Title

Environmental and Occupational Factors Associated With Chronic Myeloid Leukemia: A Case Control Study

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This research proposal is my own original work and has not been presented for a degree in any other university.

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DEDICATIONS

This work is dedicated to my parents, both victims of cancers and to my wife, Nafisa, and my children, Suheim and Sumaiya, whose never ending support, inspiration, patience and understanding enabled me to complete this work. I also dedicate this work to my brother, Iqbal Kasmani, without whose support, I would not have been what I am today.

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ABBREVIATIONS

CML	Chronic Myeloid Leukemia
AML	Acute Myeloid Leukemia
ALL	Acute Lymphocytic Leukemia
CLL	Chronic Lymphocytic Leukemia
Ph	Philadelphia Chromosome
DNA	Deoxyribonueucleic Acid
mRNA	Messenger Ribonucleic Acid
ABL	c-abl oncogene
BCR	Breakpoint Cluster Region
kb	Kilobases
bcr	Small Breakpoint Cluster Region
IARC	International Agency for Research in Cancer
EMF	Electromagnetic Field
NIEHS	National Institute of Environmental Health Sciences
kD	Kilodaltons
μT	Microtesla
MDS	Myelodysplastic syndrome
NCI	National Cancer Institute
OR	Odds Ratio
Ph +ve	Philadelphia Chromosome Positive
GIPAP	Glivec International Patients Assistance Programme
KNH	Kenyatta National Hospital
CYP	Cytochrome P450 enzyme systems
GST	Glutathione S Transferase
mSv	Milli sievert
WBC	White Blood Cells
PBF	Peripheral Blood Film

ABSTRACT

Background: The relationship between Chronic Myeloid Leukemia and broad range of exposures to occupational, environmental and lifestyle factors known to cause leukemia in general is limited. CML is by and large incurable and treatment is just palliative and life prolonging, with high case fatality rate, even in the best centers. Furthermore treatment is very expensive. Identification of leukemogenic factors is therefore important as they could easily be prevented through simple public health interventions.

Objective: The objective was to determine key environmental, occupational and lifestyle exposure factors that may be associated with Philadelphia chromosome positive chronic myeloid leukemia (CML).

Methods: A case control study involving Philadelphia positive Chronic Myeloid Leukemia cases enrolled in GIPAP clinics at the Nairobi and Aga Khan University Hospitals and two control groups for each case, matched for age and sex: a family and a hospital based control was carried out.

One hundred and eight cases with age and gender matched family and hospital based controls were recruited and a standard questionnaire was administered. Individual data on demographics, occupational, environmental and exposures to chemotherapeutic agents, use of smoking and alcohol and family history of cancer were obtained. Clinical examination was carried out in control subjects. Statistical analysis was done using bivariate and multivariate analysis to look for associations between exposure factors and CML.

Results: The median age at diagnosis of CML cases was 41.32 years with age range of 8-81 years and a male; female ratio of 1.7:1. Most of our cases were concentrated in or around Nairobi. There was no significant correlation found for exposure to benzene or pesticides. Long duration of exposure to pesticides in the family control group was significantly associated (t-test, P=0.017) with risk of CML. The use of piped water suggested a protective effect in family control with OR=0.49(95%CI=0.26-0.93 P=0.03), and a trend of risk in use of well water, (OR=1.67 95%CI=0.96-2.88 P=0.07). There was risk of CML suggested with having lived in timber houses, in the family controls, OR=1.85 (95%CI =0.98-3.50 P=0.056), while living in mud houses was suggested to be

protective in hospital controls, (OR=0.45 95%CI= 0.24-0.87 P=0.016). Exposure to abdominal radiography was positively associated with CML and attained significant levels in both groups, even after regression analysis, Family-OR=9.12 (95%CI-0.080-0.502 P=0.003), Hospital-OR 9.12 (95%CI-1.716-48.484 P=0.010), with mean duration of 5.27 ± 6.84 years, median of 3 years, prior to diagnosis of CML. More cases were exposed to radiographic investigations than controls. Family history of cancer, smoking and alcohol consumption were not found to be associated with CML.

Conclusions: Associations between exposures to organic solvents like pesticides, creosote in timber preservation, chloride compounds used in water treatment and Ph+CML were indicated but were not entirely consistent. Risk of developing CML was suggested with exposure to plain abdominal radiography. Nevertheless, for almost all cases of Ph+CML, other explanations must be sought for.

1:00 REVIEW OF LITERATURE -

1:01 INTRODUCTION: NATURAL HISTORY OF CML

Leukemias are essentially cancers of blood. They are clonal malignant disorders originating from the bone marrow. The malignant cells are disseminated through the blood circulation to almost all organs. Any tissue therefore can be involved and the symptoms likewise can be protean in nature. The leukemias have been classified as acute with survival time of about one year and chronic with survival time of 2 to 3 years or even longer. They are further sub classified into myeloid and lymphoid types, depending on the lineage of the malignant clone.[1]

Chronic myeloid leukemia (CML), also called chronic myelocytic or chronic granulocytic leukemia, is a form of leukemia whose course stays stable for several years before assuming a rapid downhill progression. It results from neoplastic proliferation of a multipotential haematopoetic stem cell. The resultant clone of leukemic cells carries out sub optimally the functions of the normal myeloid cells that they replace. CML belongs to a group of chronic myeloproliferative disorders, but the possession by leukemic cells in nearly all cases, especially in adults and children older than 2 years, of a distinct cytogenetic abnormality, the Philadelphia (Ph) chromosome, clearly distinguishes CML from the other myeloproliferative disorders. [1]

The global incidence of leukemia is 8-9/100000 people each year, with approximately 250,000 new cases occurring worldwide annually. CML accounts for 15% in adults and 5% in children of the total leukemias. [2] CML is not uncommon in Kenya, but lack of diagnostic facilities hampers its proper mapping countrywide. Even in Nairobi there are no facilities for demonstration of Philadelphia chromosome. CML cases recorded in Ministry of Health between 1998-2002 inclusive yielded a negligible number of only 28 cases registered outside Nairobi. On the other hand, records show that mean of 90.3 cases of CML were reported in Nairobi annually. [3]

A retrospective study done at Kenyatta National Hospital, Nairobi, covering 1990-2000, revealed 104 patients with CML, 55 males and 49 females. Age range was 10-72 years with median of 35 years, a decade younger than age of 45 years described among whites. Male to female
ratio of 1.1:1 is a known fact. More than 35% of cases were from Kikuyu tribe, possibly due to proximity to Nairobi.[4]

CML results from monoclonal proliferation of neoplastic hemopoetic stem cell. More than 90% of the cases of CML have Ph chromosome positive leukemic cells replacing marrow cells.[1] This chromosome is an abnormally short chromosome 22, which results from reciprocal translocations between chromosomes 9 and 22. One break occurs at band q34 near the long end of chromosome 9 and another in the upper half of chromosome 22 in the long end at band q11. The translocation breakpoint at chromosome 9 occurs near the 5' end of the c-abl oncogene (ABL), and ABL is, through these events, translocated from its normal location on chromosome 9 to chromosome 22. The summation is reciprocal translocation t(9:22)(q34:q11). The gene located at breakpoint of chromosome 22 is termed as Breakpoint Cluster Region (BCR) and measures 130 Kb. A segment of BCR in which the break occurs is referred to as bcr (small breakpoint cluster region) and measures 5.8Kb. The t(9:22)(q34:q11)gene fusion leads to a formation of a chimeric (hybrid) gene, resulting in production of chimeric mRNA which leads to production of a chimeric ABL protein product which is larger (210Kd) than the normal (145Kd). The mechanism by which this chimeric gene promotes the transition from benign state to fully malignant is still unclear but it is known that it has increased tyrosine kinase activity, leading to abnormal proliferation of myeloid cells.[5] This is usually related to chronic stable phase. After variable periods, other molecular changes occur, leading to disease progression to accelerated and blastic phases.[1] Thus CML is divided into 3 phases, based mainly on the number of immature white blood cells - myeloblasts ("blasts") - that are seen in the blood or bone marrow. Different groups of experts have suggested slightly different cutoffs to define the phases, but a common system (proposed by the World Health Organization) is described below.

Chronic Phase: Patients in this phase typically have fewer than 10% blasts in their blood or bone marrow samples.

Accelerated Phase: The standard definition of this phase is that bone marrow or blood samples have more than 10% but fewer that 20% blasts.

Blast Phase (also called acute phase or blast crisis): Bone marrow and/or blood samples from a patient in this phase have more than 20% blasts.

Possible symptoms of CML include: Fatigue, weakness, night sweats, low-grade fever, pressure under the left ribs from splenomegally, bleeding and bruising. Peripheral blood examination shows profuse neutrophil leukocytosis coexisting with increased number of basophils, esinophils and monocytes. Characteristically, there is a display of myeloid maturation spectrum with promyelocytes, myelocytes, metamyelocytes, band forms and mature elements. The bone marrow is hyper cellular with differential bone marrow and blood counts maintaining the proportion of mature and immature granulocytes that are found in normal marrow. The blast count in the marrow is usually less than 3% in the chronic stable phase at diagnosis and less than 1% after conventional chemotherapy. When they persistently remain higher than 10% after treatment, there is impending transformation.[1]

Chronic phase CML is treated with inhibitors of tyrosine kinase , the first of which was imatinib mesylate (marketed as Gleevec® or Glivec®; previously known as STI-571).[1]

Blast crisis carries all the symptoms and characteristics of either acute myelogenous leukemia or acute lymphoblastic leukemia, and has a very high mortality rate.

Prognosis: The median survival ranges from 2 to 3 years for untreated patients, 3 to 4 years with conventional chemotherapy and 5 to 6 years for those treated with interferon alpha. Five year survival rates have improved to 87% with imatinib and has been found to induce durable responses in high proportions of patients. [1]

1:02 CARCINOGENESIS

The process that brings about malignant change is called carcinogenesis, and requires various steps of cellular alterations. It is apparent that agents that bring these changes about (carcinogens) act on DNA, the molecular blueprint of the cell, causing vital alterations at various sites and stages which finally culminate into malignant cell populations.[1] Various organ and tissue functions are carried out by complex biological processes involving various gene products in accordance with the 'central dogma of molecular biology". According to this dogma, coding DNA sites (genes) code for specific mRNA which in turn determines the particular protein to be synthesized. These proteins are in many ways the functional messengers.[1]

The onset of carcinogenesis is characterized by appearance of cascade of alterations in DNA structure and gene functions. These are brought about by point mutations, amplifications, deletions or translocations. The process gets more complex as the tumor proliferates, further losing the capacity to differentiate and acquires more malignant potential and advances. The various carcinogens, viruses, chronic inflammatory processes, ionizing radiation, chemicals and drugs, atmospheric pollutants all act by altering DNA structure. This alteration in DNA structure leads to oncogene transformation and activation or Tumour Suppressor Gene (TSG) transformation and downregulation or deletion.[6]

The designation of a substance as human carcinogen is a matter of collective scientific judgment. Since 1977, working groups from IARC meet annually in Lyon, France to consider scientific information on exposures with carcinogenic potential.[6]

Exposures have been designated according to their potential for carcinogenicity in humans. The designations are as follows[7]

Group 1- Carcinogenic to humans. Sufficient evidence of information relating to humans based on epidemiological studies.

Group 2A- Probably carcinogenic to humans. Limited evidence for humans, but sufficient for animals.

Group 2B- Possibly carcinogenic to humans. Limited evidence both in humans and animals.

Group 3- Not classified as to its carcinogenicity in humans. Lack of carcinogenicity both in humans and animals.

Group 4- Probably not carcinogenic to humans. All other exposures fall here.

The estimated risk of cancer depends upon the level and duration of exposure to the population.

