HEALTH SCIENCES

1. ANTITUBERCULAR CONSTITUENTS OF *Lunasia amara* BLANCO

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Alcoholic extracts from 90 species of plants collected from the Subic forest were assayed against five microorganisms: the Gram (+) *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, the Gram (-) *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853, and the aci-fast *Mycobacterium* 607. One of the plants that exhibited promising inhibitory activity was *Lunasia amara* Blanco (Rutaceae). Results showed inhibition to *B. subtilis*, *S. Aureus* and *Mycobacterium* 607. Assay of the crude alcoholic extract against *Mycobacterium tuberculosis* H₃₇Rv resulted in 99% inhibition at 1000 mg/mL. A bioassay-directed isolation of the antitubercular constituents of *Lunasia amara* resulted in three crystalline isolates. Thin layer chromatograms of the isolates suggest their alkaloidal nature when sprayed with Dragendorff's reagent. Spectroscopic analysis revealed the alkaloids to be gravcolinine, 4-methoxy-2-phenylquinoline and kokusagine.

Keywords: alcoholic extracts, assay, crystalline isolates, alkaloids

2. BACTERIAL CARDIOLIPIN FOR SERODIAGNOSIS OF SYPHILIS

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Presently, serological diagnosis of syphilis in the Philippines makes use of commercially-prepared cardiolipin from beefheart. This study seeks to isolate and
identify certain bacteria belonging to Family Enterobacteriaceae, Halobacteriaceae, Rhodospirilliaceae and Genus Thermus which can be potential sources of lipid antigen and used for serological diagnosis of syphilis. The bacterial lipid was extracted from the microorganisms using chloroform-methanol mixture. Presence of the cardiolipin was determined using thin-layer chromatography and Fiske Subarrow method for inorganic phosphorus. Further purification of the cardiolipin was done using Sephadex LH-20. The purified bacterial cardiolipin antigen was coated onto the surface of 96-well plates. Fifty positive sera (RPR test) and fifty negative sera (RPR test) were used to test for the sensitivity of the isolated bacterial antigen and were run simultaneously with VDRL test. Plates were read at 492 nm and 620 nm (references) using LP-200/LP 300 Diagnostic Pasteur microplate reader. The absorbance reading of bacterial antigen and VDRL was compared. Results showed a very high correlation with a p value of 0.78. The study suggests the use of bacterial antigen for serological identification of elevated amount of reagin found in cases of syphilis.

**Keywords:** Syphilis, cardiolipin, lipid, antigen, serological diagnosis.

3. AN INVESTIGATION ON THE MEDICINAL QUALITIES OF *Moringa oleifera*

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This paper presents the studies on the anti-inflammatory, antimutagenic and antitumor promoting activities of isolates from the extracts of dried seeds of malunggay and the elucidation of the structures of theses bioactive isolates. Bioassay results indicated that the isolated sterol reduced inflammation in the hind paw of mice induced by carragenan and reduced the number of micronucleated polychromatic erythrocytes induced by the mutagen, mitomycin C. The glucosylsterol derivative and the acylglucosylsterol were likewise anti-inflammatory, antimutagenic and strongly inhibited the activation of the Epstein-Barr virus-early antigen, indicating strong antitumor promoting activities. A rhamnosyl thiocyanate derivative also exhibited remarkably strong antitumor promoting activity while a rhamnosyl thiocarbamate derivative exhibited antihistamine release activity, another indication of anti-inflammatory activity. Published literature showed that the same rhamnosyl thiocy-
anate derivative is antimicrobial, and the thiocarbamate derivative and a mustard oil glycoside are hypotensive. However, a rhamnosyl acetonitrile derivative is mutagenic.

**Keywords:** Moringa oleifera, malunggay, anti-inflammagens, antimitagens, antitumor promoters

### 4. POTENTIAL ANTI-PLATELET AGGREGATING AGENTS FROM MARINE SPONGES AND TUNICATES

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When stimulated, platelets will aggregate to form a hemostatic plug. This activation causes thromboembolism and inflammation. Acetysalicylic acid (aspirin) is one of the most important drugs used for the prevention and management of these diseases. An *in vitro* assay was established to screen for potential platelet aggregation inhibitors comparable to that of aspirin. A total of 113 sponges and tunicates collected from Baler, Quezon were screened qualitatively using the giemsa staining method. Ten crude methanol extracts showed 100% inhibitory activity at 2mg/ml against calcium chloride-induced platelet aggregation. Bioassay-guided isolation and purification of the potential compounds and further quantitative testing of the active samples are currently underway. This *in vitro* assay was developed on the microliter scale to allow quick screening of large number of samples of small quantities such as marine extracts.

