



Does Down syndrome occur more frequently in mothers with advanced age for patients diagnosed at Rwanda Center for Medical Genetics?

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**Does Down syndrome occur more frequently in mothers with advanced age for patients diagnosed at Rwanda Center for Medical Genetics?**

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## DECLARATION

I declare that this Dissertation contains my own work except where specifically acknowledged

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**Dr Janvier HITAYEZU**

## ABSTRACT

**Background:** Down syndrome is the most common chromosome abnormality among live born infants and the most frequent recognizable genetic cause of intellectual disability in all human ethnic groups across the world. Advanced maternal age has been identified as risk factor associated with underlying mechanism of chromosome 21 non-disjunction leading to trisomy 21, the most frequent form of Down syndrome. A shift to younger maternal age for Down syndrome births appreciated in some recent studies. Lack of sufficient research data in Sub-Saharan Africa black population, and the importance of Down syndrome pediatric patients diagnosed in Rwanda especially those born to young mothers has prompted us to conduct a research to verify the magnitude of maternal age effect as risk factor for Down syndrome pregnancy.

**Hypothesis:** the maternal age at birth of infants with Down syndrome is different from the maternal age at childbirth in the Rwanda general population.

**Methods:** A cross-sectional survey was conducted at the Rwanda Center for Medical Genetics. Cases of Down syndrome patients diagnosed over a period of more than 9 years from December 2006 till February 2016 were identified with respective maternal ages at the time of birth. Using Stata SE software version 13, the Wilcoxon signed rank test was applied to compare the maternal age for these patients with the reference median age from the 2010 Rwanda Demographic Health Survey (RDHS).

**Results:** Of 320 patients diagnosed over this period, maternal age was recorded only for 286 patients, of them 276 patients had free trisomy 21 and the mean maternal age at which they were born was 34.6 years [95% CI: 33.8-35.5]. The z test statistic calculated at the reference median maternal age gave a  $p$ -value  $< 0.0001$ .

**Conclusion:** the difference between maternal ages at birth of Down syndrome patients and childbirth in the Rwanda general population was statistically significant. Advanced maternal age was thus, until proven otherwise an important risk factor for Down syndrome births in Rwanda too.

## **KEY WORDS**

Down syndrome

Maternal age

Nondisjunction

Rwanda

## LIST OF SYMBOLS AND ACRONYMS

AFP: Alpha fetoprotein  
CHUB: Centre Hospitalier Universitaire de Butare  
CHUK: Centre Hospitalier Universitaire de Kigali  
CMHS: College of Medicine and Health Sciences  
DNA: Deoxyribonucleic acid  
DS: Down syndrome  
DSSA: Down Syndrome South Africa  
EUROCAT: European Surveillance of Congenital Anomalies  
IRB: Institutional Review Board  
MMI: Military Medical Insurance  
NDJ: Nondisjunction  
NT: Nuchal translucency  
PAPP-A: pregnancy-associated plasma protein-A  
RDHS: Rwanda Demographic and Health Survey  
RMH: Rwanda Military Hospital  
RNA: Ribonucleic acid  
RSSB: Rwanda Social Security Board  
uE3: unconjugated estriol  
UR: University of Rwanda  
US(A): United States (of America)  
WHO: World Health Organization  
 $\beta$ -hCG beta human chorionic gonadotropin

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## **Chapter 1: INTRODUCTION**

### **Background**

Down syndrome (DS), the most common chromosome abnormality among live born infants and the most frequent recognizable genetic cause of intellectual disability in all human ethnic groups across the globe, is caused by trisomy 21 due to non-disjunction during meiosis at parental gametogenesis for the majority (about 95%) of cases (Dey & Ghosh 2011). With an estimated incidence of Down syndrome of 1 in 700 live births, advanced maternal age and altered meiotic recombination have been identified as strong maternal correlates associated with underlying mechanism of chromosome 21 non-disjunction in oocyte. Earlier studies have unambiguously identified advanced maternal age (defined as age 35 years or more) as risk factor, which alone initially was used, for screening pregnancies for Down syndrome (Harris et al. 2004; Berkowitz et al. 2006). Translocation and partial trisomy, other 2 chromosomal abnormalities responsible for Down syndrome, and accounting for about 5% of all Down syndrome cases are not related to maternal age. The paternal age also has no influence on the risk (Oliver et al. 2009). A shift to younger ages for Down syndrome cases has been appreciated in some recent studies in India, and analyses of 294 cases in a retrospective study on maternal age and Down syndrome found its occurrence to be different in different age groups (Malini & Ramachandra 2006; Tajeddini 2011). The researcher recommended further studies on larger samples to more characterize this relationship. Most of epidemiological data reported on Down syndrome are statistical estimates from developed countries (Oloyede 2011). Differences (genetic, racial, environmental, etc....) between black African population and developed world make these data hardly reliable (Oloyede 2011), but very few studies and data specific for Africa can be found owing to the lack of genetic tests infrastructure, qualified personnel and poverty.

Since karyotype analyses have started in Rwanda from 2006, we have been observing number of younger mother giving birth to infants with Down syndrome; this is in part thought to be related with the fact that Rwandan mothers give birth at young age in general, or on the other hand advanced maternal age has little impact on the occurrence of DS births as it has been widely accepted in the literature. Epidemiological data for Down syndrome are insufficient at national level since there has been no mechanism of its systematic screening/detection. However, the disorder is one of the most reasons to seek genetic consultation in Rwanda and available data are

from cases seen at the center for medical genetics with an advantage of being the only institution in the country where the diagnosis can be confirmed. Those data reflect but underestimate the degree of the problem in the general population given a number of patients that may go undetected. Out of 345 patients consulted in the department of genetics up to 2010, 65 cases of Down syndrome (18.8%) were confirmed on karyotype analyses (Mutesa et al. 2010). A study done earlier in 2007 had shown young women to represent the majority of mothers with free trisomy 21 births in Rwanda (72.4% were  $\leq$  34 years old, (Mutesa et al. 2010; Mutesa L, Muganga N, Pierquin G 2007); but this was done on small sample (n=29) before to the beginning of genetic tests in the country.

The morbidity and mortality associated with Down syndrome make affected children more vulnerable; they are particularly subject of maltreatment and neglect and this may be aggravated by erroneous beliefs in some communities in developing countries like in Africa (Oloyede 2011). The management of children with Down syndrome is multidisciplinary and a child's primary health care provider, a pediatrician or at least a medical officer in resource-limited settings like Rwanda where the number of experts in specialized disciplines is limited, should play a central role in providing/coordinating optimal care for children. One of the key elements in the management is genetic counseling offered to parents and families and this can be done if one has a better understanding of the condition and is able to guide them in their future decision and good and optimal care of the affected child. The information by which advanced maternal age is risk factor for Down syndrome birth may be hard to deliver when you are in front of three or more mothers of young ages all with affected children; and statistically based figures within the same community are of great importance to deal with such situations.