Sandler and Ross[8] and Greaves[9] have extensively reviewed current etiology of leukemia. Risk factors thought to be involved in leukemia are summarized as follows:

TABLE 1: ESTABLISHED AND POTENTIAL RISK FACTORS FOR ADULT AND CHILDHOOD LEUKEMIAS

Risk Factor	**Adult Leukemia**	**Childhood Leukemia**
Genetic factors	Family history	Concordance of infant leukemia in twins
Genetic syndrome		Downs syndrome, Blooms syndrome, ataxia telengectasia, Fanconi's anaemia, Familial monosomy 7
Ionizing radiation	-atomic bombing -nuclear accident/testing -occupational exposure -radiotherapy -residential radon	-in-utero exposure to diagnostic x-rays -paternal pre-conception exposure
Chemical exposure	-benzene -petrochemicals -organic solvents -pesticides -chemotherapeutic drugs	-parental exposure to solvents/pesticides -maternal exposure to topoisomerase II inhibitors
Others	-viral infection (HTLV I) -diet -smoking	-common infections (?) -diet (maternal and child) -parental smoking -previous maternal fetal loss -maternal age and alcohol consumption -high birth weight

Assembled from Pui[10], Sandler and Ross, [8] Greaves[9]

The fact that only one of a pair of identical twins usually develops CML suggests that finding the specific cause for leukemia will be difficult if not impossible.[11] However, by studying large numbers of people all over the world, researchers have found certain factors that increase a person's risk of developing CML. Non-genetic factors may include diet, exercise, or exposure to other substances present in our surroundings

1:03 CHEMICALS OF RISK FOR LEUKEMIA CARCINOGENESIS

1:03:1 BENZENE - EXPOSURE & RISK OF DEVELOPING LEUKEMIA

Benzene is an important commercial product, with approximately 2 billion gallons produced annually in the United States. It is used mainly as a starting material in the synthesis of numerous chemicals. The main public health issue concerning benzene in the United States and other developed countries is its use as a component of gasoline and the fact that the shift to unleaded gasoline has tended to increase its benzene content.[12-15] In the United States, the current benzene content of gasoline is generally below 1%, but in other countries super unleaded gasoline can contain greater than 5% benzene. [16]

Another major source of public exposure to benzene is cigarette smoking. A pack-a-day smoker inhales approximately 2 mg/day, and nonsmokers who live, travel, or work with smokers are exposed to benzene through side-stream or second-hand smoke. [17] Because benzene is also present in many foodstuffs, the background level of benzene intake for nonsmokers has been estimated at 0.5 mg/day.[18].It is therefore difficult, if not impossible, to avoid exposure to benzene. Furthermore, benzene and solvents containing more than 1% benzene continue to be used in many countries, including China, former members of the Soviet Bloc, South America [19-22], and even Spain, where a case of benzene-induced aplastic anemia was recently described. [23]

Occupational exposure to chemicals, especially solvents containing benzene, has been associated with leukemia. [24] Workers exposed to benzene with exposures greater than 200 ppm/year have more than 20 times greater risk of developing AML than the general population. [25] Benzene's toxic effects on the marrow were first described in 1897 [26,27] and

the first case report of leukemia associated with benzene exposure appeared in 1928. [28]The ability of benzene to cause AML was first fully established in the 1970s following epidemiologic studies in Italy and Turkey.[29-31] There have been numerous reports of smoldering leukemias and preleukemias caused by benzene. These would likely be classified as myelodysplastic syndromes today. Recent studies in China, led by Hayes and Yin [32,33] and jointly sponsored by the NCI and the Chinese Academy of Preventive Medicine (CAPM), have established that benzene causes AML and MDS in humans and have also suggested that benzene exposure maybe associated with non Hodgkin's lymphoma, lymphocytic leukemia, lung cancer, and nasopharyngeal cancer.

The Philadelphia chromosome was observed by classical cytogenetics in a case of preleukemia (leucopenia) resulting from chronic exposure to benzene for 4 years without the signs of leukemia. After further 4 years without exposure, the aberration disappeared. [34] Biomarkers of the early effects of benzene, to depict possible mechanisms of leukemogenesis include hematotoxicity (complete blood cell counts), gene mutations (glycophorin A[GPA] and ras, etc.), and chromosome aberrations.[31]

Pliofilm cohort in Ohio, on which the primary epidemiologic study of people occupationally exposed to benzene was based, had an increased risk of leukemia of about 3.4.[35] Yu et al suggested a possible link between living in an area with high exposure to airborne petrochemicals (derivatives of petroleum or natural gas) and risk of developing leukemia in a study of 171 individuals with leukemia in Taiwan.[36] Among study participants under the age of 20, there was no link between increasing residential petrochemical exposure and risk of leukemia. Among study participants between the ages of 20 and 29, increasing residential petrochemical exposure did increase the risk of leukemia. Bjork et al estimated odds ratio for developing CML from benzene exposure from petrochemicals (fuel and exhaust gases) were close to unity for all categories of intensity in Ph +ve subjects. For the data on organic solvents, an effect was found for moderate or high intensity of exposure (odds ratio (OR) 3.4, and for long duration (15-20 years) of exposure (OR 2.1) [37]

Coal tars and coal tar pitches, and untreated and mildly treated mineral oils are known to be human carcinogens based on sufficient evidence of carcinogenicity in humans. Creosote is a chemical primarily used for the preservation of wood, accounting for over 97% of current coal tar production. There is also some evidence for the carcinogenicity of creosotes in humans.[38]

1:03:2 PESTICIDES AND RELATED ORGANOCHLORIDE COMPOUNDS

Aromatic organochlorides, include chlorophenoxy pesticides (DDT and its metabolite, DDE), combustion by-products such as polychlorinated dibenzo-P-dioxins (PCDDs) and dibenzofurans (PCDFs) and industrial products such as polychlorinated biphenyls (PCBs) and poly brominated biphenyls (PBBs). These compounds are chemically stable over many decades; they are passed along food chains, accumulate in fatty tissue, and are eliminated slowly from the body. Animal studies have shown that PCBs and DDT are carcinogens at high doses.[39]

Two reviews have reached similar conclusions regarding role of pesticides in childhood leukemias. Zalm and Ward from National Cancer Institute (NCI) concluded in their review of 17 case controlled studies and one cohort study that the literature supports a possible role for pesticides in development of childhood leukemias.[40] After reviewing the same literature, Daniels and colleagues in University of North Carolina concluded that leukemia association was more consistent among children whose parents had occupational pesticide exposures than among parents with residential pesticide exposure.[41]

Chlorination products like trihalomethanes and bromodichloromethanes are used in treatment of surface water and have been associated with increased incidence of cancer.[42] This was demonstrated by Kasim et al in Canada who observed a non significant increase in risk of CML with increasing years of exposure to chlorinated surface water for more than 36 year.[42] Cohn and coworkers in New Jersey also found Relative Risk of CML at

1.79(95%CI=0.90-3.55) for towns with highest stratum of trichloroethylene exposure versus towns with no detectable TCE in drinking water.[43]

1:04 SMOKING

Over the past half century, there have been numerous studies on the role played by cigarette smoking in the pathogenesis, pathophysiology and the causes of a variety of diseases in addition to lung cancer. Smoking is now known to be a cause of many types of cancers including cancers of mouth, lip, oro- and hypopharynx, larynx, esophagus, pancreas, bladder, kidney and stomach.[44]

That smoking should be a cause of so many different types of cancer should not be surprising: inhalation is a very effective way of distributing chemicals throughout the body and tobacco smoke contains at least 50 chemicals that are known to be carcinogenic in animal experiments including radioactive polonium, benzene, 2-naphthylamine, 4 amino biphenyl, and various polycyclic aromatic hydrocarbons and nitrosamines.[44] As is the case with lung cancer, risks of virtually all other smoking related cancers rise with average number of cigarettes smoked per day, degree of inhalation and years of smoking.

Smoking can increase the risk for leukemia. Fernberg and colleagues carried out a prospective cohort study to explore effect of tobacco smoke on leukemia.[45] An increased risk of AML was observed in current smokers. However, they reported current or former smokers did not have an increased risk of CML. In a study done in Sweden by Bjork et al, no relation between cumulative smoking dose (pack-years), and risk of disease was found.[37]

1:05 DRUGS

Drugs that cause or prevent cancer fall into 3 main categories:-

1) Drugs used in cancer therapy; these agents have been found to increase risk of leukemia in particular. Important classes of anticancer agents include chemicals that attach themselves to DNA in a manner that makes replication

difficult and error prone. Impaired replication may kill rapidly growing cancer cell line, but it may also lead the slower growing cells to reproduce with mutations that eventually lead to neoplasia. Chlorambucil, Cyclophosphamide, Melphalan, Busulphan, Thiotepa and Methyl-CCNU are all recognized as causing leukemia. [46]

2) Immunosuppressive agents; used to get the body to accept transplanted organs and to arrest autoimmune diseases. Since one of the functions of the immune system appears to be removal of aberrant, precancerous and cancerous cells, immunosuppresion also raises risk of cancer occurrence. [46]
3) Hormone and hormone antagonists; many of body's internal regulatory mechanisms are under hormone control and changing these with a drug appears to increase risk of some cancers and to decrease risk of others. [46]

Some of the drugs and radiation used to treat other types of cancer may increase an individual's risk of CML. Low-dose radiation used in the past to treat a variety of non-malignant conditions has been associated with an increased incidence of leukemia, of which 20-30% were CML. Various chemotherapy and immunosuppressive drugs have been associated with an increase in CML The chemotherapeutic treatment of cancer induces secondary myeloid diseases, including AML and MDS. This induction is a major clinical problem and accounts for up to 10 to 20% of all AML and MDS cases diagnosed.[47] Drugs presenting the most risk are alkylating agents, such as melphalan and busulfan, epipodophyllotoxin and other topoisomerase II inhibitors. Anthracyclines and anthracine-diones are far less leukemogenic than other topoisomerase II inhibitors. About 8% of patients treated with alkylating agents developed AML within 5 years after beginning treatment.[48] Children with ALL treated with epipodophyllotoxins have a 5 to 12% cumulative risk of AML. [49]

Watanabe and colleagues reported cases of leukemia developing in growth hormone (GH) users in twelve Japanese cases;[50] five each of AML and ALL, and one each of CML and malignant histiocytosis. The underlying diseases of these patients consisted of 8 idiopathic disease, 3 tumors and one Fanconi's anemia. Leukemia occurred during GH treatment in 9 cases and after cessation of GH in 3. The longest interval from the

cessation of GH therapy was 10 years. GH administration from a younger age tended to be linked to myeloid type of leukemia. Waller et al also reported a case of a 40-year old patient with small cell lung cancer (SCLC), treated with combined modalities including high-dose chemotherapy with subsequent autologous peripheral blood progenitor cell transplantation plus adjuvant radiotherapy.[51] The patient achieved complete remission with regards to the primary disease. After an interval of 28 months, he was diagnosed with CML

1:06 IONISING RADIATION

Just over 110 years ago in 1895, Roentgen discovered the x-ray and revolutionalised the practice of medicine. Over 60 years ago in 1945, World War II was brought to an end after atomic bombs were dropped on Hiroshima and Nagasaki. The first nuclear power plant began operating in 1957 and now, nearly 20% of electricity produced each year in USA is from nuclear energy. In some countries such as France, over 70% of electrical power is from nuclear sources.[52] Over the course of a century, radiation has become pervasive in our world, sometimes with deleterious consequences. The Chernobyl nuclear reactor accident, for example, occurred in 1986 and spewed radioactivity throughout Europe and Asia.[52] The beneficial uses of radiation have also been widespread, most notably in treatment of cancer and diagnosis of disease.

We live in a sea of low level invisible radiation. Odourless, colourless, ionizing radiations continually bombard our bodies throughout life, and it is the release of ionizing energy within cells that can cause cancer.[52]

Conclusive evidence that radiation can cause cancer comes from studies of Japanese atomic bomb survivors, pioneering radiologists and patient populations. While the single most important study is of the survivors of atomic bombs, there are well over 100 studies of patient population linking radiation to cancer which confirm and extend our knowledge of radiation effects.[52] The major unanswered questions revolve around magnitude of the risk at low levels of exposure, the ameliorating effect of spreading

exposure over time, and lifetime risk following exposure in childhood. The amount of radiation needed to double the risk of cancer is quite large and of order of 2000 mSV, nearly a 1000 times the annual exposure received from natural background sources.[52]

Some types of radiation are more effective in causing cancer than other types, for example, alpha particles, which are emitted during decay of radon and radon progenies and neutron, which can be experienced during high altitude air travel. Children and females appear somewhat more sensitive to effects of radiation than adults and males.[52]

Leukemia can occur in excess within 2 years after exposure to ionizing radiation, but risk appears to return to near normal levels after 20-30 years have passed. Other cancers take 10 or more years after exposure before excesses can be detected and risk appears to remain high throughout life.[52] Age at exposure can modify risk to cancer, for example, radiogenic thyroid cancer is not apparent among adults exposed after age of 20, and radiogenic breast cancer is not seen among women exposed after menopause. Major radiation induced cancers are leukemia, female breast cancer, thyroid and lung cancer.

Watch-dial painters in early part of 20th century used radium containing paint to make the dial glow in dark. The painters, mostly women, would twirl the brush on the tongue, to make a point in the brush. The radium that they ingested via this route was concentrated in their bones and osteogenic sarcoma was a relatively common result. Also radiologists who practiced in early 20th century had an elevated rate of leukemia as a result of their occupational exposure to radiation.[53]

Leukemia in adults is strongly associated with occupational exposure to ionizing radiation. One of the greatest risks to astronauts in traveling to Mars or beyond may be leukemia from cosmic radiation exposure. There is little evidence, however, that non-ionizing radiation such as electro-magnetic fields (EMF) induces leukemia. Indeed, two recent studies have shown that EMF exposure is not a major risk factor for leukemia in children[54] or in adults.[55]

Radioactive iodine is used in medicine to treat and diagnose disease; it is released during nuclear reactor operations and it is a component of radioactive fall out from weapons testing.[56,57] The thyroid gland usually absorbs most of the radioactive iodine ingested or injected and the adult thyroid gland appears relatively immune to carcinogenic effects of radiation and other organs receive much lower doses.

