae account for 70-80% of cases, with the distal femur and proximal tibia being the most common location. They are considered of reactive nature, and about 50% of them are secondarily associated with other entities such as giant cell tumor. All aneurysmal bone cysts showed involvement of chromosome segments 17p11-13 and/or 16q22.[13,14,15] In our study, a 23 year old male with aneurysmal bone cyst in the distal tibia showed a karyotype of hypotetraploidy, chromosomal gains involving 10p and 14p and the presence

of a marker.

Giant cell tumor. Giant cell tumor of bone is one of the most common benign primary bone neoplasm derived from stromal cells.[16] These stromal cells have the ability to recruit and harbor macrophage and multinucleated osteoclast-like giant cells. Despite being often considered benign, giant cell tumor of bone can be a difficult neoplasm because of its aggressiveness and unpredictable response to treatment. Cytogenetically, it is characterized by telomeric associations and by a high frequency of telomeric fusion of chromosomes 13, 14 and 21, a process which has been implicated in the production of chromosome instability and tumorigenesis.[13,14,15, 17, 18] In another study, a karyotype of giant cell tumor showed hypodiploid, hypotetraploid, and multiploid, with more than 200 chromosomes per mitosis present in some cells. Other chromosomal aberrations observed included ring chromosomes, double minutes, translocations, multiple fragments, and multiradials.[19] In our study, a 20 year old female with giant cell tumor showed a normal karyotype.

Lipoma. Lipomas are benign neoplasms of adipose tissue and represent one of the most common mesenchymal neoplasms in humans.[20] Cytogenetic studies in a classic lipoma showed a t(3;12)(q27;q14-15).[17, 21, 22] An intramuscular lipoma in a child showed t(1;4;12)(q25;q27;q15).[23] Other studies showed abnormalities involving chromosome region 12q13-15 specifically translocations with 3q27-28, 1p32-34, 21q21-22, 2p21-23, and other non-recurrent rearrangements.[14, 15, 17, 20, 21] Abnormalities not involving 12q13-15 include lipomas with deletion 13q, ring chromosomes, and rearrangements of 6p21-23, 11q13, 1p36, and 13q12-q22.[17, 20, 21] In our study, a 28 year old female with a lipoma on the proximal thigh showed a karyotype of chromosomal gain involving chromosome 10p and a marker (unidentifiable chromosome).

Classic Osteosarcoma. Classic osteosarcoma is the most common primary malignant sarcoma of bone, occurring at a peak incidence in the second decade of life.[16] The consensus findings, based on a number of classical cytogenetic surveys, are frequent structural alterations at chromosome bands or regions 1p11-13, 1q11-12, 1q21-22, 11p15, 12p13, 17p11-13, 19q13, and 22q11-13 and common numerical abnormalities +1, -9, -10, -13, and -17.[24] Conventional cytogenetic studies have shown that osteosarcomas are often highly aneuploid, with a large number of both structural and numerical chromosomal alterations. Chromosomal gain of 1p21-31, 1q21-24, 4q12-13, 4q28-31, 5p13-14, 7q31-32, 8q23-24, 8q21, and 17p11-13; chromosomal losses of 1p, 5q, 7p, 10q, 13q,16, 16qter, 17p, 17q, 19, 19q, 20q; and rearrangement of 20q were identified.[4,5,6] In our study, a 17 year old female with osteoblastic osteosarcoma showed chromosomal losses at 10q24, 2p11 and 1q32; chromosomal gains at 14p and 15p and the presence of a marker (unidentifiable chromosome). Another patient is a 14 year old male with a karyotype of polyploidy. A case of fibroblastic osteosarcoma in a 12 year old male showed normal karyotype.

Surface osteosarcoma. A case of parosteal osteosarcoma in a 33 year old male showed translocation t(11;14), chromosomal loss at 9q and the presence of a marker (unidentifiable chromosome).

Chondrosarcoma. Chondrosarcoma is the second most frequent primary malignant tumor of bone, representing approximately 3.6% of all primary osseous neoplasms.[16] They have complex karyotypes (multiple numerical and structural chromosomal aberrations).[14, 15] Other cytogenetic findings include nonrandom rearrangements of 1p36, 1p11, 1q21, 5q13, 11p15, 12q13-15, 15p11, 19p13, and 20q11. The most frequent numerical changes are trisomy for chromosomes 7 and 20 and monosomy 10.[17] Extraskeletal chondrosarcomas, less common than skeletal chondrosarcoma, exhibit a broad morphological spectrum and may be myxoid, mesenchymal or rarely, well differentiated. Only the myxoid type has been demonstrated to contain specific chromosomal alterations such as reciprocal t(9;22)(q22;q11-12), t(9;17)(q22;q11), t(9;15)(q22;q21), t(9;22;15)(q31;q12;q25).[14, 14, 26, 27] Our patients, a 33 and a 32 year old male, both with classic skeletal chondrosarcoma had normal karyotypes.