### **Problem Statement**

The absence of epidemiological data made it difficult to know whether observed number of children with Down syndrome from young mothers in Rwanda was related to the fact that mothers in the general community give birth at young age or if other risk factors than advanced maternal age may explain this situation as opposed to what is generally known in other communities especially in developed world. Figures from the small sample study done on Rwandan patients in 2007 and showing a large proportion of young mothers (Mutesa L, Muganga N, Pierquin G 2007) were not sufficient to provide us with a true picture in the general population and no comparison was made to allow inferential statistics. However, without any

further studies, it was hard to know whether the same trend is still present to date. The knowledge/information from that study, together with the regularly diagnosed DS patients born to young mothers have led to the thought that probably the advanced maternal age plays a little to DS births for cases diagnosed at the center for medical genetics in Rwanda. Thus, there was need to verify this with statistical evidence and carry out a comparative analysis with the general population.

The present project wanted to examine whether DS births occur more frequently as the maternal age increases in Rwanda, which may guide in genetic counseling on the subject and setting up prenatal diagnosis programs and other policy/decision-making.

### **Objectives of the study**

The main objective of the study was to evaluate maternal age distribution at birth for children diagnosed with DS at the Center for Medical Genetics and compare this with mean maternal age at birth in the Rwandan population

Specific/secondary objectives of the study were:

- To identify cases of DS diagnosed at the Centre for Medical Genetics in Rwanda
- To describe the trend in number of patients diagnosed with DS at the Centre for Medical Genetics in Rwanda over the 9- year-period
- To compare distribution of maternal age at birth for DS infants with maternal age for childbirth in the general population

### **Research question**

How is maternal age at birth for infants with DS who consult the Center for Medical Genetics compared to maternal age at birth in the Rwanda general population?

### **Hypotheses**

Null hypothesis (H<sub>0</sub>): The maternal age at birth of infants with DS is similar to that in Rwanda general population

Alternative hypothesis (H<sub>1</sub>): the maternal age at birth of infants with DS is different from the maternal age at birth in Rwanda general population.

## **Chapter 2: REVIEW OF THE LITERATURE AND RESEARCH**

In this chapter, important information related research findings on DS and maternal and other possible etiologic risk factors are summarized. It also gives an overview on general information regarding epidemiology, morbidity and clinical manifestations, diagnostic procedures and care of Down syndrome.

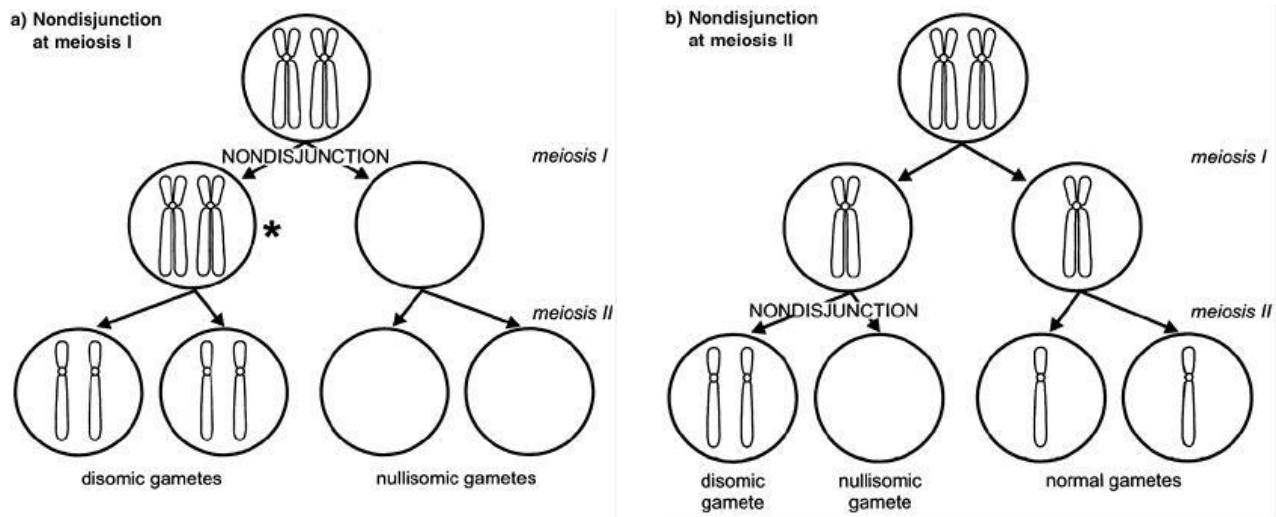
### **Introduction**

DS owes its name to an English physician, John Langdon Down at London hospital who, for the first time described the clinical appearance of this condition in his work entitled “Observation on an ethnic classification of idiots” published in 1866 (Oloyede 2011; Chen 2006). DS was the first medical condition recognized to result from a chromosome abnormality when, in 1959 the French physician Jerome Lejeune et al. identified that DS is caused by trisomy 21 (Oloyede 2011; Chen 2006; Gardner, R. J. McKinlay; Sutherland 2004). It is the most common chromosome abnormality among liveborn infants and the most frequent recognizable genetic cause of intellectual disability in all human ethnic groups across the globe; it is estimated to occur in 1 in 800 to 1 in 600 livebirths (Malini & Ramachandra 2006; Oloyede 2011). In their research, Lejeune and colleagues had found the majority (about 95%) of trisomy 21 cases is due to non-disjunction (NDJ) i.e., failure of chromosomes to segregate properly during meiosis at parental gametogenesis and this was also observed in subsequent studies; this gives rise to full/free trisomy 21, with the remainder [about 5%] resulting from mosaicism and translocations (Dey & Ghosh 2011; Malini & Ramachandra 2006). Extensive researches have been conducted to find out the etiologic factors associated with the underlying mechanism of NDJ of chromosome 21. Around 90% of errors occur during maternal meiosis, of which majority [three-quarters] happens at meiosis I, and one-quarter at meiosis II [figure1], although the latter probably have been initiated at meiosis I (Yoon et al. 1996; Gardner, R. J. McKinlay; Sutherland 2004; Dey & Ghosh 2011). Among errors of paternal origin (about 10%), the proportions due to meiotic I and meiotic II errors are almost equal (Gardner, R. J. McKinlay; Sutherland 2004).