**Keywords:** sponges, tunicates, platelets, aggregation, aspirin, calcium chloride, inflammation, giemsa, anti-platelet, marine.

### 5. PURIFICATION AND CHARACTERIZATION OF HUMAN CHORIONIC GONADOTROPIN ALPHA SUBUNIT: A POTENTIAL LUNG CANCER MARKER

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Initial studies on the distribution profile of alpha and beta subunits of urinary human chorionic gonadotropin in lung cancer, using an ion-exchange chromatogra-
phy revealed an unusual increase in concentration of the alpha subunit in the non-small cell lung carcinomas (NSCLC). SDS-PAGE under reducing conditions was performed to assess the purity of the frozen-dried isolated subunits. A distinct band of about 20 kD was significantly observed in the alpha subunits from non-small cell carcinoma of the lung. When subjected to Western blotting, the 20 kD protein band reacted strongly with the monocional antibody against the hCG alpha subunit. Further purification of the subunits was performed using repeated reverse phase HPLC (Supelcosil C18) until a single peak reactive against hCG alpha antibody was obtained. The purified and characterized hCG alpha subunit can be used in the production of an anti-hCG immunoassay kit for nonsmall cell lung cancer. The immunoassay kit can serve as an easy, fast and inexpensive screening test for that type of lung cancer.

**Keywords:** lung cancer, urinary human chorionic gonadotropin, cell carcinoma, Western blotting, immunoassay

6. **STUDIES ON Prospopis vidaliana NAVES AS A POTENTIAL SOURCE OF DRUG MATERIAL**

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A large number of drugs being used today are derived from plants and the search for bioactive compounds from plants is still an ongoing process. As a result of a field screening in the Zambales rainforest, a plant has been identified to have a great potential as a source of a new drug material. *Prospopis vidaliana* Naves (Leguminosae), locally known as "aroma" was collected from the forest and subjected to chemical and biological studies. Antimicrobial activity of the crude ethanolic extract was evaluated using ATCC bacterial strains and 19 bacterial wound isolates. Results obtained from disc diffusion assay indicate that it inhibited both Gram (+) and Gram (-) bacteria, as well as species of *Staphylococcus* and *Escherichia coli* from wound isolates. Cytotoxicity assay using the brine shrimp assay and showed an LC₅₀ - 35.69 µg/mL. Cytotoxicity assay using the microculture tetrazolium (MIT) method indicated a comparable activity against both the human cancer cell lines and murine fibroblasts. The Ames test showed that it is nonmutagenic against *Salmonella typhimurium* TA 98 and TA 100. No DNA damaging properties were observed up to a dose level of 400
The crude ethanolic extract was subjected to bioassay-guided isolation. The antimicrobial activity was monitored using disc diffusion method and bioautography and the cytotoxicity was monitored using brine shrimp assay. Two antimicrobial and cytotoxic alkaloids were isolated and their structures were elucidated using different spectroscopic methods.

Keywords: aroma, Leguminosae, Prosopis vidaliana, crude ethanolic extract

7. THE ANALGESIC ACTIVITY OF THE ALKALOIDS FROM THE SEEDS OF Ipomoea muricata JACQ. CONVOLVULACEAE

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Indolizidine alkaloids were previously isolated from the seeds of Ipomoea muricata for which analgesic activity of the seeds was attributed to. In separate studies, the analgesic activity was reported for the alkaloid ipomine and ipalbidine. This study compared the analgesic activity of four indolizidine alkaloids from the seeds of Ipomoea muricata. The analgesic activity of three tertiary indolizidine alkaloids, ipomine, ipalbidine, ipalbine and a quaternary indolizidine alkaloid designated as EL, were tested on mice by the hot-plate method. All alkaloids showed analgesic effects at dose levels of 1 mg/kg-BW, 5 mg/kg-BW and 10 mg/kg-BW, except for standard drug indomethacin at 5 mg/kg-BW. The order of analgesic activity of the alkaloids was EL > ipalbidine > ipomine > ipalbine. By relating the structures of the individual alkaloids to their activities, it appeared that the presence of the quaternary nitrogen in the structure of EL contributed to its enhanced activity. EL can be a lead compound in the development of a potent analgesic agent.