^{131}I also has an eight day half life which means that the radiation released during decay is protracted over time, which might allow repair of radiation damage more readily than if the dose were received all at once. Very large studies of primary adult populations given ^{131}I in medical settings have failed to find consistent increases in any cancer, including leukemia and thyroid cancer, despite substantial exposures.[58,59]

Radioactive iodine treatment of thyroid cancer is associated with an increased incidence of CML in some case reports and series. CML has also been reported after heart transplants where radiation therapy was given. Walgraeve et al from Department of Hematology, University Hospital Gasthuisberg, Leuven, Belgium, reported a case of a patient who developed Philadelphia chromosome-positive CML 5 years after successful treatment for thyroid carcinoma with 131 radioactive iodine.[60]

Chap and coworkers. from Department of Medicine, UCLA School of Medicine, reported development of CML 11 years after radiation therapy for Histiocytosis X.[61] Frist and colleagues in Tennessee, reported a case of recurrent cardiac rejection in a heart transplant recipient successfully treated with total lymphoid irradiation. Five years after transplantation chronic myelogenous leukemia was diagnosed in this patient.[62]

1:07 MEDICAL RADIOGRAPHY AND X-RAYS

The 1st report that pre-natal x-ray exposures were associated with increased risk of leukemia and solid cancers during childhood were published in 1950s.[56,57] Evidence for causal association comes almost entirely from case-controlled studies, whereas

practically all cohort or prospective studies, including atomic bomb survivors exposed in utero, find no association.[63]

Most of the case-controlled studies of medical exposure to diagnostic x-rays during pregnancy are consistent with 40-50% increased risk of leukemia, lymphoma, Wilms tumour, neuroblastoma or brain cancer.[63] Such similarity in excess relative risk estimate for each type of cancer, suggests possible underlying bias that has not been identified. Nonetheless, the medical profession has acted prudently and pelvimetry x-rays have been largely replaced by ultrasound procedures, which produce images from sound waves and do not involve ionizing radiation.

Overall, the leukemia risk increased about 7% for every 10 mSv exposure. (The amount of radiation is measured in grays; the estimated biological effects of radiation are measured in Sieverts [Sv]. 1 Sv = 1,000 milliSieverts [mSv]). All of us are exposed to small amounts of background radiation — about 2 mSv per year (the amount will vary depending on where you live). For most people, exposure to radiation is highest when they receive medical procedures. However, the amount of radiation exposure is very small. The amount of radiation exposure is about 0.01 mSv with dental X-rays, 0.02 mSv with a chest X-ray, 0.7 to 1.3 mSv for a body X-ray (hip, spine, abdomen, etc.), 8 mSv with a chest CT, and 10 mSv with a CT scan of the abdomen or pelvis.[64]

Preston-Martin and colleagues conducted a study of 136 Los Angeles County residents aged 20-69 with CML diagnosed from 1979 to 1985 and 136 neighborhood controls.[65] During the 3-20 years before diagnosis of the case, more cases than controls had radiographic examinations of the back, gastrointestinal (GI) tract and kidneys, and cases more often had GI and back radiography on multiple occasions. The association was strongest for the period 6-10 years before diagnosis, and the effects of radiation exposure during this period remained significant after consideration of other risk factors in a logistic regression analysis.

1:08 HEREDITARY OR GENETIC FACTORS

Familial susceptibility has been found virtually in every form of cancer in humans, including tumours classified as carcinomas, sarcomas, brain tumours, leukemia/lymphomas. Some of this aggregation may be due to shared exposure to carcinogens, the rest is presumably due to inherited susceptibility. In general, a person who has a parent or sibling with cancer at a young age has about two-fold or a higher risk of developing cancer.[66]

There are no clear hereditary factors associated with CML. Identical twins of patients with CML are at no greater risk of developing CML than other siblings. This strongly suggests that environmental factors are much more important than genetic factors in the development of CML. It is a scientific mystery as to why only one of a pair of identical twins will develop CML, since the genetics are identical and environmental exposures are similar, if not the same.[11]

HLA is the histocompatibility system that is used to match people for bone marrow, liver and kidney transplants. One study has found that a specific HLA type, DR4, is associated with a lower incidence of CML; however researchers have not yet identified the reason for this decrease.[11]

Loeffler et al described relationship between *BCR-ABL*$^{+}$ CML and genetic polymorphisms in the *CYP1A1, GSTM1,* and *GSTT1* genes.[67] Their data indicate a reduced risk for CML in individuals carrying the mutant allele *CYP1A1*2A.* This is the first report of a protective role of this allele, which is a risk factor for childhood ALL according to Krajinovic et al.[68] The latter finding is explained by the elevated metabolizing activity associated with CYP1A1*2A, which results in enrichment of reactive intermediates of some carcinogens, for example, polycyclic aromatic hydrocarbons (PAHs), in phase I of metabolism.[69] These intermediates must be detoxified

by the phase II enzymes such as GSTs. Accordingly, homozygous *GSTM1* or *GSTT1* deletions are risk factors for childhood ALL and several other neoplasia.[68,69] In contrast, the relevant carcinogens for CML seem to be detoxified by *CYP1A1*. This should mean that the phase II metabolism is not needed for detoxification of these carcinogens, which is in accordance with the observation that there is no association between *GSTM1* or *GSTT1* deletions and CML risk.[67]

The result that *CYP1A1*2A* is a protective factor against CML means (1) that genetic susceptibility may be relevant for CML risk, (2) that environmental carcinogens seem to play a role in the etiology of CML, and (3) that the carcinogens relevant for CML risk might differ from carcinogens relevant for other malignancies, for example, PAHs. Moreover, even different hematological malignancies seem to be preferentially attributed to different chemical carcinogens. Taken together with other results, the available knowledge of inherited genetic and environmentally acquired susceptibility might be relevant for predicting individual risk patterns for hematological and other malignancies

2:0 JUSTIFICATION

For many types of cancer, progress in the areas of cancer screening and treatment has offered promise for earlier detection and higher cure rates. The risk factors are different for different types of cancer. An awareness of these risk factors is important because 1) some risk factors can be changed (such as smoking or dietary intake or some environmental exposures), thus decreasing the risk for developing the associated cancer; and 2) persons who are at high risk for developing a cancer can often undergo regular screening measures that are recommended for that cancer type. Researchers continue to study which characteristics or exposures are associated with an increased risk for various cancers, allowing for the use of more effective prevention, early detection, and treatment strategies. Because the average age at diagnosis is over 45 years, it is suspected that an environmental exposure over a long period of time is required to cause CML

CML is by and large incurable and treatment is just palliative and life prolonging. Furthermore it is very expensive. Identifying the causes of leukemia is therefore an important public health concern, as it could lead to an eventual prevention, and early detection of this disease. Epidemiologic studies concerning this disease have not been carried out locally and in Africa as a whole, though cases of CML are quite prevalent in our country. This study intended to look at sociodemographic details of patients diagnosed with CML and already on treatment, in attempt to link any factors that may be associated with occurrence of this disease. The risk factors so far established as leukemogens are found locally and part of lifestyle, for example chemicals like benzene, pesticides and radiation, and this study will determine any links or associations between the selected exposure factors and CML in our study population.

3:0 NULL HYPOTHESIS

The null hypothesis was that there is no relationship between exposure variables and Chronic Myeloid Leukemia.

4:0 OBJECTIVES

4:1 GENERAL OBJECTIVE

The general objective was to determine key environmental, occupational and lifestyle exposure factors that may be associated with Philadelphia chromosome positive (Ph+) chronic myeloid leukemia (CML).

4:2 SPECIFIC OBJECTIVES

The specific objectives were;

1. To determine characteristics of exposure factors, specifically of Chronic Myeloid Leukemia patients and controls (sociodemographic, occupational, environmental);

2. To describe exposure to carcinogenic treatment modalities in patients with chronic myeloid leukemia and controls and

3. To determine associations between Chronic Myeloid Leukemia and sociodemographic, occupational and environmental exposure factors.

5:0 STUDY VARIABLES

Dependent study variable was whether the study participant was Philadelphia chromosome positive CML or not, whereas the independent variables were the exposure factors.

Demographics were looked at in terms of age and sex of study participant, date and age of diagnosis of CML, residence of the study participant and the BMI.

Occupational exposure to benzene and organic solvents was either by working in petroleum, plastic, paint, oil and motor repair industries and the duration of the exposure. Exposure to pesticides and herbicides was by working in farms or gardens and using these substances at least for more than one year.

Occupational exposure to cytotoxics was by working in medical field administering chemotherapeutic agents, whereas occupational exposure to radiation was by working in radiology and radiotherapy units.

Environmental exposures were investigated by inquiring the kind of residential housing material, the source of drinking water and the kinds of fuel used for cooking.

Lifestyle risk factors were exposure to cigarette smoking and consumption of alcoholic beverages and exposure to carcinogenic treatment modalities was by ever being treated with cytotoxic or immunosuppressive agents or exposure to radiotherapy or diagnostic radiography.

6:0 STUDY METHODOLOGY

6:01 STUDY DESIGN

The study was a case control study in which cases were matched by age with gender familial and hospital control population.

6:02 STUDY SITES

The study sites were Glivec International Patients Assistance Programme (GIPAP) clinics which run at the Nairobi and Aga-Khan University Hospitals. This is an assistance programme which provides Ph chromosome positive patients with Imatinib Mesylate (marketed as Gleevec® or Glivec®) at no cost. It began in 2002 in Kenya, supported by Novartis Pharma, The Max Foundation and Axios International (the latter two are international organizations which run donor programmes). All CML patients in Kenya who can afford to do chromosomal studies for Philadelphia Chromosome are eligible to join the programme, the majority being referred from Kenyatta National Hospital. The patients also continue to be seen in the KNH heamatoncology clinic but at further time spans. Some patients are directly referred by their private doctors to any of these clinics, especially at Aga Khan University Hospital, which runs its own clinic. As at February 2008, since inception, there were 112 patients enrolled in the programme at The Nairobi Hospital clinic and 33 patients at Aga Khan University Hospital. The cases and familial controls were recruited at these clinics.

The hospital controls were recruited from the KNH medical outpatient and specialized clinics.

6:03 STUDY POPULATIONS

6:03:1Cases

The cases comprised of patients with diagnosis of Philadelphia chromosome positive CML, enrolled and on follow up with GIPAP programme.

6:03:2Controls

Two control groups were selected for each case, matched with respect to gender and age: one being a hospital based control, sourced from Kenyatta National Hospital's outpatient clinics, and the other a family member of the case, being a 1st, 2nd or 3rd degree relative..

6:04 CASE DEFINITIONS

Diagnosis of Chronic Myeloid Leukemia was based on a peripheral blood film features of profuse neutrophil leukocytosis coexisting with increased number basophils, easinophils and monocytes with display of full maturation spectrum of myeloid series- promyelocytes, myelocytes, metamyelocytes, band forms and mature elements, confirmed by hyper cellular bone marrow with myeloid hyperplasia. All patients were cytogenetically confirmed as Philadelphia chromosome positive, which was determined by FISH (fluorescent in situ hybridization) method. (Appendix 1) This information was obtained from the patients clinical records.

Hospital controls were patients with a non malignant, non-hematological disease with physical examination not suggestive of any malignancy or hematological disorder and a normal haemogram parameters- WBC<11*109/L and normal morphology of WBC on PBF. They were patients from medical out patient clinics in KNH, with any other medical condition.

6:-05 INCLUSION CRITERIA

All Philadelphia chromosome positive CML patients on follow up in GIPAP programme were included and family members who are 1st, 2nd, or 3rd degree relative of the patient were included as familial controls.

6:06 EXCLUSION CRITERIA

Those that decline to consent for interview and controls suffering from any other malignancy or with abnormal FHG or PBF were excluded.

6:07 SAMPLE SIZE

The sample size of 106 per group was obtained according to the formula for case control studies.[70]

$$n = \frac{(Z_{1-\alpha/2}\sqrt{\{2P_2(1-P_2)\}} + Z_{1-\beta}\sqrt{\{P_1(1-P_1)+P_2(1-P_2)\}})^2}{(P_1-P_2)^2}$$

Where:

n= sample size

$Z_{1-\alpha/2}$= 1.96 (5% significance level)

$Z_{1-\beta}$= 0.84 (power at 80%)

P_1= proportion of exposed cases

P_2= proportion of exposed controls

This was by using OR of 7.6 with exposure ratio in cases of 3.1% and in controls of 0.8% for working as a painter to develop CML in a study by Mele and coworkers in Italy.[71]

6:08 STUDY MATERIALS AND TOOLS

Each recruited subject was administered a detailed standard questionnaire. Patient's particulars and demographic history were obtained in terms of residential details; geography of the residential surroundings, kind of house lived in, water and fuel source prior to diagnosis. A lifelong occupational history was obtained, focusing on all jobs held for at least 1 year, including work task, department, and duration of employment. Evidence about specific exposures were enquired about exposure to benzene and organic solvents by working in petrochemical, paint, plastic or motor repair industries;

application of pesticides by working as a gardener, horticulturist, farmer, or farmhand; handling of cytotoxics by working as a chemotherapist, nurse or pharmacist, exposure to radiation by working in radio imaging or radiotherapy units. Furthermore, details about smoking and alcohol consumption habits were also obtained. The questions on medical history before the time of diagnosis focused on chemotherapy and radiotherapy as well as treatment with isotopes and exposure to x-rays were asked.. Body Mass Index (BMI) was calculated for all subjects according to the formula weight in kilograms divided by square of height in meters. The information was recorded in the questionnaire by the principal investigator or the trained assistant.