### **Effect of advanced maternal age**

The developmental differences of gametogenesis in women and men make oogenesis more vulnerable to mal-segregation than spermatogenesis; the meiosis initiated in fetal ovary at about

11-12 weeks of gestation gets arrested at late prophase I for many years (10 to 50 depending on the time of ovulation) to resume at puberty when meiosis I (MI) is completed and the process progresses through metaphase of the second meiotic division (MII) where the follicle pauses again until it is fertilized and then the full meiotic process is completed (Ghosh et al. 2009; Dey & Ghosh 2011). Spermatogenesis, however, initiates at puberty and dividing cells enter one meiotic stage to the other without any delay (Sherman et al. 2005; Sherman et al. 2007). It is believed, as the maternal age advances, changes in the ovarian and uterine environment and functions (accumulation of toxic effects, degradation of meiotic machinery, hormonal signaling) around an oocyte in the arrested state lead to non-disjunction (Sherman et al. 2005). Altered pattern of meiotic recombination is, aside from maternal age another known factor associated with maternal NDJ (Dey & Ghosh 2011; Sherman et al. 2005; Oliver et al. 2008). Based on results from 2 US and Indian studies, chromosomal NDJ is thought to be a complex and multi-factorial event of which underlying mechanisms are, on one hand, associated with age independent factors and with age-dependent risk factors on the other (Sherman et al. 2005; Oliver et al. 2008; Ghosh et al. 2009).



**Figure 1: Classical view of the mechanics of nondisjunction** (Gardner, R. J. McKinlay; Sutherland 2004)

## Other etiologic hypotheses and risk factors:

Despite the discovery of underlying chromosomal abnormalities for Down syndrome half a century ago with related extensive researches, the exact etiology of the disorder is still unknown (Cuckle 2005; Dey & Ghosh 2011). As mentioned above, advanced maternal age and altered meiotic recombination have been recognized as risk factors for chromosome 21 NDJ. Number of other etiologic factors for DS have been studied some with conflicting results, others with not enough evidence (Dey & Ghosh 2011; Cuckle 2005; Sherman et al. 2005). Risk factors for NDJ can be categorized as “age-dependent” and “age-independent”(Sherman et al. 2005). Other hypotheses include:

*Gene polymorphism in folate metabolic pathways:* the hypothesis is based on the essential role of folate in nucleic acids (DNA and RNA) synthesis and methylation; abnormal metabolism can lead to DNA hypo-methylation, instability, mal-segregation and aneuploidy. Results have been conflicting (Patterson 2008; Cuckle 2005)

### *Production line hypothesis*

By this hypothesis, the ovulation order of oocytes in a woman follows their production order in fetal life, with those formed later having fewer chiasmata and more univalents, hence more susceptible to non-disjunction. There was no clear and consistent supportive evidence in animal model experiments (Cuckle 2005)

### *Susceptible chiasma formation*

Unusual chiasma placement (close to centromere or telomere), which is normally at the middle, was found to be another risk for chromosome 21 NDJ because of the instability it provokes, with increased susceptibility to random segregation (Dey & Ghosh 2011; Ghosh et al. 2009). In US and Indian population-based studies, single telomeric exchange was found to be more prevalent among young women (<29 years) with chromosome 21 NDJ, and the authors thought this might be an age independent risk factor (Ghosh et al. 2009; Dey & Ghosh 2011)

### *Biological aging hypothesis*

This is based on the idea that physiological ageing of ovary, and not the chronological age of women, may be more important for increasing rate of meiotic errors and subsequent aneuploid birth (Dey & Ghosh 2011; Cuckle 2005). Different views exist on this hypothesis, e.g. insufficient hormone signals with aging ovary, limited oocyte pool, senescence of meiotic



machinery (spindle function, sister chromatid adhesive proteins, microtubule motor proteins, etc) (Freeman et al. 2000; Cuckle 2005; Dey & Ghosh 2011).

#### *Genetic aging hypothesis*

At the same chronological age, some of the mothers who have DS baby are genetically older than the mothers of euploid baby and this may be the underlying cause of biological aging; this was observed in young but not old mothers (Dey & Ghosh 2011).

#### *Compromised microcirculation hypothesis*

This can result in abnormally low pH in the oocyte leading to NDJ following a cascade of events. There are still of controversy (Cuckle 2005).

#### *Relaxed selection hypothesis*

With advancing maternal age, the tendency to miscarry for affected fetuses might decrease but no consistent results/ evidence for this hypothesis when abortuses with normal karyotype were compared to those with NDJ (Cuckle 2005)

#### *Delayed fertilization and sperm ageing hypotheses*

Higher incidence of spindle defects may happen following ageing or over-ripeness in the secondary oocyte remained in MII metaphase and waiting for fertilization in the Fallopian tube; this increase the chance of non-disjunction (Cuckle 2005); this, with sperm ageing theory were epidemiologically linked with infrequent coitus (Cuckle 2005)

#### *Mitochondrial (mt) DNA mutation hypothesis*

Its effects to non-disjunction of chromosomes may be mediated through a decline in ATP level and increased production of free radicals as a result of mtDNA mutations and affect cell division and recombination processes. These mutations in oocyte increase with age and have been identified in some disorders relatively frequent in affected families like Alzheimer's disease, diabetes and hypothyroidism (Cuckle 2005)

### **Epidemiology, morbidity and clinical manifestations of Down syndrome**

The incidence of Down syndrome averages 1 in 700 live births. Some data come from population-based studies, while others are hospital/institution-based (Sherman et al. 2007; Molteno 1997; Oloyede 2011). Available data are mainly from the developed world and have generally focused on birth prevalence (Loane et al. 2013; Sherman et al. 2007). In the US, based on data obtained from 11 surveillance systems and accounting for about 22% of the live births in

the country, maternal age-adjusted prevalence of DS was 13.65 [95% confidence intervals (CI): 13.22–14.09] per 10,000 live births, or 1/732 (Sherman et al. 2007). In this study, prevalence ratio differences were noted for ethnic groups, being higher in Hispanic and lower in Non-Hispanic Black compared to Non-Hispanic Whites (Sherman et al. 2007). Recent study in Europe shows a total prevalence (i.e. including live births, fetal deaths and pregnancy termination cases) of trisomy 21 per 10 000 births of 22.0 (95% CI 21.7–22.4) or 1/455 while the live birth prevalence is 11.2 (95% CI 10.9–11.5) or 1/893; these data are from twenty-one population-based EUROCAT (European Surveillance of Congenital Anomalies) registries covering 6.1 million births obtained over a 20 – year period between 1990 and 2009 (Loane et al. 2013). In this study, analysis of data has shown a steady increase for the proportion of births to older mothers (35 years and above) in EUROCAT registries from 13% in 1990 to 19% in 2009. Researchers have noted the live birth prevalence remained stable overtime while it showed a more than three-fold variation between countries and regions (Loane et al. 2013). These variations are in part attributable to variation in maternal age profile and differences in pregnancy termination practices/legal acceptability within European countries. The 2012 annual report of the National DS Cytogenetic Register for England and Wales issued 1,982 diagnoses of Down syndrome, majority of which (64%) were made prenatally, a rate of 2.7 per 1,000 births or 1/370. The live birth rate was 1.1 per 1,000 live births with over 90% of women opting for pregnancy termination after receiving a prenatal diagnosis (Morris et al. 2014).