Well-differentiated liposarcoma. Liposarcoma is one of the most common soft tissue sarcomas accounting for approximately 20% of all mesenchymal malignancies.[28] The tumor occurs most often in the lower extremities, particularly the thigh and leg.[20] Liposarcomas are divided into 3 major categories: well-differentiated, myxoid and round-cell and pleomorphic.[29] Well-differentiated liposarcoma simulates lipomas closely. Cytogenetically, it is characterized by telomeric associations, supernumerary ring, ring form of chromosome 12, amplification of 12q13-15, t(12;16)(q13;p11), giant marker chromosomes, which may contain homogenously staining regions and tiny spherical chromatin bodies of a few mega-base pairs of size (dmin). [14, 15, 17, 21, 30-32] In our study, a 23 year old female with a well differentiated liposarcoma showed a normal karyotype.

Significance of Abnormal Chromosomal Findings

Aneuploidy and chromosomal instability have been found to be characteristic among human cancers and are linked to the progressive development of high-grade invasive tumors.[33,34] In tumorigeneis, aneuploidy is frequently preceded by tetraploidy, a state of having more than two sets of homologous chromosomes.[34] This is supported by findings of tetraploid cells among pre-malignant conditions and in early and mature stages of certain types of cancers.[35] And in a recent study by Fujiwara et al., tetraploidy enhanced the frequency of chromosomal alterations and promoted tumor development in *p53* mouse mammary epithelial cells, a direct experimental test of the tumorigenic potential of tetraploid cell.[36] Tetraploidy can

arise by several mechanisms including mitotic slippage, cytokinesis failure or viral-induced cell fusion.[35] Another study has demonstrated that it can arise from nondisjunction of chromosomes during mitosis through mitotic cleavage failure.[37]

G- and R-banding are classic banding techniques used in cytogenetic investigations to identify numerical and structural chromosomal abnormalities. It is commonly used for screening for chromosome level abnormalities because the procedure is simple and robust and is not costly. However, use is limited because of its low resolution and labor-intensive analysis with low efficacy in highly rearranged karyotypes.[38] New methods have recently been developed to advance cytogenetic investigations, the fluorescence in situ hybridization (FISH) and comparative genome hybridization (CGH). FISH relies on hybridization with probes that identify specific chromosomal structure, unique sequence probes, or probes that react with multiple chromosomal sequences. It can be used on both dividing and interphase cells, including archival material, however it is too specific. CGH, on the other hand, gives an overview of DNA sequence copy number changes (losses, deletions, gains, amplifications) in a tumor specimen. It avoids the specificity problem inherent in FISH and other probe-dependent molecular techniques and is therefore a good screening method. However, it gives no information about balanced rearrangements, it reflects a theoretical average of the tumor sample and the potential heterogeneity among cells is not directly evident. Though both FISH and CGH are powerful, none of these techniques can supplant banding cytogenetics as a screening method for the detection of chromosome-level acquired mutations in tumor cells.[17,38]

The treatment of musculoskeletal tumors has improved dramatically over the past two decades. Limb saving surgeries have replaced amputations, and medical and oncologic management have resulted in better control of both local and systemic disease. However, mortality remains significant and novel forms of treatment are being studied. The study of cytogenetics in bone and soft tissue tumors will play an important role in providing information for diagnosis, management and prognostication.

Conclusion

This is the first study that looks at chromosomal abnormalities in a series of Filipino patients with musculoskeletal tumors. Despite the relatively high rate of contamination among our specimens, the finding of significant abnormalities in this series raises the importance of continuing to obtain chromosomal studies in all of our patients with bone and soft tissue tumors

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APPENDIX A

Tissue biopsy requirements

1. Specimen requirements: Tissue – approximately 1 cu cm in size
2. Collection: Medium provided by the laboratory, Cytogenetics Laboratory, Medical Genetics Unit, National Institutes of Health, University of the Philippines Manila.
    a. Label specimens with patient's full name.
    b. Physician's request must accompany the specimen. The request must include the patient's name, relevant clinical details, and physician's name, hospital and contact number.
3. Transport:
    a. Keep specimen cool, not frozen, during transport to laboratory.
    b. Do not immerse tube in water to avoid contamination.
    c. Specimen should be submitted 24 hours from time of collection.
4. Results: Results are made available 3-4 weeks from submission of specimen.