6:09 SCREENING AND RECRUITMENT

The GIPAP clinic at Nairobi Hospital is held every alternate Saturday, and at AKUH, on Tuesday, Thursday and Friday afternoons. Patients were selected from both clinics. The PI and the research assistant visited these clinics to recruit the cases and their accompanying familial controls. All patients attending these clinics had the details of the study explained to them and if they consented, they were recruited and administered the questionnaire. The systematic recruitment continued until the desired sample size was achieved. If the patient was accompanied by an appropriate familial control subject, he/she was also recruited in the same fashion; otherwise the patient was requested to bring an appropriate family member on the next visit.

On average, 2-4 patients attended AKUH GIPAP clinic per clinic day. The Nairobi Hospital GIPAP clinic had a higher attendance of about 10-15 patients per clinic day. 3-5 patients and their accompanying relatives were recruited per clinic day. One week before the data collection began, the research assistant was trained on how to fill the questionnaire.

6:10 CLINICAL METHODS

6:10:1 Subject recruitment

1) Patients

Every patient attending GIPAP clinic with diagnosis of CML was eligible for the study. The patients had the study explained to them and if they consented and signed a consent form, they were considered recruited and a questionnaire was administered. Recruitment was repeated on each clinic day until desired sample size was achieved.

2) Control Groups

Patients accompanied by eligible family members were also explained the study and if they consented, were recruited, and the questionnaire administered to them. Patients who came alone or not accompanied by a family member eligible to be a familial control subject were explained the criteria for the ideal familial control subject and requested to be accompanied by one on their following clinic visit. Transport was refunded to these subjects.

Each week, a similar number of age and gender matched hospital controls were also picked from medical outpatient and specialized clinics. The PI went over the patient files to select appropriate age and gender matched individuals. Once a control subject was identified, the study was explained to him/her, and if consent was obtained, the questionnaire was administered. If no consent was given, another control was identified in the same way and the procedure repeated.

6:10:2 Clinical Evaluations

Physical examination was performed on all recruited control subjects to rule out any definite hematological or oncological disorder, with special emphasis on weight loss, lymphadenopathy, splenomegaly or any masses.

After the interview, 2mls of venous blood was drawn from cubital veins of the controls for haemogram and peripheral blood film. This was put in EDTA tubes and analyzed within 8 hours in the hematology unit using the Cell Diyn 1300® model of automated cell counts.

The result of the blood analysis was communicated to the subject by phone. Subjects with abnormal result were guided to receive appropriate medical attention and subsequently excluded from the study.

6:11 DATA MANAGEMENT AND ANALYSIS

Data was entered into MS Access, cleaned and verified. Statistical analysis of data was undertaken using Statistical Package for Social Sciences (SPSS) version 11.5. Data was presented in form of tables, graphs and pie charts. Descriptive statistics such as means, medians and standard deviation were determined where applicable. Duration of exposure, age and BMI were calculated as continuous variables. For purposes of diagrammatic presentation, the number of persons per district and age were used as categorical variables. Exposure variables were categorized in a binary format as ever or never exposed. To assess the significance of differences in continuous data, the t-test was used and for categorical data, chi-square was used. Comparison of results using Odds Ratio with 95% Confidence Interval was done. Multivariate risk analysis was done using logistic regression. P values of <0.05 was considered significant. P values in the OR tables were obtained from chi square tests computed for 2*2 tables with appropriate statistical correction where necessary. Data was analyzed and both sets of analysis presented separately for each control groups to avoid non-differential bias.[72]

7:0 ETHICAL CONSIDERATIONS

Ethical approval to carry out this study was obtained from Kenyatta National Hospital/UON Ethics and Research Committee and patients were enrolled for study only after giving informed written consent. All information obtained from the study had been handled in confidence and used only for intended purpose.

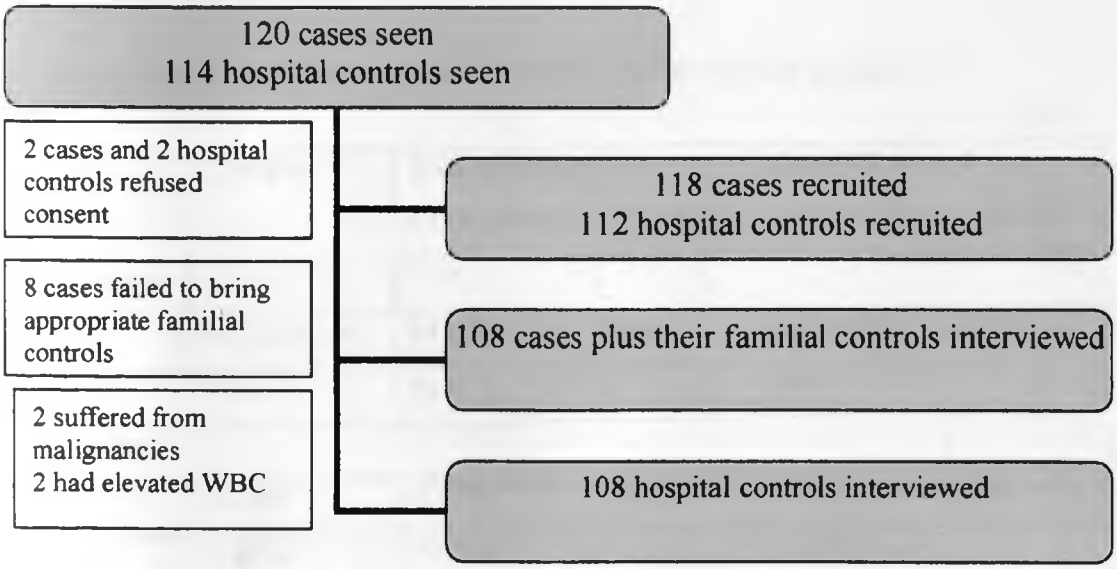
The only invasive procedure was collection of blood for full hemogram in the control population. The results of blood test were communicated to the subjects. Incase of abnormal results in the FHG of control population, they were guided for the appropriate clinical interventions. No extra financial cost was borne by the patients or controls as they were interviewed when they came for their routine clinic. The familial controls requested to accompany the patients were reimbursed their transport costs. Questionnaire was in simple English which could easily be translated into Kiswahili or local languages.

8:0 RESULTS

A total of 120 cases, 108 familial controls and 112 hospital controls were recruited between August 2008 and January 2009.

Twelve cases were excluded for various reasons: two did not give consent and ten failed to bring appropriate familial control subjects. Four hospital controls were excluded: two had a history of suffering from previous malignancy; one from breast cancer and other one from Kaposi's sarcoma and two had elevated WBC counts. One hundred and eight cases with two controls for each were analyzed. Full hemogram and peripheral blood film was done for all controls.

FIGURE 1: PATIENT FLOW CHART



8:01 DEMOGRAPHIC CHARACTERISTICS

Patients' age ranged from 8-81 years with the median age of 40.5 and mean age of 41.31± 15.33. Mean age in males was 40.35±15.74 while in females was 42.97±14.69. The mean age in familial and hospital control groups was 41.07±15.21 and 41.2±14.83 with medians of 41.0 and 40.0 respectively.

There were 68 (63%) males and 40 (37%) females were enrolled into the study, with Male: Female ratio of 1.7:1, which is comparable with previous literature.[4]

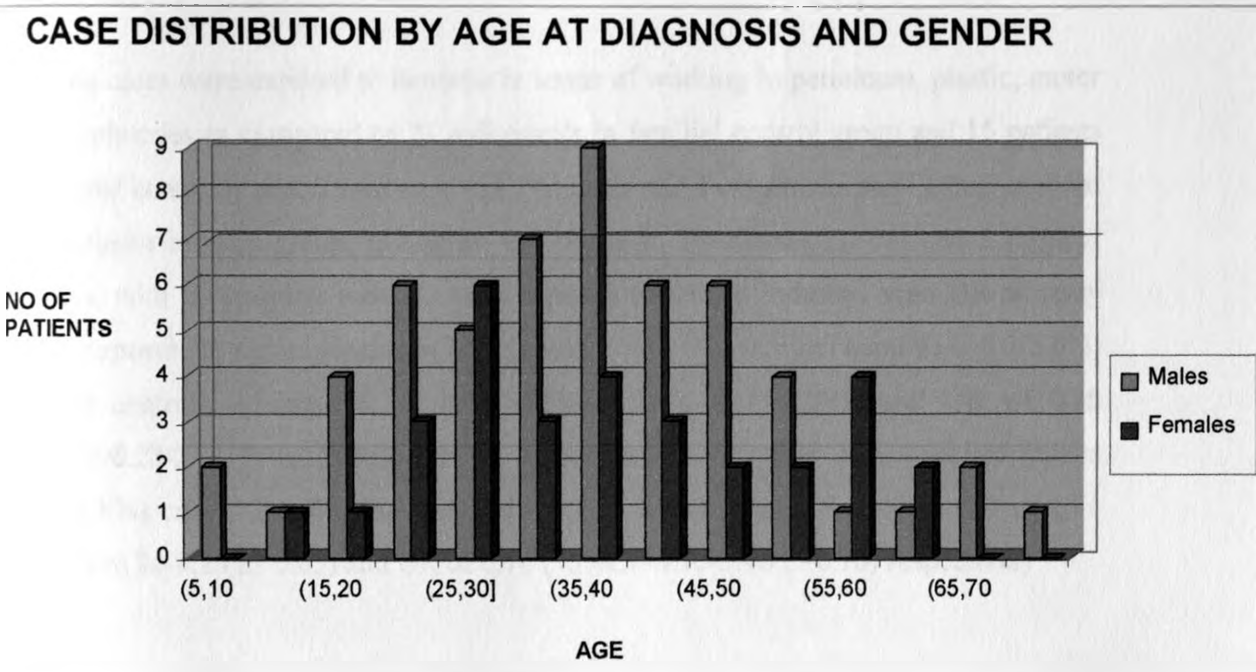
The most prevalent age group for the cases was in age bracket of 35-40 years, which was also the most prevalent age group in males, while in females it was 25-30 years. Cases with extremes of age, the youngest and oldest patients were also males. (Figure 2 and 3)

The results of the demographic characteristics and BMI for the 3 study groups are summarized in the table below:

TABLE 2: DEMOGRAPHIC CHARACTERISTICS OF STUDY SUBJECTS

	CASES	**FAMILAIAL CONTROL**	**P VALUE**	**HOSPITAL CONTROL**	**P VALUE**
Age in years					
Mean	41.32±15.34	41.07±15.21	0.90	41.2±15.09	0.95
Median	40.5	41.0		40.0	
Occupation					
Indoor	63.3%	73.5%	0.11	56.3%	0.33
Out door	36.7%	26.5%		43.7%	
BMI					
Mean	25.26±6.25	25.04±5.69-	0.70	23.16±4.41	0.08
Median	23.93	23.84		22.42	

FIGURE 2: GRAPH TO SHOW CASE DISTRIBUTION BY AGE AND GENDER



Cases and control groups were more involved in indoor occupations rather than outdoor, although the hospital control group was more equally distributed (indoor=56.3% and outdoor=43.7%). The familial control group had more indoor occupations, at 73.5% of the whole group than the rest. (Table 2)

Majority of patients seen came from the vicinity of Nairobi city, which was the study site, and its immediate environs. There were also a high number of patients from Mombasa, which is the second largest city in Kenya. Otherwise, the cases were mainly from the southern, more populated part of Kenya except 2 patients who came from North Eastern province. Nine patients had lived out of Kenya at some points in their life. (Figure 4)

8:02 EXPOSURE TO BENZENE

Thirteen cases were exposed to benzene in terms of working in petroleum, plastic, motor repair industries as compared to 21 individuals in familial control group and 15 patients in hospital control group. There were 4(3.7%) cases and 4 controls in each group exposed to petroleum in each group, giving an OR of unity. Two cases (1.9%) and 2 familial controls, with no hospital control, were exposed to plastic industry, with OR at unity again. Exposure to paints yielded 6(5.6%) cases, 13(12.0%) familial controls and 7(5.6%) hospital controls, giving OR of 0.43 (95%CI0.16-1.18 P=0.093) and OR of 0.85 (95%CI=0.28-2.61 P=0.76) respectively. Exposure to solvents in motor vehicle repairs has 3(2.8%) cases, 2(1.9%) familial and 4(3.7%) hospital controls, giving OR of 1.51 (95%CI=0.24-9.25 P=0.65) and OR of 0.74 (95%CI=0.16-3.40 P=0.70) respectively

8:03 EXPOSURE TO PESTICIDES

There were 46(43.0%) cases exposed to pesticides in terms of working on farms, gardens or as horticulturalists, either as an occupation or hobby, compared to 36 (33.3%) in familial control group and 46 (42.6%) patients in hospital control group. This was found to be non significant with OR of 1.51 (95%CI=0.87-2.62 P=0.15) in comparison to familial controls and OR 1.02 (95%CI=0.59-1.75 P=0.95) in comparison to hospital control group.