The estimated prevalence Down syndrome in 11 states of the South-East Asia region ranges from 0.8 per 1, 000 live births in DPR Korea to 2.1 per 1, 000 live births in Bhutan, Nepal and Timor-Leste (WHO-Regional Office for South-East Asia 2013; Christianson et al. 2006).

Few data/publications on DS are available in Africa, especially Sub-Saharan Africa. The condition was initially believed to be common in all ethnic groups but rare among Africans; it is not until the first description of black African children with DS in 1955 by Luder and Musoke that further research activities about the condition have started in this region, but it is only in 1982 when the first reliable data on incidence of DS in Black African children were published by Adeyokunnu (Luder & Musoke 1955; Christianson 1996; Oloyede 2011); in a retrospective study over a 9-year period at the academic hospital, Ibadan, South Western Nigeria, Adeyokunnu reported an incidence of 1.16 per 1000 live births or 1/862.

Added to this “ignorance”, and probably the most important today in this part of the Third World, are socio-economic and traditional/cultural factors with limited quality medical care (Oloyede 2011; Christianson 1996).

Considerable advances in surveillance and management and/or advocacy of persons with Down syndrome are observed in South Africa. Down Syndrome South Africa (DSSA) is a national non profit organization formed in 1986 for the constitutional rights of persons with Down syndrome and other intellectual disabilities and for parent advocacy (DSSA 2011; Molteno 1997). The organization currently counts 12 regional branches/support/outreach groups throughout the country to serve these vulnerable people and their families. According to the organization, the incidence of Down syndrome in the country is about 1 in 500 live births (Oloyede 2011), and three separate hospital-based prospective studies published in 1990s reported incidences of 1.33, 2.09 and 1.67 per 1000 livebirths in a Pretoria urban academic hospital, a rural hospital and at an academic hospital in Johannesburg respectively (Christianson 1996; Oloyede 2011). In all the three studies, the proportion of DS infants born to mothers aged 35 years and older was  $\geq 52\%$ . In a 20-year birth prevalence of Down syndrome study in Cape Town, from 1 January 1974 and 31 December 1993, the overall (deliveries plus pregnancy terminations following prenatal diagnosis) prevalence rate was 1.49 per 1000 (white 1.88, coloured 1.54 and black 1.29 per 1000) or 1 in 672 (Molteno 1997; Oloyede 2011). A total of 784 DS pregnancies were noted over that period. The study confirmed an increasing risk with advancing maternal age and noted racial discrepancy vis-à-vis prevalence and terminations of pregnancy; the rate was the highest for whites, but there was a marked decline over the 20 years as it was the case for the total group but not for the black population, which had the lowest rate. The rate of pregnancy termination was the highest in Whites (18.3) and the lowest in Black (1.4%), while it was carried out in 5.8% of colored pregnancies; the trend over the 20 years was not significant (Molteno 1997; Oloyede 2011).

Nowadays, the prevalence of DS in Rwanda is not known. Available data are from cases diagnosed at the center for medical genetics. It was not possible until late 2006 when the department and laboratory of medical genetics opened at the University of Rwanda that cytogenetic analyses were able to be carried out in the country. Out of 345 patients consulted in the department of genetics up to 2010, sixty five cases of Down syndrome (18.8%) were confirmed on karyotype analyses, making it, by far the most identifiable genetic anomaly at the

center (Mutesa et al. 2010). Of the 65 patients, there were 63 cases of free trisomy 21 or 96.9%. In our study about pattern of congenital heart diseases in Rwandan children with genetic defects, out of 125 cases identified over a 2-year period from May 2010 through May 2012, DS patients were 89 i.e. 71.2% (Teteli 2014). In a descriptive study on 29 DS children from Rwanda published in 2007, young mothers at the time of birth represented the majority; there were 3 mothers aged 20-24 years, 8 mothers aged 25-29 years, 10 mothers between 30-34 years i.e. 21/29 (or 72.4%) were between 20 and 34 years of age (Mutesa L, Muganga N, Pierquin G 2007; Mutesa et al. 2010). As above mentioned, DS is the most frequent recognizable genetic cause of intellectual disability in all human ethnic groups across the globe; epidemiological studies for DS syndrome started in the 1800s before its genetic basis was later identified; thanks to characteristic facial dysmorphism and other physical stigmata, physicians had started to recognize and distinguish Down syndrome patients from the heterogeneous group of people with intellectual disabilities and it is J.Langdon Down, in 1866 who emphasized the set of clinical findings in those individuals constitute a distinct entity (Sherman et al. 2007).

DS is generally easily recognized with its almost universal characteristic facial dysmorphism and other distinctive phenotypic traits (round face, small nose, upslanting palpebral fissures, epicanthus, flat neck, bilateral single palmar crease); all the characteristic morphological features are not necessary present in an individual patient and may be mild; intellectual disability is universal and muscle hypotonia (which improves with age) and joint laxity nearly constant (Sherman et al. 2005; Sherman et al. 2007; Karen Summar 2011)

In addition to the characteristic morphological and developmental features individuals with DS are more prone to potential malformations and complications including congenital heart defects (e.g. atrioventricular septal defects, ventricular septal defects, etc) in about 50% of cases, congenital and acquired digestive anomalies (duodenal atresia, Hirschsprung disease), endocrine and auto-immune disorders (hypothyroidism, diabetes mellitus, gluten intolerance, alopecia), problems with hearing and vision like congenital cataract, and other comorbidities and abnormalities like seizures, megakaryoblastic leukaemia, sleep apnea, premature aging and Alzheimer disease early in the 4<sup>th</sup> decade and infertility. The life expectancy for DS children approximates 50 to 55 years, some living into their 70s (Moran 2014; Karen Summar 2011)

## Prenatal screening and diagnosis

Maternal age was the initial single risk factor for Down syndrome used for screening pregnant women; a consensus to use a "threshold" of 35 years to offer invasive testing was reached mainly because this was considered to carry the same risk as that of procedure-related pregnancy loss from obtaining fetal specimen for karyotype

These assumptions have been subject of controversy since many other factors are considered to come into play and, with the current state of technological advances and use of noninvasive screening methods, they are obsolete today (Harris et al. 2004; Berkowitz et al. 2006).

All women, regardless of maternal age may be offered prenatal screening in different parts of the world and under different criteria from a country to another.