Keywords: analgesic, alkaloids, Ipomoea muricata
Biomedical studies on nuclear fallout effects showed that whole-body exposure to relatively low doses of ionizing radiation (5-10 Gy) induces the clinically broad spectrum of the Hematopoietic Syndrome characterized by severe anemia, immunodeficiency, followed by death within 10-30 days. Whole thymus explants derived from intraphameral 30-day old inbred male ICR mice were cultured in vitro after enzymatic dissociation and removal of thymic macrophages. This thymocyte model is applied in many cell death researches and had been found to undergo a morphologically and molecularly distinct p53-based cascades of apoptotic events with exposure to DNA-damaging insults, such as some anti-cancer agents, hyperthermia, hypoxia and radiation. Exogenous application in culture of a radiation protecting agent GC-2112, a herbal extract derived from water-soluble components of garlic bulbs (Allium sativum), improved total cellular survival for various observation periods concomitantly shifting the LD50/24 (lethal dose) from 17 Gy (control) to 21 Gy (with radioprotection). Target-based analyses revealed time-dependence of radioprotection process which exhibited shifting in both a and b parameters and clustering of thymocyte deaths into three distinct modes. This increase in total cell survival of GC-2112-treated thymocytes, however, failed to correlate with the prevention of apoptosis-associated internucleosomal DNA scissions. DNA fragmentation in the absence of irradiation and with treatment of GC-2112 was induced despite the overall improvement of total cellular survival of thymocytes exposed to radiation. The mean dose reduction factors (DRFs) ranged from 10.7-1.1. Mechanisms to the observed radiomodification may possibly involve a mixture of bioactive poly-thiols found rich in garlic bulbs. The findings presented a paradoxical correlation between apoptosis, cell survival and radioprotection which may be explained by the differential reactivities of the bioactive compounds in GC-2112 and the variabilities in radiosensitivities of the primary thymocyte culture. The differential effect on radio protection showed potential in the...
application of herbal drugs as dietary prophylaxis against clinical morbidities arising from either medical, occupational or environmental exposures to ionizing radiation.

**Keywords:** radioprotectors, apoptosis, thymocytes, radiation-induced death, herbal medicine, target theory, immunity, radiation biology, applications in radiotherapy

### 9. CULTIVATION OF MAMMALIAN CELLS FOR LABORATORY ASSAY SYSTEMS

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The developed techniques of culturing cells *in-vitro* led to the production of specific cells. These serve as tools to the understanding of the cell's biological nature contributing further to the proper diagnosis and search for the therapeutic modalities. The study cultivated mammalian cells for laboratory assay systems. Freshly explanted surgical tissues were immersed in Hank's Balance Salt Solution. The tissue explants were enzymatically digested and cultured in RPMI 1640 culture medium supplemented with 10% fetal bovine serum. Obtained cells were incubated at 37°C in 5% CO₂ and fed every other day until they showed confluent growth and characteristic morphology. Cells were harvested and subcultured for various laboratory assays and cryopreservation. Results of the study showed the culture of the following cells: normal urothelial cells, transitional and squamosal carcinoma of the urinary bladder, sphenoid wing meningioma and tumor cells of the posterior fossa of human brain. Preliminary studies showed that these cells demonstrate variable growth behavior, growth rate, characteristic morphology and viability in culture medium. Laboratory assays also showed promising results for the search of therapeutic agents from natural product extracts and the search of homografts for the reconstructive surgery.