FIGURE 3: MAP OF KENYA SHOWING DISTRIBUTION OF CML CASES AS OBSERVED IN THE STUDY

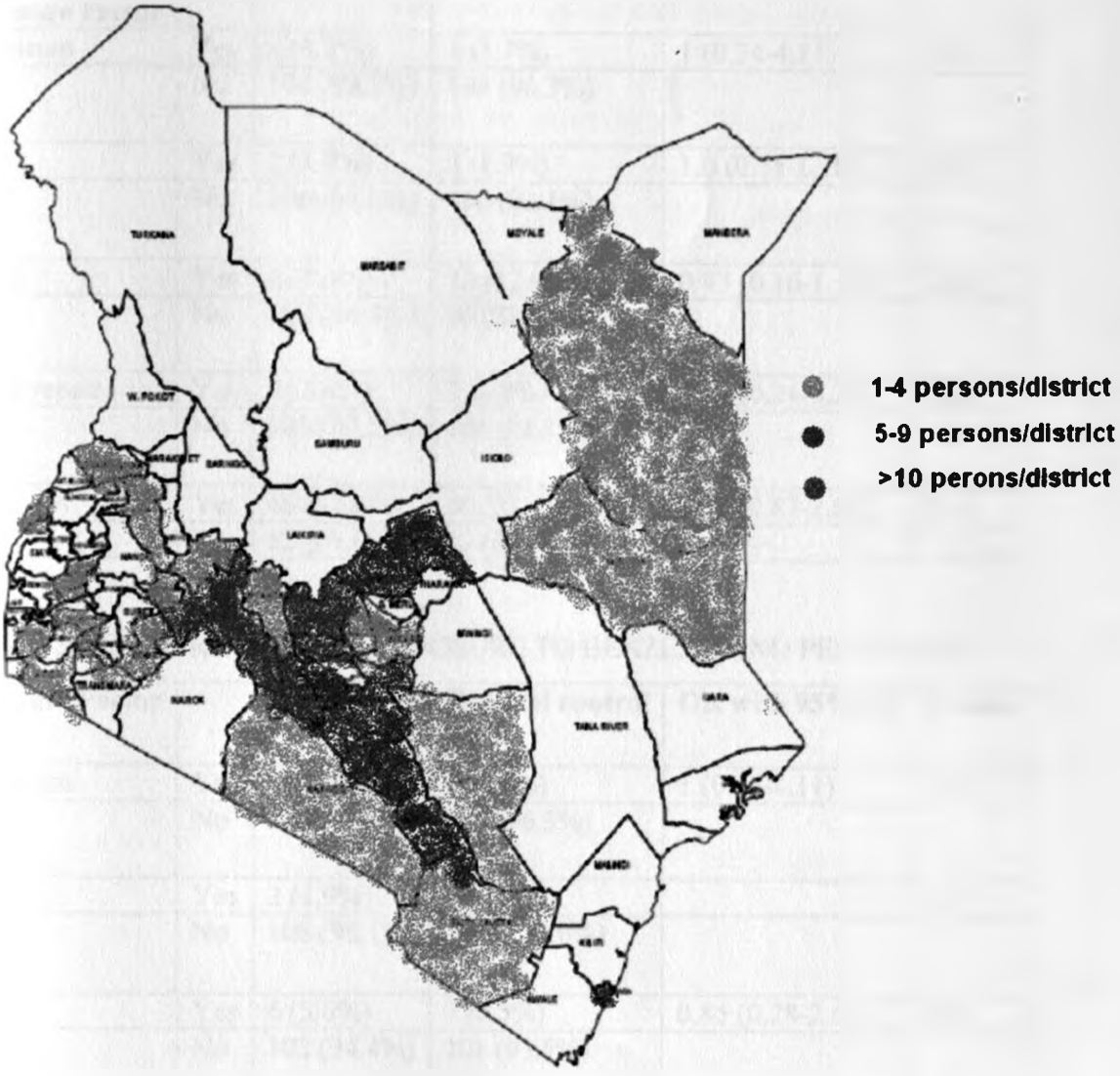


TABLE 3a: ODDS RATIOS FOR EXPOSURE TO BENZENE AND PESTICIDES

Exposure Factor		Cases	Familial control	OR with 95% CI	P value
Petroleum	Yes	4 (3.7%)	4 (3.7%)	1 (0.24-4.11)	1.00
	No	104 (96.3%)	104 (96.3%)		
Plastic	Yes	2 (1.9%)	2 (1.9%)	1.0 (0.14-1.18)	1.00
	No	106 (98.1%)	106 (98.1%)		
Paints	Yes	6 (5.6%)	13 (12.0%)	0.43 (0.16-1.18)	0.093
	No	102 (94.4%)	95 (88%)		
Motor repair	Yes	3 (2.8%)	2 (1.9%)	1.51 (0.24-9.25)	0.65
	No	105 (97.2%)	106 (98.1%)		
Pesticides	Yes	46 (43.0%)	36 (33.3%)	1.51 (0.87-2.62)	0.15
	No	62 (57.0%)	72 (66.7%)		

TABLE 3b: ODDS RATIOS FOR EXPOSURE TO BENZENE AND PESTICIDES

Exposure Factor		Cases	Hospital control	OR with 95% CI	P value
Petroleum	Yes	4 (3.7%)	4 (3.7%)	1 (0.24-4.11)	1.00
	No	104 (96.3%)	104 (96.3%)		
Plastic	Yes	2 (1.9%)	0 (0.0%)		0.15
	No	106 (98.1%)	108 (100.0%)		
Paints	Yes	6 (5.6%)	7 (6.5%)	0.85 (0.28-2.61)	0.76
	No	102 (94.4%)	101 (93.5%)		
Motor repair	Yes	3 (2.8%)	4 (3.7%)	0.74 (0.16-3.40)	0.70
	No	105 (97.2%)	104 (96.3%)		
Pesticides	Yes	46 (43.0%)	46 (42.6%)	1.02 (0.59-1.75)	0.95
	No	62 (57.0%)	62 (57.4%)		

The cases had a mean of 11.38±9.65 years (95%CI=6.23-16.52) of exposure to benzene, compared to 7.95±8.97 years (95%CI=3.63-12.27 P=0.288) in familial control group and 6.00±4.35 years (95%CI=3.49-8.51 P=0.058) in hospital control group. None of these reached statistically significant levels.

Duration of exposure to pesticides in the cases was a mean of 16.49±17.31 years (95%CI=11.41-21.57) compared to hospital control mean duration of 16.09±16.06 years (95%CI=11.26-20.91), but a statistically significant difference with P=0.017 with familial control mean duration of 9.50±8.54 years (95%CI=6.77-12.23)

FIGURE 4: DURATION OF EXPOSURE TO BENZENE AND PESTICIDES AGAINST STATUS

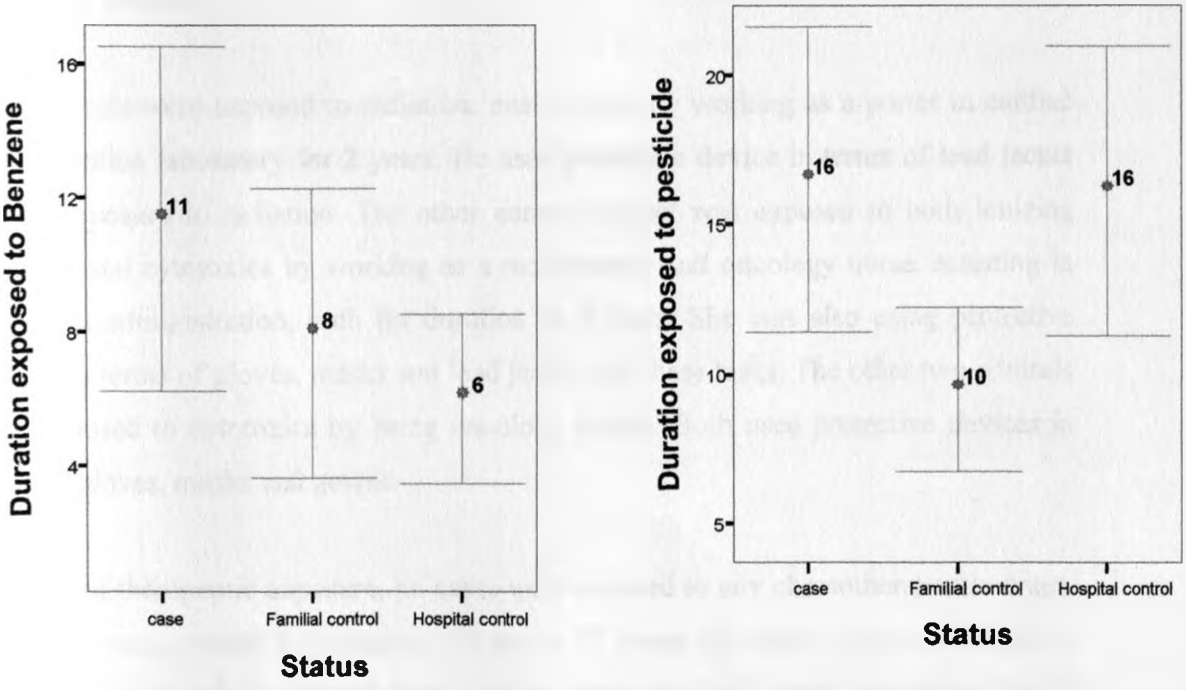


TABLE 4: DURATION OF EXPOSURE TO BENZENE AND PESTICIDES

Exposure	Cases (Mean years)	Familial control (Mean years)	T test significance	Hospital control (Mean years)	T test significance
BENZENE	11.38	7.95	0.288	6	0.058
PESTICIDES	16.49	9.5	0.017	16.09	0.909

8:04 EXPOSURE TO CYTOTOXICS AND RADIATION

There were two cases and three controls occupationally exposed to cytotoxics and one case and two controls occupationally exposed to ionizing radiation. These numbers were too low to permit analyses. The case was a 58 years old female, exposed to both ionizing radiation by working as a radiotherapy assistant nurse for 10 years and to cytotoxics by mixing drugs as an oncology nurse for duration of 1 year. She used protective devices during these tasks in terms of lead jackets during radiation exposure and gloves, gowns, masks and fume chambers during cytotoxic preparations. She was living out of Kenya during this time. The other case was a 36 years old male probably exposed to cytotoxics by working as a casual laborer in a pharmaceutical industry packing drugs, part of them may have been cytotoxics, for 6 years; he also used protective devices in terms of gloves, masks and gowns.

Two controls were exposed to radiation, one of them by working as a porter in cardiac catheterization laboratory for 2 years. He used protective device in terms of lead jacket during exposure to radiation. The other control subject was exposed to both ionizing radiation and cytotoxics by working as a radiotherapy and oncology nurse, assisting in cytotoxics administration, both for duration of 7 years. She was also using protective devices in terms of gloves, masks and lead jackets for these tasks. The other two controls were exposed to cytotoxics by being oncology nurses. Both used protective devices in terms of gloves, masks and gowns.

In terms of therapeutic exposure, no cases were exposed to any chemotherapeutic drugs. One case was exposed to radiation. He was a 57 years old male, who had resided in Nairobi his whole life, suffered from a brain cancer and had cranial irradiation for 30 days, 3 years prior to onset of CML. He also had 3 brain CT scans and 5 skull and chest x-rays done during this period.

8:05 HOUSING MATERIAL

Majority of cases and controls had ever lived in block houses; 59(54.6%) cases compared to 72(66.7%) familial controls and 67(62.0%) hospital controls, giving OR of 0.60 (95%CI=0.35-1.05 P=0.07) and OR of 0.78 (95%CI=0.43-1.27 P=0.27) respectively. There were 10(9.3%) cases, 19 (17.6%) familial and 13 (12.0%) hospital controls who had ever lived in brick houses, giving OR of 0.48 (95%CI=0.21-1.08 P=0.07) in familial control group and OR of 0.75 (95%CI=0.31-1.78 P=0.51) in hospital control group. The number of cases who had ever lived in timber walled houses was 32(29.6%), compared to 20(18.5%) familial control group and 23(21.3%) in hospital control group with OR=1.85 (95%CI=0.98-3.50 P=0.056). More patients from hospital control group had ever lived in mud houses than familial control group, 33(30.6%) and 14(13.0%) respectively, compared to 18(16.7%) cases giving OR of 0.45 (95%CI=0.24-0.87 P=0.016) in the hospital control group and OR of 1.34 (95%CI=0.63-2.86 P=0.44) in the familial control group. The number of cases who had ever lived in iron sheet houses was 23 (21.3%) compared to 21 (19.4%) familial controls and 26 (24.6%) hospital controls, giving OR of 1.12 (95%CI=0.58-2.18 P=0.74) and OR of 0.85 (95%CI=0.45-1.62 P=0.63) respectively.