Despite the development of screening tests, maternal age is still an important factor and considered to have an "*a priori*" risk of Down syndrome which is used, together with screening results, to estimate a patient specific risk (Khalil & Pandya 2006). There are important differences between countries and within individual countries regarding prenatal screening and diagnosis. These variations are due many factors like the availability of different resources and required expertise, cost effectiveness, termination of pregnancy laws and social, cultural and religious convictions (EUROCAT 2010). Any medical decision should be patient-centered, individualized and from informed "parent-to-be" choices/preferences. Available noninvasive screening tests for Down syndrome include measurement of maternal serum marker levels which are interpreted and morphological ultrasound findings (Resta 2005). Based on results and maternal age, the risk of having a child with Down syndrome is calculated allowing parents to make an informed decision about invasive diagnostic procedures (amniocentesis or Chorionic villus sampling, CVS). Maternal markers used are alpha fetoprotein (AFP), pregnancy-associated plasma protein-A (PAPP-A), free or total beta human chorionic gonadotropin (beta-hCG), unconjugated estriol (uE3), and inhibin A. ultrasound screening consists mainly in determining nuchal translucency (NT) and gestational age (by crown-rump length); other markers for Down syndrome like nasal bone hypoplasia need further evidence to become part of routine screening sonographic findings (Khalil & Pandya 2006). Ultrasound is also beneficial in detecting other common birth defects associated with Down syndrome and other disorders (Khalil & Pandya 2006)

Different options exist in both the 1<sup>st</sup> and 2<sup>nd</sup> trimesters. The first trimester combined test (free

$\beta$ -hCG, PAPP-A, NT and maternal age) is performed between 9 and 13 weeks.

Second trimester biochemical screening include the Double (Age + AFP + hCG), Triple(Age + AFP + hCG + uE3) and Quadruple (Age + AFP + hCG + uE3 + inhibin A) Tests. Integrated test combines maternal age and serum markers with (full integrated test) or without ultrasound (Serum integrated test), in both the first and second trimesters; nuchal translucency and PAPP-A are measured at 10 to 13 weeks, while AFP, uE3, hCG, and inhibin A are obtained at 15 to 18 weeks. Sequential and contingent testing have been developed to differentiate women at very low risk from those at a higher risk who may need immediate invasive prenatal diagnosis. (Khalil & Pandya 2006). No screening test has a maximum detection rate and there is always a percentage of false positive results (Khalil & Pandya 2006).

The next generation genomic sequencing technology has been clinically validated as a Secondary maternal plasma-based screening test used to detect free fetal DNA in women who are screen-positive by any current primary screening test (Sparks et al. 2012; Ashoor et al. 2012). Confirmation of results with an invasive test is still needed.

### **Management of Down syndrome**

There is no cure for Down syndrome but an organized multidisciplinary approach aiming at evaluating and monitoring for associated abnormalities is needed for optimal care and prevention of common disorders in patients with this condition (Bull 2011; Roizen & Patterson 2003; Weijerman & De Winter 2010). One of the essential elements is the evaluation for congenital heart disease with an echocardiogram for all newborns to detect abnormalities that may not be symptomatic or apparent on physical examination. Other important elements requiring special evaluation/follow-up include hearing and otitis media, growth, ophthalmologic disorders, endocrine and hematological disorders, etc. Research on pharmacotherapies (e.g. pentylentetrazole) targeting mainly intellectual disabilities have been undertaken in animal models but further studies and evidence are needed for their efficacy and safety in children with DS. Supplementation with antioxidant nutrients for the treatment of DS (e.g. zinc, megavitamins, minerals, etc.) has not shown its benefits over placebo (Bianchi et al. 2010; Blehaut H, Mircher C, Ravel A, Conte M, de Portzamparc V 2010). Counseling is an important and key element in the management from the time of prenatal diagnosis

## **Chapter 3: METHODOLOGY**

It has been difficult to know without specific epidemiological data whether observed number of children with DS from young mothers in Rwanda is related to the fact that mothers in the general community give birth at young age or if other risk factors than advanced maternal age may explain this situation as opposed to what is generally known in other communities especially in developed world.

With the present project we wanted to examine trends of maternal age for DS births in Rwanda, which may guide in genetic counseling on the subject and setting up prenatal diagnosis programs.

The hypothesis was: “the maternal age at birth of infants with Down syndrome is below the maternal age at birth in Rwanda general population”. The study proposal was submitted to and approved by the University of Rwanda, college of Medicine and Health Sciences Institutional Review Board (Approval Notice: No 036/CMHS IRB/2016).

This chapter includes a summary of the process used to access and review related literature and research, population and sample, instrumentation for data gathering, data collection process, analysis of collected data and ethical considerations.

Online literature search was conducted mainly using pubmed free access through hinari. Articles from different studies on DS were selected and downloaded for detailed consultation; principal key words used are Down syndrome, maternal age, and risk factors. Further articles on general epidemiological & clinical information about Down syndrome were also downloaded for a general overview on the condition.

### **Study design, site and period**

A cross-sectional survey was conducted at the Rwanda Center for Medical Genetics. The center is unique in such a way that it is the only institution in the country that can offer karyotype testing thanks to its medical genetics laboratory located in Butare (school of Medicine/University of Rwanda), southern province. The study, mainly retrospective with some cases traced prospectively from December 2015 when the project was started, covers a period of more than 9 years for data collection since the beginning of genetic testing, mainly karyotype in Rwanda from December 2006 till February 2016. The study per se lasted 5 months from December 2015 till April 2016 with the completion of this report. The center is still young with currently 2



medical doctors, geneticists (PhD holders) doing genetic outpatient clinics in 3 referral hospitals (the University Teaching [CHUK] and Rwanda Military Hospitals in Kigali capital city and the University Teaching Hospital of Butare [CHUB] in the South province); outpatient clinic has not but started in 2014 at the Rwanda Military Hospital, RMH and it has been irregular in other hospitals especially at CHUB when the second medical geneticist graduated in July 2015 was still in training. The medical genetics laboratory has two permanent and experienced A0 laboratory scientists; they have benefited from special workshops on genetic testing processes and the unit is under the medical geneticist head and supervision. It can currently only perform karyotype locally while DNA is extracted and sent to partner laboratories (mainly at the University of Liege Center for Human Genetics, Belgium) for molecular analyses when needed; with basic equipment now available, molecular analyses are expected to start in the near future at the center locally.

### **Study population and sample**

The target population consisted of all patients with the diagnosis of DS confirmed on standard karyotype or further testing. All the cases of Down syndrome confirmed at the center constituted the sampled population for the present study project. It was a convenience sample consisting of all available cases during the study period of 9 years plus 3 months; based on 65 cases of DS that were diagnosed on a period of 3 years (actually 39 months, from December 2006 till March 2010) i.e. 22 patients per a year on average, a sample size of at least 200 cases was expected.