**Keywords:** Mammalian cells, assay systems
10. ALBUMIN EXCRETION RATES IN FILIPINO PATIENTS WITH INSULIN-DEPENDENT DIABETES MELLITUS: A CASE COHORT

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Diabetic kidney disease is an important cause of morbidity in insulin-dependent diabetes mellitus (IDDM). The 1991 guidelines issued by the American Diabetes Association included assessment of albumin excretion rate in the urine as a sign of progression of the disease process. The early detection of microalbuminuria [20-200 ug/min] by immunoturbidimetric assay (ITA) can be an important tool for early diagnosis and the intervention to prevent irreversible kidney damage in the population at risk. This study aimed to determine the significant differences in glycosylated hemoglobin levels and albumin excretion rates within 6 months in this study group of IDDM patients. It also aimed to examine the relationship between MA and the following clinical characteristics: age at onset of IDDM, duration of IDDM, height, weight, body mass index (BMI), insulin requirement per day, glycosylated hemoglobin, systolic and diastolic blood pressure. In March 1996, 31 Filipino diabetic children and adolescents were studied for early signs of diabetic nephropathy. Eight patients were identified as having microalbuminuria (MA). All 31 subjects were followed up for six months. In the initial ITA, microalbuminuria was identified in eight patients. On repeat ITA, two of the eight patients showed normal albumin excretion. In six patients in whom the microalbuminuria persisted, the AER had decreased. One patient who had a normal AER initially became microalbuminuric. In the normoalbuminuric group, the male: female ratio and mean age was 1:1.3 and 12.73 +/-5.0 respectively while in the microalbuminuric group, it was 1:7 and 14.5 +/-5.0. The mean difference in AER between the first and second ITA in those with microalbuminuria showed a trend towards a decrease in excretion rates. However, this was not statistically significant (p=0.08). Among variables studied, only diastolic blood pressure (p=0.05) was found to be significantly correlated with microalbuminuria.

Keywords: albumin, excretion, diabetic kidney disease, insulin-dependent diabetes mellitus, and microalbuminuria.
11. C-PEPTIDE IN FILIPINO CHILDREN WITH INSULIN-DEPENDENT DIABETES MELLITUS: A MEASURE OF RESIDUAL B-CELL FUNCTION

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Children with insulin-dependent diabetes mellitus (IDDM) have minimal or absent secretion of insulin, thereby requiring exogenous insulin as therapy. Studies have shown that C-peptide concentration is a valid index for residual B-cell function, unaffected by exogenous insulin and insulin antibodies. This study was done to determine the residual B-cell function as measured by C-peptide in Filipino children with IDDM and to correlate this with variables including sex, weight, age at onset of IDDM, duration of illness, serum insulin, dose of insulin, blood glucose (FBS), and glycosylated hemoglobin (HBA1C). Among 50 IDDM patients studies, 18% (n=9) had residual B-cell function, the age, duration of diabetes, insulin does of patients and HBA1C were not significantly different from those with abnormal levels. Patients with abnormal C-peptide levels were noted to have significantly lower age of onset (p<0.0015) and weight (p<0.0084). There was no significant correlation between the duration of diabetes and glycemic control.

Keyword: Insulin-dependent diabetes mellitus, residual cell function, C-peptide concentration, exogenous insulin, insulin antibibodies

12. DETERMINATION OF MICROALBUMINURIA (MA) USING SEMIQUANTITATIVE METHOD (MICRAL-TEST) IN FILIPINO PEDIATRIC PATIENTS WITH INSULIN DEPENDENT DIABETES MELLITUS (IDDM)

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The hallmark of the diabetic kidney is persistent proteinuria and progressive renal impairment. Several years prior to the appearance of proteinuria, subclinical elevation of urinary albumin excretion or microalbuminuria (MA) can be detected.
MA is albumin excretion rate of 20-200 μg/minute. MA is highly predictive of diabetic nephropathy, hypertension and cardiovascular disease. This study was done to determine the prevalence of MA using Micral-Test (MT) in Filipino patients with IDDM, to determine the diagnostic properties of MT (validity and accuracy) and to compare it with quantitative immunoturbidimetric assay (ITA). MT was determined by semiquantitative dry immunochemical method using a 24 hour urine sample. The same sample was used for ITA. Thirty one diabetics were tested for MT. There were 24 normo albuminuric and 7 microalbuminuric patients. In the former group, the male:female ratio and mean age were 1:2 and 12 +/- 5.0 (+/-SD) respectively while in the latter group, it was 1:6 and 14 +/- 5.3 (+/-SD). The prevalence of MA in the study was 22.58%. MA has a sensitivity of 85.51%, specifically of 9%, negative predictive value of 94.11% positive predictive value of 42.68% and accuracy of 71%. The study disclosed 77.42% of Filipino diabetics were correctly classified as either having or not having elevated urinary albumin excretion using ITA as the gold standard. The Micral-Test provided a sensitive and specific test for clinic screening of microalbuminuria.