TABLE 5a: ODDS RATIOS FOR HOUSING MATERIAL

Exposure factor		**Cases**	**Familial control**	**OR with 95% CI**	**P value**
Blocks	Yes	59 (54.6%)	72 (66.7%)	0.60 (0.35-1.05)	0.07
	No	49 (45.4%)	36 (33.3%)		
Bricks	Yes	10 (9.3%)	19 (17.6%)	0.48 (0.21-1.08)	0.07
	No	98 (90.7%)	89 (82.4%)		
Timber	Yes	32 (29.6%)	20 (18.5%)	1.85 (0.98-3.50)	0.056
	No	76 (70.4%)	88 (81.5%)		
Mud	Yes	18 (16.7%)	14 (13.0%)	1.34 (0.63-2.86)	0.44
	No	90 (83.3%)	94 (87.0%)		
Iron sheet	Yes	23 (21.3%)	21 (19.4%)	1.12 (0.58-2.18)	0.74
	No	85 (78.7%)	87 (80.6%)		

TABLE 5b: ODDS RATIOS FOR HOUSING MATERIAL

Exposure factor		Cases	Hospital control	OR with 95% CI	P value
Blocks	Yes	59 (54.6%)	67 (62.0%)	0.78 (0.43-1.27)	0.27
	No	49 (45.4%)	41 (38.0%)		
Bricks	Yes	10 (9.3%)	13 (12.0%)	0.75 (0.31-1.78)	0.51
	No	98 (90.7%)	95 (88.0%)		
Timber	Yes	32 (29.6%)	23 (21.3%)	1.56 (0.84-2.89)	0.16
	No	76 (70.4%)	85 (78.7%)		
Mud	Yes	18 (16.7%)	33 (30.6%)	0.45 (0.24-0.87)	0.016
	No	90 (83.3%)	75 (69.4%)		
Iron sheet	Yes	23 (21.3%)	26 (24.6%)	0.85 (0.45-1.62)	0.63
	No	85 (78.7%)	82 (75.9%)		

8:06 KIND OF HOUSEHOLD FUEL EVER USED

Use of firewood as a source of fuel was reported by 79 (73.1%) cases, 71 (65.7%) familial controls and 85 (78.7%) hospital controls giving odds ratios of 1.42 (95%CI=0.79-2.54 P=0.24) and 0.74 (95%CI=0.39-1.38 P=0.34) respectively. Electricity was used as a mode of fuel by 24(22.2%) cases, 18 (16.7%) familial controls and 6 (5.60%) hospital controls with OR of 1.43 (95%CI=0.72-2.82 P=0.30) in familial controls and OR 4.86(95%CI=1.90-12.44, P-<0.001) in hospital control group. More controls had ever used paraffin as a source of fuel than cases, 72(66.7%) of both familial and hospital controls and 58(53.7%) of cases. Similar OR of 0.58 (95%CI=0.33-1.01, P-0.052) was attained in both groups for use of paraffin as a source of fuel. Charcoal use had been reported by 72 (66.7%) cases, 81 (75.0%) familial controls and 73 (67.6%) hospital controls with OR of 0.67 (95%CI+0.37-1.20 P=0.178) and OR of 0.96 (95%CI=0.54-1.69 P=0.885). Gas had been used as mode of fuel by 54 (50.0%) cases, 66 (61.1%) familial controls and 40 (37.0%) hospital controls with ORs of 0.64 (95%CI=0.37-1.09 P=0.1) and 1.70 (95%CI=0.99-2.93 P=0.055).

TABLE 6a: ODDS RATIOS FOR KIND OF HOUSEHOLD FUEL USED

Exposure Factor		Cases	Familial control	OR with 95% CI	P value
Firewood	Yes	79 (73.1%)	71 (65.7%)	1.42 (0.79-2.54)	0.24
	No	29 (26.9%)	37 (34.3%)		
Electricity	Yes	24 (22.2%)	18 (16.7%)	1.43 (0.72-2.82)	0.30
	No	84 (77.8%)	90 (83.3%)		
Paraffin	Yes	58 (53.7%)	72(66.7%)	0.58 (0.33-1.01)	0.052
	No	50 (46.3%)	36(33.3%)		
Charcoal	Yes	72 (66.7%)	81 (75.0%)	0.67 (0.37-1.20)	0.178
	No	36 (33.3%)	27 (25.0%)		
Gas	Yes	54 (50.0%)	66 (61.1%)	0.64 (0.37-1.09)	0.1
	No	54 (50.0%)	42 (38.9%)		

TABLE 6b: ODDS RATIOS FOR KIND HOUSEHOLD OF FUEL USED

Exposure Factor		Cases	Hospital control	OR with 95% CI	P value
Firewood	Yes	79 (73.1%)	85 (78.7%)	0.74 (0.39-1.38)	0.34
	No	29 (26.9%)	23 (21.3%)		
Electricity	Yes	24 (22.2%)	6 (5.60%)	4.86(1.90-12.44)	<0.001
	No	84 (77.8%)	102 (94.40%)		
Paraffin	Yes	58 (53.7%)	72(66.7%)	0.58 (0.33-1.01)	0.052
	No	50 (46.3%)	36(33.3%)		
Charcoal	Yes	72 (66.7%)	73 (67.6%)	0.96 (0.54-1.69)	0.885
	No	36 (33.3%)	35 (32.4%)		
Gas	Yes	54 (50.0%)	40 (37.0%)	1.70 (0.99-2.93)	0.055
	No	54 (50.0%)	68 (63.0%)		

8:07 SOURCE OF DRINKING WATER

Use of piped water as a source of drinking water was reported by 74 (68.5%) cases, 88 (81.5%) familial controls and 78 (72.2%) hospital controls. Odds Ratio of 0.49 (95%CI=0.26-0.93, P-0.03) was attained in the familial control group for use of piped drinking water, and OR of 0.84 (95%CI=0.47-1.50, P-0.56) in the hospital control group. Wells and boreholes formed the majority of surface water as a source for drinking water, with positive response from 49 (45.4%) cases, 36 (33.3%) familial controls and 38 (35.2%) hospital controls with ORs of 1.67 (95%CI=0.96-2.88 P=0.07) and 1.53 (95%CI=0.89-2.64 P=0.13) respectively. Rain water was used by 13 (12.0%) cases and 13 (12.0%) familial controls with OR at unity, and 11 (10.2%) hospital controls with OR of 1.21 (95%CI=0.51-2.83 P=0.66). Dam water was used by 3 (2.8%) cases, 4 (3.7%) in each familial and hospital control groups with OR of 0.74 (95%CI=0.16-3.40 P=0.7). Use of river water was reported by 34 (31.5%) cases, 26 (24.1%) familial controls and 47 (43.5%) hospital controls with ORs of 1.45 (95%CI=0.80-2.64 P=0.22) and 0.60 (95%CI=0.34-1.04 P=0.07) respectively, whereas use of spring water was reported by 7 (6.5%) cases, 4 (3.7%) familial and 3 (2.8%) hospital controls with ORs of 1.80 (95%CI=0.51-6.34 P=0.35) and 2.43 (95%CI=0.61-9.64 P=0.19).

8:08 SMOKING AND ALCOHOL USE

History of cigarette smoking was found in 27 (25.0%) cases, 26 (24.1%) familial controls and 29 (26.9%) hospital controls, while 36 (33.3%) cases had history of regular use of alcohol compared to 43 (39.8%) and 48 (44.4%) familial and hospital control groups respectively. The OR for ever having smoked cigarettes was 1.05 (95%CI=0.5-1.95 P=0.87) when compared to familial control and 0.91 (95%CI=0.49-1.67 P=0.76) when compared to hospital controls. The Odds ratios for regular consumption of alcohol were 0.76 (95%CI=0.43-1.32 P=0.32) and 0.63 (95%CI=0.36-1.09 P=0.094) when compared with familial and hospital control groups respectively.

TABLE 7a: ODDS RATIOS FOR SOURCE OF DRINKING WATER

Exposure Factors		Cases	Familial control	OR with 95% CI	P value
Piped	Yes	74 (68.5%)	88 (81.5%)	0.49 (0.26-0.93)	0.03
	No	34 (31.5%)	20 (18.5%)		
Well	Yes	49 (45.4%)	36 (33.3%)	1.67 (0.96-2.88)	0.07
	No	59 (54.6%)	72 (66.7%)		
Rain Water	Yes	13 (12.0%)	13 (12.0%)	1.0 (0.44-2.27)	1.00
	No	95 (88.0%)	95 (88.0%)		
Dam	Yes	3 (2.8%)	4 (3.7%)	0.74 (0.16-3.40)	0.70
	No	105 (97.2%)	104 (96.3%)		
River/Stream	Yes	34 (31.5%)	26 (24.1%)	1.45 (0.80-2.64)	0.22
	No	74 (68.5%)	82 (75.9%)		
Spring	Yes	7 (6.5%)	4 (3.7%)	1.80 (0.51-6.34)	0.35
	No	101 (93.5%)	104 (96.3%)		

TABLE 7b: ODDS RATIOS FOR SOURCE OF DRINKING WATER

Exposure Factors		Cases	Hospital control	OR with 95% CI	P value
Piped	Yes	74 (68.5%)	78 (72.2%)	0.84 (0.47-1.50)	0.56
	No	34 (31.5%)	30 (27.8%)		
Well	Yes	49 (45.4%)	38 (35.2%)	1.53 (0.89-2.64)	0.13
	No	59 (54.6%)	70 (64.8%)		
Rain Water	Yes	13 (12.0%)	11 (10.2%)	1.21 (0.51-2.83)	0.66
	No	97 (88.0%)	95 (86.1%)		
Dam	Yes	3 (2.8%)	4 (3.7%)	0.74 (0.16-3.40)	0.7
	No	105 (97.2%)	104 (96.3%)		
River/Stream	Yes	34 (31.5%)	47 (43.5%)	0.60 (0.34-1.04)	0.07
	No	74 (68.5%)	61 (56.5%)		
Spring	Yes	7 (6.5%)	3 (2.8%)	2.43 (0.61-9.64)	0.19
	No	101 (93.5%)	105 (97.2%)		

TABLE 8a: ODDS RATIOS FOR EXPOSURE TO SMOKING

Exposure Factors		Cases	Familial control	OR with 95% CI	P value
Ever smoked	Yes	27 (25.0%)	26 (24.1%)	1.05 (0.5-1.95)	0.87
	No	81 (75.0%)	82 (75.9%)		

TABLE 8b: ODDS RATIOS FOR EXPOSURE TO SMOKING

Exposure Factors		Cases	Hospital control	OR with 95% CI	P value
Ever smoked	Yes	27 (25.0%)	29 (26.9%)	0.91 (0.49-1.67)	0.76
	No	81 (75.0%)	79 (73.1%)		

TABLE 9a: ODDS RATIOS FOR EXPOSURE TO REGULAR USE OF ALCOHOL

Exposure Factors		Cases	Familial control	OR with 95% CI	P value
Regular alcohol use	Yes	36 (33.3%)	43 (39.8%)	0.76 (0.43-1.32)	0.32
	No	72 (66.7%)	65 (60.2%)		

TABLE 9b: ODDS RATIOS FOR EXPOSURE TO REGULAR USE OF ALCOHOL

Exposure Factor		Cases	Hospital control	OR with 95% CI	P value
Regular alcohol use	Yes	36 (33.3%)	48 (44.4%)	0.63 (0.36-1.09)	0.094
	No	72 (66.7%)	60 (55.6%)		

8:09 EXPOSURE TO RADIOGRAPHY AND X-RAYS

The mean number of x-rays done by cases was 3.08 ±2.89, whereas the mean number done by familial and hospital controls were 2.61 ±2.31 and 2.03 ±1.44 respectively. More cases had abdominal radiography, 13 (20.0%) compared to 3 (4.5%) and 2 (2.5%) in familial and hospital control groups respectively. This showed significant levels in cases when compared to either controls (P=0.06 for familial and 0.001 for hospital controls). This remained statistically significant after logistic regression analysis, with OR 7.29 (95%CI-2.002-26.536 P-0.003) in familial control group and OR 9.12 (95%CI-1.716-48.484 P-0.01) in hospital control group. The mean duration of abdominal radiography prior to diagnosis of CML was 5.27 ± 6.84 years, whereas for other radiological investigations it was more than 10 years prior to diagnosis. (Table 10).