### **Instrumentation and Data collection**

Information regarding the diagnosis and laboratory confirmation of DS was verified in registers, soft data, and patients' files/lab test request forms in the genetic laboratory archives as well as using clinical files in hospitals; the patient date of birth, date of consultation/test, parents date of birth as well as other relevant socio-demographic and clinical data were recorded on hard copy of a pre-designed data collection form for each patient. A soft database was created using Epidata 3.1 software for data entry and Stata SE 13 software for data analysis. Treatments of text and manuscript preparation were carried out using Microsoft Office Word 2010.



## Data analysis

With descriptive statistics Stata SE 13 software was used to determine frequencies of DS cases according to different maternal age/age groups and the mean maternal age at birth for this group of children. Since there was no control group, to test whether the maternal age at birth of these DS patients is different from that in the general population we used data from the 2010 Rwanda Demographic Health Survey (RDHS). During the 2010 RDHS, 13,671 women in the reproductive age from 15 years to 49 years were interviewed, of them 8,094 women had given birth to at least one child with a total of 32,639 children. The mean and median ages at which each woman gave birth were calculated from different ages when her respective children were born and from individual maternal mean and median ages, we were able to compute the mean and median ages for the whole population of women enrolled in the survey. The mean maternal age is 27.1 years, while the median is 26.3 years. When these parameters were calculated for the last childbirth, the mean maternal age is found to be 28.9 years, while the median is 29.3 years. After testing the normality, data in our sample were found to be not normally distributed and a non-parametric test (the Wilcoxon signed-rank test) applied to compare the maternal age in our study sample to reference medians in the 2010 RDHS. At a significance level  $\alpha=0.05$ , the test statistic calculated has allowed statistical decision and conclusion to reject the Null hypothesis.

## Ethical considerations

### *Confidentiality*

No confidential information related to study participants was disclosed during the study process and no names will appear in any publication/reports.

### *Informed consent*

The study nature presents no (or may only present minimal) risks to the participants. A waiver of informed consent was sought from the University of Rwanda College of Medicine and Health Sciences Institutional Review Board (UR, CMHS IRB).

### *Ethical approval*

The study proposal was submitted to and approved by the UR, CMHS IRB. Approval Notice: No 036/CMHS IRB/2016 (see appendix E)

## Chapter 4: RESULTS

This chapter presents important study findings. The research was mainly interested in maternal age at the time of giving birth to a child with DS but other important variables were also collected.

Over a period of 9 years and 3 months (from December 2006 till end February 2016), 1560 karyotypes were performed in the medical genetics laboratory of which 320 cases of DS (or 20.5%) were identified

### Characteristics of patients

Of the 320 patients with Down syndrome, the male over female sex ratio was almost 1:1 (or 163 males over 157 females). One patient was seen at age 17 years (1 patient). The mean age at the time of testing was 20.5 months (615.6 days, 95% CI: 505.6- 725.5); 30 babies (9.4%) were tested by age 29 days, 173 (or 63.64% ) by their 1<sup>st</sup> anniversary, and 246 (or 86.52%) by age 3 years, while 3.13%(or 10 patients) were aged above 10 years. The younger age at the time of consultation/test was the birth date for 1 baby, while the oldest was 17 years