**Keywords:** Microalbuminuria, semi-quantitative method, Insulin-dependent diabetes mellitus

### 13. A CHROMOGENIC DIAGNOSTIC KIT FOR THE RADIOSENSITIVE TYROSINASE ENZYME IN ORIENTAL FRUIT FLY Bactrocera philippinensis

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A biochemical marker for radiation injury in Oriental fruit fly, *Bactrocera philippinensis*, was identified and isolated in denaturing polyacrylamide gel electrophoresis (SDS-PAGE). The biomarker, Gs-protein, is a tyrosinase enzyme whose gene locus is found to be radiosensitive even at a very low radiation dose of 25 Gy as demonstrated by the loss of Gs-protein band from SDS-PAGE gel analysis of pupal homogenates from irradiated larvae. Isolated Gs-protein has a tyrosinase enzyme activity capable of converting tyrosine into an intermediate product, DOPA. It also exhibit the copper-containing property of mushroom tyrosinase as shown in the preliminary TXRF analysis. An apparent molecular weight of 109 kDa was calculated from SDD-PAGE preparation of the Gs-protein with high molecular weight proteins as standards. A chromogenic test based on the reaction of tyrosinase with 2-methyl-DOPA was developed for *B. philippinensis* for use as a field quarantine kit. Irradiated fruit fly has been shown in earlier studies to lose its tyrosinase enzyme. The presence
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14. CLINICAL AND LABORATORY PROFILE OF PEDIATRIC PATIENTS WITH NONTYPHOIDAL SALMONELLA BACTEREMIA IN PGH AND RITM FROM 1991-1995

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Morbidity and mortality from Salmonellosis rank high among children. The study aimed to describe the clinical and laboratory profile of patients with Salmonella bacteremia. Children from 0-18 years old with Salmonella bacteremia admitted in PGH and RITM from 1991-1995 were used in the study. Fifty percent of records of 117 patients with Salmonella bacteremia were retrieved. Results showed that in infants 1 year and below, common symptoms were watery nonbloody diarrhea, fever and vomiting. Anemia and dehydration were complications noted in 52% and 38% respectively. CHC showed either leukopenia, leukocytosis or thrombocytopenia. Fecal leukocytes was seen in only 28%. Group B Salmonella, being the predominant pathogen, was sensitive to Chloramphenicol in 57% of isolates. In older children, abdominal pain together with fever and diarrhea were frequently observed. 32% developed dehydration during confinement. Group C Salmonella was the most common isolate, with 69% sensitive to Chloramphenicol. 67% clinically improved after antimicrobial and supportive treatment.

Keywords: Nontyphoidal Salmonella bacteremia, Salmonellosis, PGH
15. GENOTYPE ANALYSIS OF HEPATITIS C VIRUS AMONG BLOOD DONORS AND INMATES IN METRO MANILA, THE PHILIPPINES

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A genotype analysis of Hepatitis C virus among blood donors and inmates in Metro Manila was done. Antibodies against Hepatitis C virus (HCV) were detected in 18 (2.3%) of 800 sera from commercial blood donors and 23 (4.6%) of 502 sera from inmates in Metro Manila. The difference in the antibody prevalence between the two groups was statistically significantly (P<0.05). HCV RNA was detected in 14 (78%) of the 18 antibody-positive sera from blood donors and 19 (83%) of the 23 antibody-positive sera from inmates. Genotype analysis revealed that HCV-1b (50%) was most common among blood donors, followed by HCV-1a (36%) and HCV-2a (7%). Among inmates, on the other hand, HCV-1a (68%) was most common, followed by HCV-1b (11%), HCV-2b (5%). Overall, HCV-1a and HCV-1b appeared to be predominant among them. Thus, the genotype prevalence in the Philippines was distinct from those in other Southeast Asian countries such as Thailand, Vietnam and Indonesia, and also distinct from those in the Far East including Taiwan, Mainland China and Japan.