Barium meals were done on 4 (5.1%) cases, 7 (10.3%) familial controls and only 2 (2.5%) hospital controls with ORs of 0.56 (95%CI=0.16-2.02 P=0.37) and 2.55 (95%CI=0.45-15.37 P=0.27). More controls had various Computerized Tomography scans done; 10 (14.9%) familial controls and 17 (21.3%) hospital controls, compared to 6 (9.2%) cases, with OR of 0.58 (95%CI=0.20-1.70 P=0.32) and OR of 0.38 (95%CI=0.14-1.02 P=0.05) respectively. The number of cases who had chest radiographs done were 36 (53.7%) compared to 35 (52.2%) familial and 54 (66.7%) hospital controls, with ORs of 1.06 (95%CI=0.54-2.09 P=0.86) and 0.58 (95%CI=0.30-1.13 P=0.11). Limb radiographs were done on 18 (27.3%) cases, 26 (38.8%) familial controls and 20 (25.0%) hospital controls with OR of 0.59 (95%CI=0.29-1.23 P=0.16) and OR of 1.13 (95%CI=0.54-2.36 P=0.76). Skull radiographs were done in 9 (13.8%) cases, 6 (9.0%) familial controls and 6 (7.5%) hospital controls, with ORs of 1.63 (95%CI=0.55-4.88 P=0.21) and 1.98 (95%CI=0.67-5.89 P=0.21), whereas 5 (7.7%) cases had spinal radiographs compared to 10 (14.9%) familial controls and 7 (8.8%) hospital controls, with ORs of 0.87 (95%CI=0.26-2.88 P=0.19) and 0.87 (95%CI=0.26-2.88 P=0.82)

TABLE 10: MEAN DURATION OF X-RAY PRIOR TO DIAGNOSIS OF CML

Radiographic Investigation In Cases	Time before Diagnosis (Years) ± SD
Abdominal X-ray	5.27 ± 6.84
Skull x-ray	12.25 ± 17.68
Limb x-ray	12.65 ± 17.68
Spinal x-ray	13.00 ± 12.81
CT Scans	18.83 ± 23.21

TABLE 11a: OR FOR EXPOSURE TO X-RAYS

Exposure		Cases	Familial control	OR with 95% CI	P value
Barium meal	Yes	4 (5.1%)	7 (10.3%)	0.56 (0.16-2.02)	0.37
	No	62 (93.9%)	61 (89.7%)		
CT scans	Yes	6 (9.2%)	10 (14.9%)	0.58 (0.20-1.70)	0.32
	No	59 (90.8%)	57 (85.1%)		
Abdominal x-ray	Yes	13 (20.0%)	3 (4.5%)	5.33 (1.44-19.22)	0.06
	No	52 (80.0%)	64 (95.5%)		
Chest x-ray	Yes	36 (53.7%)	35 (52.2%)	1.06 (0.54-2.09)	0.86
	No	31 (46.3%)	32 (47.8%)		
Limbs	Yes	18 (27.3%)	26 (38.8%)	0.59 (0.29-1.23)	0.16
	No	48 (72.7%)	41 (61.2%)		
Skull	Yes	9 (13.8%)	6 (9.0%)	1.63 (0.55-4.88)	0.38
	No	56 (86.2%)	61 (91.0%)		
Spine	Yes	5 (7.7%)	10 (14.9%)	0.48 (0.15-1.48)	0.19
	No	60 (92.3%)	57 (85.1%)		

TABLE 11b: OR FOR EXPOSURE TO X-RAYS

Exposure		Cases	Hospital control	OR with 95% CI	P value
Barium meal	Yes	4 (5.1%)	2 (2.5%)	2.55 (0.45-15.37)	0.27
	No	62 (93.9%)	79 (97.5%)		
CT scans	Yes	6 (9.2%)	17 (21.3%)	0.38 (0.14-1.02)	0.05
	No	59 (90.8%)	63 (78.8%)		
Abdominal x-ray	Yes	13 (20.0%)	2 (2.5%)	9.75 (2.11-45.01)	0.001
	No	52 (80.0%)	78 (97.5%)		
Chest x-ray	Yes	36 (53.7%)	54 (66.7%)	0.58 (0.30-1.13)	0.11
	No	31 (46.3%)	27 (33.3%)		
Limbs	Yes	18 (27.3%)	20 (25.0%)	1.13 (0.54-2.36)	0.76
	No	48 (72.7%)	60 (75.0%)		
Skull	Yes	9 (13.8%)	6 (7.5%)	1.98 (0.67-5.89)	0.21
	No	56 (86.2%)	74 (92.5%)		
Spine	Yes	5 (7.7%)	7 (8.8%)	0.87 (0.26-2.88)	0.82
	No	60 (92.3%)	73 (91.3%)		

8:10 FAMILIAL HISTORY OF CANCER

More cases than controls had a history of cancer in 1st or 2nd degree relative, 16 cases with 1st degree and 10 cases with 2nd degree relatives having suffered from some cancer, compared history of cancer in 8 1st degree and 10 2nd degree relatives in the familial control group. Hospital control group had history of cancer in 6 1st degree and 5 2nd degree relatives, giving OR of 2.0 (95%CI=0.59-6.78 P=0.26) and 1.33 (95%CI=0.32-5.55 P=0.69) compared to familial and hospital controls respectively.

TABLE 12a: FAMILY HISTORY OF CANCER

Exposure	Cases	Familial control	OR with 95% CI	P value
1st degree relative	16 (61.5%)	8 (44.4%)	2.0 (0.59-6.78)	0.26
2nd degree relative	10 (38.5%)	10 (55.6%)		

TABLE 12b: FAMILY HISTORY OF CANCER

Exposure	Cases	Hospital control	OR with 95% CI	P value
1st degree relative	16 (61.5%)	6 (54.5%)	1.33 (0.32-5.55)	0.69
2nd degree relative	10 (38.5%)	5 (45.5%)		

Further stratification to deal with multiplicity of exposure was attempted but was not possible because the numbers were too small to permit analysis.

9:00 DISCUSSION

9:01 DEMOGRAPHICS

Our study age range for CML cases was 8-81 years, with median age of 41.32. Abinya et al found a similar age range of 10-72 years in KNH in 2000.[4] They found median age of 35 years at diagnosis whereas Bjork in Sweden found a median age of occurrence of CML at 51 years, almost 10 years younger than our population.[37] Our male to female ratio was 1.7:1, which is comparable to what Abinya and colleagues[4] found at 1.1:1 and Bjork et al at 1.23:1,[37] which is in keeping with the general observations.

Our peak age of occurrence of CML is 10 years younger than in the West. The peak for females is even at a younger age of 25-30 years, but the number is too small to make a firm statement. Similar median age of 40 years for CML was found in Nigeria[73] reflecting generally younger age of occurrence of CML in Africa.

Other cancers in Africans have also found to be occurring at a younger age group, for example, median age of occurrence of non-Hodgkin's lymphoma in Kenya is only 32 years against the mean age of 50 years internationally.[1] It is not clear why we have earlier age of occurrence of CML and further studies are required to probe into the etiologic possibilities of this significant difference. The important contributing factors could be genetic, environmental, and socioeconomic.

Majority of the patients came from environs of Nairobi and 44.5% of our patient came from or had at one time lived in Nairobi at least for a year. The patient population was concentrated proximal to health facility, particularly Nairobi, where expertise, health personnel and diagnostic facilities are available, and also ease of communication. Clinicians and physicians outside Nairobi may not be aware of the GIPAP programme and may not be referring patients to the programme. The only far away pocket was 7 patients coming from Mombasa district on the Coast of Kenya. This is the second largest city in Kenya with a high population density and good infrastructure and links to Nairobi. The pocket of patients from North-Eastern Kenya could be explained by links of people from the province with Nairobi, particularly in the Eastleigh area. Some areas in southern Kenya yielded no cases and that could possibly be explained by the health care seeking behavior of the communities living in those regions and possible belief in traditional and

herbal modes of treatment. Abinya et al also had more than 35% of cases being from Kikuyu tribe, and they thought it was possibly due to proximity to Nairobi.[4] This is reflected in other chronic disease outpatient clinics in KNH and is thought to be due to the patient catchment area.

9:02 BENZENE

We found the odds ratio for benzene exposure from petrochemicals plastic and rubber industries and exposure to paint were close to unity in Ph +ve subjects. Failure to stratify by intensity or duration may have failed to show association of CML with benzene. Longer duration of exposure, as found in the cases, may probably be contributing to risk of developing CML. The findings by Bjork et al in Sweden were similar.[37]

This also agrees well with a combined cohort study of petroleum workers in China, who were exposed to petrochemicals—such as benzene at low concentrations and gasoline.[74] At higher concentration exposures, non-significant effects of occupational exposure to benzene on leukaemias other than AML have, however, been reported.[75]

Yu et al suggested a possible link between living in an area with high exposure to airborne petrochemicals (derivatives of petroleum or natural gas) and risk of developing leukemia in a study in Taiwan.[36] We only had one case in our study, residing in close proximity to an oil refinery.

9:03 PESTICIDES

The association between farming and leukemia in general, which has been studied in numerous epidemiological settings, is likely to be weak, if present at all.[76] Our data did not indicate risk of CML associated with agricultural life, manifested by OR estimates almost at unity for typical agricultural exposures such as farming occupations and pesticides. The odds ratio was just above unity when compared with familial controls. Duration of exposure to pesticides was similar with hospital control but significantly

lower in familial group. We think this may be due to general nature of agricultural lifestyle in the hospital control group, who had a homogenous distribution in the country.

A meta analysis by Belgian researchers to determine if occupational exposure to pesticides was associated with a higher risk of CML Overall, the case-control studies indicated that the Relative Risk of 1.38(95%CI 1.06-1.79) of developing CML among farmers and agricultural workers exposed to pesticides.[77] However Bjork found the OR for exposure to pesticides for Ph+ve CML patients in Sweden was 0.75(95%CI 0.42-1.3).[37]

9:04 HOUSING MATERIAL

Living in timber houses suggested a trend of risk for development of CML in the familial controls with OR 1.85 (95%CI =0.98-3.50 P=0.056), and protection from disease was suggested by living in mud houses in the hospital control group with OR 0.45 (95%CI= 0.24-0.87 P=0.016).

This was attributed to creosote, which is the chemical primarily used for the preservation of wood, accounting for over 97% of current coal tar creosote production. There is evidence for the carcinogenicity of creosotes in humans.[38]

9:05 KIND OF HOUSEHOLD FUEL USED

Although the OR for use of electricity as a mode of fuel was statistically significant (P=<0.001) for hospital control group, the numbers exposed were very small. The same was not statistically significant in familial control group (P=0.30).

There are some large studies investigating the association between magnetic fields in homes and leukemia in the long term and no consistent association has been observed and studies of electric appliance use did not support an association with leukemia risk.[78]

Interestingly OR for use of paraffin as a mode of fuel fell significantly below unity (OR=0.58 95%CI=0.33-1.01, P-0.052). We have no explanation for except that it is a

random finding, as combustion products have been classified as carcinogenic.[39] It may also be due to the fact that paraffin is the most common source of fuel used in our setup.

9:06 SOURCE OF DRINKING WATER

Protection was suggested by use of piped water, with statistical significance being achieved in familial controls (OR=0.49 95%CI=0.26-0.93 P=0.03), with same trend although not significant in hospital control (OR=0.84 95%CI=0.47-1.50 P=0.56). There was also a trend in risk suggested with use of well water (Familial OR=1.67 95%CI=0.96-2.88 P=0.07), (Hospital OR=1.53 95%CI=0.47-1.50 P=0.56).

This could probably be explained by chlorination products like trihalomethanes and bromodichloromethanes, used in surface water, which have been associated with increased incidence of cancer,[42] although physical verifications and measurements of chemicals were not done in this study.

Kasim et al in Canada also demonstrated a non significant increase in risk of CML with increasing years of exposure to chlorinated surface water for more than 36 year.[42] Cohn and coworkers in New Jersey found Relative Risk of CML at 1.79(95%CI=0.90-3.55) for towns with highest stratum of trichloroethylene exposure versus towns with no detectable TCE in drinking water.[43]

9:07 SMOKING AND ALCOHOL USE

In this study, we found no association between smoking and CML. This is in keeping with previous studies. Smoking can increase the risk for leukemia. Fernberg and colleagues carried out a prospective cohort study to explore effect of tobacco smoke on leukemia.[45] An increased risk of AML was observed in current smokers by Fernberg et al, however, they also reported current or former smokers did not have an increased risk of CML. In a study done in Sweden by Bjork et al, no relation between cumulative smoking dose (pack-years), and risk of disease was found.[37]

Thus, the suggestion that tobacco smoking is a risk factor for myeloid leukemia in general[80-82] may not be applicable for Ph+CML, stressing the need for including detailed morphological and genetic features in epidemiological investigations of leukaemias.

There was no association found with use of alcohol either. If anything, our study found the Odds Ratios for alcohol use below unity which suggests it may be protective, however, Gorini and colleagues, in Italy, found a non-significantly positive association for all levels of total alcohol and wine intake, and a significant positive linear trend effect for CML (P = 0.03).[83]

9:08 RADIATION EXPOSURE

We had 1 case who developed CML 3 years after cranial irradiation. Other case reports of CML occurring after exposure to irradiation have also been published. Chap and coworkers, from UCLA School of Medicine, reported development of CML 11 years after radiation therapy for Histiocytosis X.[61] Frist and colleagues in Tennessee reported a case of recurrent cardiac rejection in a heart transplant recipient successfully treated with total lymphoid irradiation. Five years after transplantation chronic myelogenous leukemia was diagnosed in this patient.[62]

Ionizing radiation has been found as a risk factor for AML, ALL, and CML but not for CLL. In the Life Span Study (LSS), Pierce et al evaluated atom bomb survivors from 1950 to 1990. Among the 86,572 persons studied 249 leukemia deaths were attributable to radiation exposure.[84] Preston et al. found 50% of all leukemias were attributable to radiation between 1950-1987 in Hiroshima and Nagasaki.[85] Although high dose radiation exposure increases leukemia rates, low dose of radiation has limited role in the etiology of leukemia.[86]

9:09 RADIOGRAPHY AND X-RAYS

In our study more cases than controls had abdominal radiography with the mean of 5.27 ± 6.84 years prior to diagnosis, and the mean number of x-rays done is more in cases than controls. However, due to small numbers, it is difficult to make conclusive remarks on effect of abdominal radiography on Ph+ve CML, and further follow-up studies would be required. The suggestion that the abdominal radiography was done as part of investigative work-up for CML may not be an adequate explanation, as the mean duration prior to the investigative procedure would have led to blastic transformation of CML.