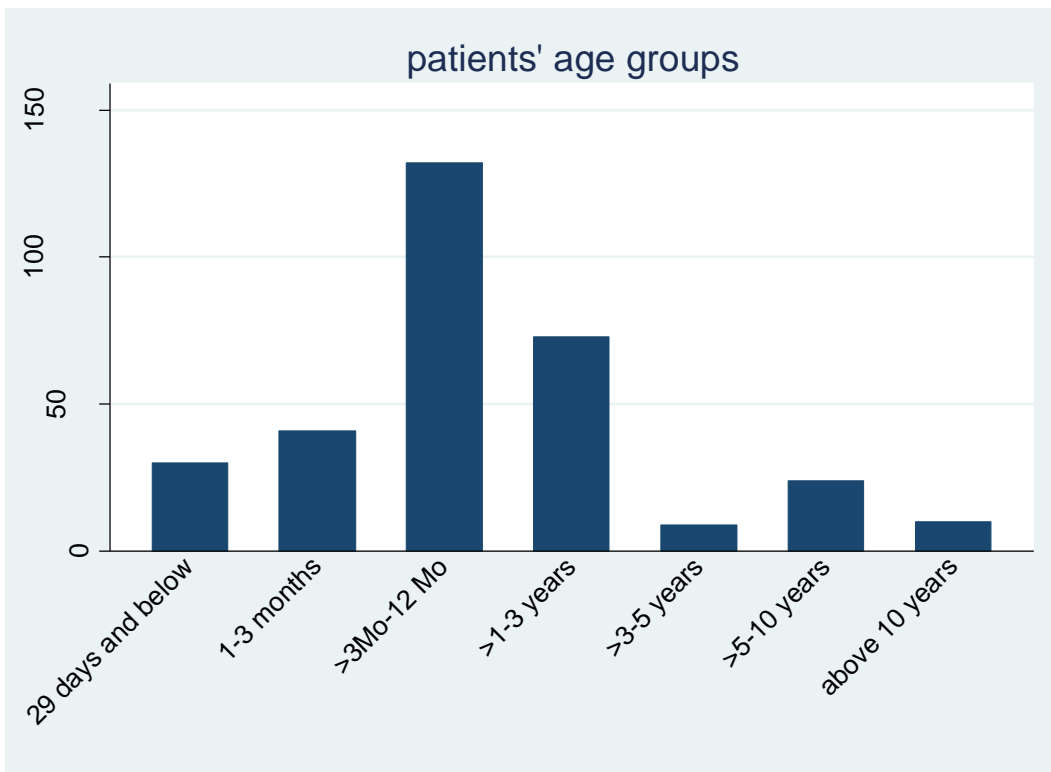


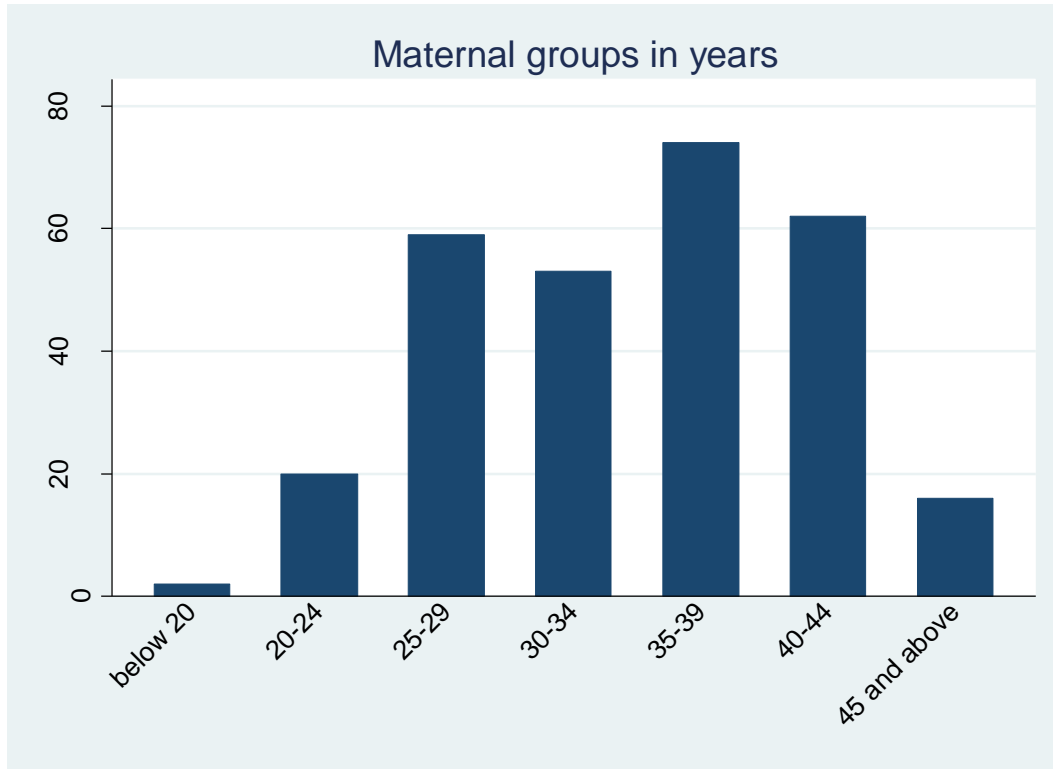
Figure 2: Number of Down syndrome cases in different age groups

The birth order was recorded for 265 patients and ranged from the first to the twelfth born child. The second born children were more represented with 45 patients (or 16.98%) while only one patient (or 0.38%) was the 12<sup>th</sup> born in order (see appendix B).

Similarly, parity for patients mothers (recorded for 263 patients) varied from primiparas through grand multiparity of as high as 12 births. Multiparity with 2 births represented 17.11% (i.e. 45 mothers) while one mother (0.38%) had reached her 12<sup>th</sup> births (see appendix C). The number of live and/or stillbirths were not specified.

Maternal age/date of birth was recorded for only 286 patients. The youngest gave birth to a DS child at 16 years of age, while the oldest was aged 53 years. The mean maternal age in the whole group was found to be 34.5 years [95% CI: 33.7- 35.3]. 46.85% were aged 34 and below, 44.4% were between 30 and 39 years, while 27.27% were aged between 40 and 53 years. When calculated only for those with free trisomy 21 DS (276 patients), the mean maternal age is 34.6 years [95% CI: 33.8-35.5].

Using the Wilcoxon signed-rank test, the z test statistic calculated at the median maternal age of 26.3 years was 12.9 [ $p > |z| = 0.0000$  or  $p \text{ value} < 0.0001$ ] for those patients with free trisomy 21 DS; positive and negative observations were 233 and 43 respectively. Similar findings were obtained when comparison was made to the median maternal for the last born; the z test statistic was 10.3 [ $p > |z| = 0.0000$  or  $p \text{ value} < 0.0001$ ], while positive and negative observations were 200 and 76 respectively.



**Figure 3: Age groups for mothers of DS patients**

It was mentioned for 266 patients whether they use a health insurance or not. Of the 261 patients with insurance, 190 (or 72.8%) had community based health insurance commonly known as “Mutuelle de Santé”; users of Rwanda Social Security Board, RSSB (43 patients) and Military Medical Insurance, MMI (8 patients) together represented 19.5%.

Three hundred and four patients had their origin registered. Only 8 patients were foreigners (mainly from refugee camps in Rwanda. The remaining 296 patients were coming from 29 (out of 30) districts of Rwanda; only Rutsiro district in the Western province was not represented. Kigali City (with its 3 districts, Gasabo, Kicukiro and Nyarugenge) had 134 patients (or 45.2% of those from Rwanda), 42 patients (14.2%) were from Eastern province, 25 patients (8.5%) were from Northern province, 71 patients (24%) were from Southern province, while 24 patients (8.1%) were from West.

## Genetic results

Free trisomy 21 was found in 308 patients (or 96.2%) with one of them having double trisomy (chromosomes 21 and X: 48, XXX, +21). Translocation cases were found in 11 patients (or 3.4%), 10 of them being robertsonian translocations (6 cases of isochromosome 21, two cases of translocations between chromosomes 21 and 22, one case between chromosomes 15 and 21 and one case between 14 and 21) with a rare translocation between chromosome 21 and the Y sex chromosome determined using FISH techniques performed in the USA. One patient was found to have 2 cell lines with a standard karyotype 47, XY, +21 and a robertsonian translocation involving chromosomes 13 and 21. There was also one patient with free/standard trisomy 21 associated with inversion of chromosome 9 segments [47, XY, + 21 inv9 (p11; q13)]. 136 patients were seen at CHUK, 113 were from RMH and 71 were consulted at CHUB. More than 50% of patients were diagnosed only between 2013 and 2015.

Karyotype	Freq.	Percent	Cum.
47, XY, +21	157	49.06	49.06
47, XX, +21	151	47.19	96.25
46, XY, rob t(21;21)(q10;q10)	3	0.94	97.19
46, XX, rob t(21;21)(q10;q10)	3	0.94	98.13
Other	6	1.88	100
Total	320	100	

**Table 1: karyotype results<sup>1</sup>.**

<sup>1</sup> One patient with double trisomy 48, XXX, +21 was intentionally registered as 47, XX + 21 for statistical purposes

Year of test	Freq.	Percent	Cum.
2007	18	5.63	5.63
2008	10	3.13	8.75
2009	13	4.06	12.81
2010	57	17.81	30.63
2011	33	10.31	40.94
2012	21	6.56	47.5
2013	35	10.94	58.44
2014	47	14.69	73.13
2015	79	24.69	97.81
2016	7	2.19	100
Total	320	100	

**Table 2: Number of Down syndrome patients diagnosed in different years**

Associated disorders/defects: It was documented for 172 patients that echo was performed and 113 of them (or 65.7%) were reported to have abnormal findings i.e. congenital heart defects. Other birth defects were reported for 10 patients. Four patients had gastrointestinal tube-related disorders ( 1 case of imperforate anus, 2 cases of Hirschsprung disease, 1 case of duodenal atresia) and 1 patient had congenital cataract.