Keywords: Hepatitis C virus, genotype, blood donor, inmate

16. HEPATITIS C VIRUS INFECTION AND RISK FACTORS AMONG INMATES AT MUNTINLUPA, METRO MANILA: AN EMERGING CONCERN IN THE COMMUNITY

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The Hepatitis C virus infection and risk factors among inmates at Muntinlupa, Metro Manila were studied. The seroprevalence of hepatitis C virus (HCV) infection was 5.0% in 502 inmates examined at the Camp Sampaguita, Bureau of Prisons,
Muntinlupa, Metro Manila. The practice of homosexual activities and parenteral drug use were found to be significantly associated with HCV infection a prevalence of 12.9% and 35.0%, respectively. Though the prevalence of anti-HCV was higher among those with tattoos, who had blood transfusion, pierced ears, "bulitas" implants, and self-inflicted wounds or "paandar" these were not statistically significant. Local baseline data of HCV infection in the general population is needed to determine the magnitude of the disease and to determine risk factors.

**Keywords:** Hepatitis C virus, Hepatitis C, Muntinlupa, Metro Manila, homosexual activities

17. PULMONARY PARAGONIMIASIS IN NON-RESPONDING TUBERCULOSIS PATIENTS IN IROSIN, SORSOGON

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In the province of Sorsogon, pulmonary tuberculosis remains as a leading cause of mortality and morbidity. In the municipality of Irosin, of the 157 patients listed in the TB registry in 1993, an estimated 40-50% are nonresponders to short course chemotherapy. As a prelude to a bigger survey of TB patients not responding to chemotherapy and to document pulmonary paragonimiasis among this set of patients, a study of nonrespondents was done. Patients clinically diagnosed to have pulmonary tuberculosis by the Rural Health Unit in Irosin, Sorsogon and nonresponding to at least six months of anti-tuberculosis treatment were studied by the research team in October 1994. Clinical histories were reviewed, patients were examined, and an early morning sputum specimens were collected and examined for the presence of acid-fast bacilli and Paragonimus westermani ova. Nine patients were seen and examined, and all had clinical histories consistent with pulmonary tuberculosis. Six had history of hemoptysis, and eight had abnormal pulmonary findings at the time of examination. Four were found to be positive for AFB, while five had Paragonimus eggs in their sputum. Three patients with AFB also had Paragonimus ova in their sputum. Pulmonary paragonimiasis exists in Irosin and should be considered in patients suspected to have pulmonary tuberculosis. Routine screening for Paragonimus in endemic areas may be necessary in patients clinically diagnosed to have pulmonary tuberculosis and in those who are responding to anti-TB treatment.

**Keywords:** pulmonary paragonimiasis, tuberculosis
18. THE PHILIPPINE GENERAL HOSPITAL EYE INJURY REGISTRY (PRELIMINARY REPORT): DEMOGRAPHICS OF EYE INJURY CASES SEEN FROM JANUARY- JUNE 1996

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Ocular trauma is one of the major causes of monocular blindness in the Philippines with a 13.6%-28.5% incidence in population-based surveys. In the Philippine General Hospital (PGH), 26% of all new eye cases are due to eye injuries. In an effort to establish a national registry of ocular injuries for all major centers in the country, the PGH Eye Injury Registry (PGH EIR) was initiated. Initial data of 1,071 cases of eye injuries included in the PGH EIR from January-June 1996 were reviewed. Cases were analyzed for demographic information. Mean age of the patients reviewed is 26.99 yrs. 63.3% of the patients are in working age group. Mostly are males (80%). Workplace accounted for 36% of all injuries. Majority of the patients (48.1%) are laborers and technicians; half of whom are metal and building trade workers. Serious eye injury (injury involving permanent and significant structural change) account for 17.0% of all injuries. Visual activity at initial consult was Hand Movement to Light Perception in 38.9% of these patients. The incidence of eye injuries requiring hospitalization 15.1%. Annual medical costs were estimated conservatively at 3.1% million pesos for this hospital alone and approximately 681,000 man-hours were lost. Work-related eye injuries are frequented and may lead to loss of vision and man-hours. The development of preventive programs and the reduction of ocular morbidity need to be addressed.

Keywords: ocular trauma, monocular blindness, eye injury