Preston-Martin and colleagues in Los Angeles, had more cases than controls who had radiographic examinations of the back, gastrointestinal tract and kidneys, and cases more often had gastrointestinal and back radiography on multiple occasions. The association was strongest for the period 6-10 years before diagnosis, and the effects of radiation exposure during this period remained significant after consideration of other risk factors in a logistic regression analysis. We had almost similar findings in this study regarding exposure to abdominal radiography.

9:10 HEREDITARY AND GENETICS

There are no clear hereditary factors associated with CML. Identical twins of patients with CML are at no greater risk of developing CML than other siblings. This strongly suggests that environmental factors are much more important than genetic factors in the development of CML. It is a scientific mystery as to why only one of a pair of identical twins will develop CML, since the genetics are identical and environmental exposures may also be similar, if not the same.[11]

However, we had more cases than controls who had a family history of cancer, especially in the 1st degree relatives but none reaching statistically significant value.

10:0 CONCLUSIONS

Associations between exposures to organic solvents like pesticides, creosote as used in timber preservation, water treatment by chlorinated chemicals and Ph+CML were indicated but were not entirely consistent. However our study population was small. Risk of developing CML was suggested with exposure to plain abdominal radiography. Nevertheless, for almost all cases of Ph+CML, other explanations must be sought for.

11:0 RECOMMENDATIONS

A more detailed follow up of this study with physical verification of actual environmental variants needs to be carried out, with a larger sample size as this was a pilot study of leukemogens, some not studied previously as risk factors specifically regarding CML. Larger study also needs to be done to verify association of CML with abdominal radiography. Studies should also be carried out in hospitals in all provinces to enable generalization of results. A nationwide registry for all CML cases needs to be established to enable recruitment of participants for larger future studies.

12:0 STUDY LIMITATIONS

Recall bias may have affected data collection. Selection bias may also have been present as only patients enrolled in the GIPAP programme were interviewed. Many of the patients were coming from neighboring areas of Nairobi, thus geographic concentration of the study population may have been limited. This was a questionnaire based study and definite measurements of exposure to chemicals and radiation were not carried out and environmental check was not done.

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APPENDIX 1

Fluorescent in situ hybridization method

Fluorescent in situ hybridization refers to using fluorescently labeled probe to hybridize to cytogenetic cell preparations.

In addition to standard preparations FISH can also be performed on:

- bone marrow smears
- blood smears
- paraffin embedded tissue preparations
- enzymatically dissociated tissue samples
- uncultured bone marrow
- uncultured amniocytes
- cytospin preparations

Slide preparation

The slide is aged using a salt solution usually consisting of 2X SSC (salt, sodium citrate). The slides are then dehydrated in ethanol, and the probe mixture is added. The sample DNA and the probe DNA are then co-denatured using a heated plate and allowed to re-anneal for at least 4 hours. The slides are then washed to remove excess unbound probe, and counterstained with 4',6-Diamidino-2-phenylindole (DAPI) or propidium iodide.

Analysis

Analysis of FISH specimens is done by fluorescence microscopy by a clinical laboratory specialist in cytogenetics (CLSp(CG)). For oncology generally a large number of interphase cells are scored in order to rule out low level residual disease, generally between 200 and 1000 cells are counted and scored.[87]

APPENDIX 2

QUESTIONNAIRE

□ **CASE** □ **FAMILIAL CONTROL** □ **HOSPITAL CONTROL**

1.NAME.....(If control, name of case).....

2.AGE..... 3.SEX □ MALE □ FEMALE

4.PHONE CONTACT .....

5.DATE OF DIAGNOSIS.....

6.AGE AT DIAGNOSIS.....

7.PHASE OF DISEASE: □ CHRONIC □ ACCELERATED □ BLASTIC

8.DIAGNOSIS (HOSPITAL/FAMILIAL CONTROL).....

9.OCCUPATION: CURRENT ..... YEARS.....

(from time of diagnosis) PREVIOUS..... YEARS.....

PREVIOUS..... YEARS.....

10.RESIDENCE:

	DISTRICT	DIVISION	LOCATION	SUBLOCATION	YEARS OF RESIDENCE
RES 1 - CURRENT					
RES 2 - PREVIOUS					
RES 3 - PREVIOUS					

OCCUPATIONAL EXPOSURES:

11.Exposure to Benzene and Organic Solvents □ YES □ NO

Working in	DURATION	WORK TASK	DEPARTMENT
-Petroleum industry			
-Plastic industry			
-Paint and oil industry			
-Motor repair			
-Other (Specify)			

12.Application of pesticides/Herbicides by working as:- ☐ YES ☐ NO

DURATION	WORK TASK	DURATION
Gardener		
Horticulturalist		
Farmer		
Farm Hand		
Other (Specify)		

13.Handling of cytotoxics by working as: ☐ YES ☐ NO

	TASK	WORK DURATION
-Medical practitioner		
-Nurse		
-Nurse aid		
-Pharmacist		
-Other (Specify)		

14.Handling of ionizing radiation by working as: ☐ YES ☐ NO

	TASK	WORK DURATION
-Radiologist		
-Radiographer		
-Radiotherapist		
-Radiology nurse		
-In radiology department		
-Other (Specify)		

FOR QUESTIONS 12, 13,14

DO YOU USE PERSONAL PROTECTIVE DEVICES? ☐ YES ☐ NO

IF YES, WHICH ONE?

☐ GLOVES ☐ MASKS ☐ GOWNS ☐ OTHERS(specify).....

ENVIRONMENT

16.WHAT KIND OF HOUSE DO YOU OCCUPY? (Indicate type of material used to build the house in the box)

	RES 1	RES 2	RES 3
PERMANENT			
SEMI-PERMANENT			
TEMPORARY			
OTHER (SPECIFY)			

17. WHAT KIND OF COOKING FUEL DO YOU USE AT HOME? (TICK THE RIGHT COLUMN)

	Residence 1, 2 or 3?	ALWAYS	MOST OF THE TIME	OCCASIONALLY
FIRE WOOD				
PARAFFIN/KEROSENE				
ELECTRICITY				
CHARCOAL				
LPG/NATURAL GAS				
BIOGAS				
DUNG				
OTHER (SPECIFY)				

18. WHAT IS THE MAIN SOURCE OF DRINKING WATER FOR YOUR HOUSEHOLD?

	RES 1	RES 2	RES 3
PIPED			
OPEN WELL/BOREHOLE			
CLOSED WELL/BOREHOLE			
RAIN WATER			
BOTTLEDWATER			
SURFACE WATER→DAM/STANDING LAKE/RIVER/STREAM, SPRING WHICH ONE? (NAME)			
OTHER (SPECIFY)			

OTHER EXPOSURES

19. HAVE YOU EVER RECEIVED CHEMOTHERAPY OR CYTOTOXICS OR STEROID OR OTHER IMMUNOSUPPRESIVE AGENTS?

☐ YES ☐ NO

-IF YES, WHICH ONES? (record all conditions suffered by patients prior to diagnosis of CML and other co-morbid conditions and concurrent treatments)

CONDITIONS	TREATMENT/MEDICATIONS	WHEN/DURATION

20. HAVE YOU EVER RECEIVED RADIOTHERAPY?

☐ YES ☐ NO

IF YES, FOR WHAT CONDITION? .....

HAVE YOU EVER BEEN TREATED WITH RADIOISOTOPES?

☐ YES ☐ NO

IF YES, FOR WHAT CONDITION? .....

21. HAVE YOU EVER BEEN X-RAYED ☐ YES ☐ NO

WHAT X-RAYS WERE YOU DONE?	HOW MANY TIMES?	AT WHAT AGE?

22. HAS ANY OF YOUR RELATIVES SUFFERED FROM CML OR ANY OTHER LEUKEMIA ☐ YES ☐ NO

-DOES ANY OF YOUR RELATIVES SUFFER FROM ANY CANCER?

☐ YES ☐ NO

IF YES, WHO? .....

WHICH KIND OF LEUKEMIA/CANCER?.....

23. DO YOU SMOKE? □ YES □ NO

□ CURRENT □ PREVIOUS

STICKS PER DAY..... YEAR STARTED.....

YEAR STOPPED.....

ARE THERE OTHER PEOPLE AT HOME/WORKPLACE CONSTANTLY SMOKING? □ YES □ NO (circle place)

24. DO YOU TAKE ALCOHOL? □ YES □ NO

IF YES, WHAT TYPE(S)?.....

□ CURRENT □ PREVIOUS

YEAR STOPPED.....

DURATION CONSUMED.....YEARS

Number of bottles: ..... /day, started in year.....

..... /week, started in year.....

..... /month, started in year.....

25. PHYSICAL EXAMINATION

□ WEIGHT LOSS □ LYMPHADENOPATHY □ PALLOR

□ SPLENOMEGALLY □ MASSES

HEIGHT.....CMS WEIGHT.....KGS

BMI.....

26. CURRENT THERAPY.....

27. HAEMOGRAM RESULTS

WBC COUNT..... DIFF: N..... L..... E..... M.....

PBF.....

APPENDIX 3

CONSENT EXPLANATION

My name is Dr Riaz Kasmani. I would like to tell you about the study I am carrying out. I intend to look at environmental and occupational factors associated with a form of leukemia called chronic myeloid leukemia (CML). Leukemia is basically a cancer of blood. CML is a form of leukemia that stays stable for several years before assuming a downhill progression. Its investigations and treatment are very expensive and so far just palliative and life prolonging.

Despite the fact that a lot is known about this disease, its cause is not known. The fact that this disease occurs later in life at about age of 45 years, it is believed that some factors associated with environmental or occupational exposures may be responsible. In order for us to understand this disease better, I would like to ask you some questions about your environment, occupation and family. You shall also be examined physically to make sure you do not suffer from any cancer or other blood diseases, and some blood (2 mls) will be collected from your arm to confirm the same. The results of the blood test will be communicated to you by phone, and incase you should need any medical attention from the same, you will be guided accordingly.

You may be asked to participate in this study either because you suffer from this disease or as a person to form a comparison group to patients suffering from this disease. Our aim is to compare the factors between patients suffering from CML and those not suffering so as to evaluate how these factors relate to the disease.

You will not be coerced to respond to the questions that will be asked. Participation is purely voluntary. Your participation may however, enable us to understand this disease better and help other people who may develop it later. If you have any further questions, you are free to ask them any time. If you consent to be enrolled into the study, then you will be required to sign or put a thumb print in the space provided.

MAELEZO KHUHUSU IDHINI (KUKUBALI)

Jina langu ni Dr. Riaz Kasmani. Ningependa kukueleza kuhusu mradi wa utafiti tunaoendeleza. Tunataka kuangalia ni mambo yapi katika mazingira, kikazi au kwa maisha yetu ambazo zinaweza kusababisha ugonjwa wa saratani ya damu (Chronic Myeloid Leukemia). Ugonjwa huu una mtindo wa kuanza polepole na baadaye kuenea mwili na kuangamiza maisha. Utafiti wa ugonjwa na matibabu ni ghali kwa kipesa na pia matibabu haina uthibitisho wa tiba.

Licha ya kuwa mengi yaeleweka kuhusu ugonjwa huu, chanzo chake haijulikani. Tuna amini ya kuwa sababu fulani za kimazingira ndio chanzo cha ugonjwa huu. Ningependa kukuuliza maswali kadha kuhusu mazingira, kazi na maisha yako. Pia tutakupima kimwili kuhakikisha kwa huna saratani yoyote ama ugonjwa wowote wa damu ambao itahakikishwa kwa kutolewa damu kidogo ya kiasi 2 mls kwa mkono. Majibu ya damu utaelezwa kwa simu, na zikiwa na kosoro yoyote, utaelekezwa kupata usaidizi.

Unaweza kushugulika na utafiti huu kwa sababu wewe uko na ugonjwa wa CML ama kwa sababu tunataka kulinganisha mazingira na eneo la makazi yako na ya wale ambao wako na ugonjwa.

Huta shurutishwa kujibu maswali ambao utaulizwa. Kushiriki ni kwa hiari yako. Kushiriki kwako ingawaje yaweza kutuelewesha ugonjwa huu vyema na kusaidia wengine watakao ugua baadaye. Ukiwa na maswali zaidi kuhusu utafiti huu, uko huru kuyauliza wakati wowote. Ikiwa utaidhinisha kuandikishwa kwenye mradi basi utahitajiwa kuweka sahihi ama dhibitisho kwa kidole cha gumba kwenye sehemu iliyotengwa.

APPENDIX 4

CONSENT FORM

I _____ do hereby consent freely, without any form of coercion or inducement to take part in the above study and to be interviewed. Its purpose and nature has been fully explained to me by _____ and I understand that I can withdraw at any time should I change my mind.

Signed: _____ Date: _____

Witnessed By: _____ Date: _____

IDHINI

Mimi _____natowa papa hapa idhini mwenyewe bila aina yoyote ya kushurutishwa au kulazimishwa kushiriki katika utafiti uliotajwa hapa juu na kwa kuhojiwa. Nimeelezwa kikamilifu kuhusu madhumuni na hali yake na _____

naelewa kuwa naweza kujiondoa wakati wowote iwapo nitabadilisha mawazo.

Sahihi_____ Tarehe_____

Shahidi_____ Tarehe_____