## CHAPTER 5: DISCUSSION

This chapter is discussing the main findings/results of the study, trying to make comparison with other studies done before. Conclusions and recommendations drawn from those findings are presented here.

Advanced maternal age was identified as an important risk factor for Down syndrome, the most common chromosome abnormality among live infants and most frequently recognized genetic cause of intellectual disability (Oliver et al. 2009). A shift to younger maternal ages for Down syndrome cases has been appreciated in some recent studies (Malini & Ramachandra 2006); earlier data also showed a big proportion of young mothers giving birth to Down syndrome infants for patients diagnosed at the Center for Medical Genetics in Rwanda (Mutesa L, Muganga N, Pierquin G 2007). These were the main reasons that prompted us to conduct a study on Down syndrome patients so far diagnosed in Rwanda to see, on one hand, whether they are frequently born to mothers of more advanced age as it is already widely accepted and, on the other hand, provide the medical professionals and general public with specific data and information that are useful for management strategies and policy making. The study hypothesis was that infants with Down syndrome diagnosed at the Center for Medical Genetics are born to younger mothers compared to maternal age at birth in the Rwanda general population.

Findings of this study show that large proportion(53.15%) of infants with Down syndrome diagnosed at the center for Medical Genetics were born to mothers with advanced age ( 35 years old and above) and statistically, the maternal age for these children is significantly higher than the maternal age at childbirth in the Rwanda general population [p value < 0.0001]

The majority of patients are users of the community based health insurance “Mutuelle de santé”. The mean age at which Down syndrome patients are tested with a karyotype is 20.5 months; some can be tested as early as their birthday while others are seen and tested later when they are teenagers. No case of prenatal diagnosis was found! The birth order for children with DS is almost similar to the level of parity for their mothers when cross-linked and analyzed together. For instance, 42 third and 38 fourth born children were born from para 3 and para 4 mothers respectively. These patients are from all the provinces and corners of the country (except one district, Rutsiro that was not represented) with around 45% of them from the city of Kigali and the southern province is the second highly represented with 24% for those from Rwanda.

DS continues to be the most common identified genetic conditions in Rwanda as it is the case worldwide (Dey & Ghosh 2011; Oloyede 2011; Sherman et al. 2007). The average number has increased overtime since the beginning of genetic services with now 35 patients per year (320 patients over 9 years and 3 months) while it was 22 patients per year in 2010. This probably results from increasing awareness of medical professionals and population. Standard trisomy is the most frequent form of Down syndrome in about 96% of cases; this is consistent with what is described in literature (Antonarakis 1991; Gardner, R. J. McKinlay; Sutherland 2004; Chen 2006). It is clear that after analyzing data from this study DS patients diagnosed at the Center for Medical Genetics are more frequently born to mothers with advanced maternal age. The median age at which DS children were born to these mothers was found to be significantly higher than the median maternal age at the time of giving birth to a child in the Rwanda general population; the hypothesis by which DS children diagnosed at the Center for Medical Genetics were born at the similar maternal age than in the general population was rejected.

The results/findings of this study differ from those found earlier in 2007 at the same center (Mutesa L, Muganga N, Pierquin G 2007); the mean maternal age was 31.6 years in the 2007 study while it is 34.5 years for the current one [for all the types of DS karyotypes, and not for standard trisomy alone] ; while the mothers  $\geq$  34 years and younger represented 72.4% in 2007, we found only 46.8% in this study. These findings differ largely because the sample size was much smaller in 2007 than in the current study (29 mothers in 2007 vs 286 mothers today). The current findings are thus more reliable and change the perception that Down syndrome infants diagnosed at the Center for Medical Genetics in Rwanda are more frequently born to young mothers. This perception has been there since the findings in the 2007 study and probably continued because of a “recall bias”. Since it was already known that advanced maternal age is an important risk factor we, medical professionals probably tend to remember more those Down syndrome infants “unusually” born to young mothers because they have marked our memories. It may seem normal or it is expected to see a Down syndrome infant born to a 40-year old mother and this may not bring about much attention; on the contrary however, it is unusual/not expected to give birth to baby with Down syndrome at 21 years of age for instance; this may be an emotional and more likely to be remembered experience than when it was an old mother. Even without the “ recall bias” it would be difficult, without statistical



figures to know which age group ( below vs above 35 years) was more frequent because they are almost equal ( 46.85% vs 53.15% ). The 2007 study findings in Rwanda are comparable to those found in India in 2006 where, on a sample of 69 patients with Down Syndrome, the majority was born to young mothers with 75% aged 18-29 years (Malini & Ramachandra 2006). The authors thought it was probably due to young age of women at their marriage but the mean age for Indian mothers was not specified. Interestingly in that Indian study, the effect of maternal grandmother was found to be of statistical significance with an increase in odds by 30% per extra year; that is women born to aged mothers had an increased chance of giving birth to DS child compared to controls and therefore, advanced maternal grandmother age at the time of birth for the child's mother was found to be a risk factor for DS births in studied patients (Malini & Ramachandra 2006). The maternal grandmother age was not studied in our 2 studies in Rwanda and its effect cannot be appreciated. As for the 2007 Rwandan study, this Indian study was done on a smaller sample than our current study. In contrast to findings in our current study again, another Indian study, this time with a relatively higher sample size similar to ours, the percentage of infants with DS born to mothers aged 35 years and above was 15% i.e. 44 over a total of 294 patients studied, and the researcher recognized number of other Indian studies with similar findings (Tajeddini 2011).

The proportion of advanced maternal age in our study is comparable to findings in South African hospital-based birth prevalence studies; in Pretoria urban academic hospital, a rural hospital and Johannesburg academic hospital studies, DS infants born to mothers aged 35 years and above were 52%, 56% and 55% respectively (Christianson 1996). Similarly in the 20-year birth prevalence study in Cape Town, from available data on maternal age between years 1987 and 1993, DS infants born to mothers aged over 35 years were 35%, 52% and 60% for whites, coloreds and blacks respectively; here, the researchers confirmed the increasing risk for Down syndrome with advancing maternal age (Moltano 1997).

In the 2012 annual report in England and Wales, the mean age for women at birth of their infants with a postnatal diagnosis of Down syndrome was 35.3 (95% CI: 34.8 – 35.9), with an overall ( pre and postnatally diagnosed) 65% or 1163/1786 of the women aged 35 or older (Morris et al. 2014); these results are comparable/consistent with our current findings.

The mean age at which the diagnosis of Down syndrome is confirmed with a karyotype (20.5 months) is markedly lower in this study compared to that in the 2007 study at the same center

(9.2 years) (Mutesa L, Muganga N, Pierquin G 2007). This may partly result from improved awareness of medical care providers vis-à-vis Down syndrome and use genetic services which may explain a big percentage (>50%) of cases diagnosed only over the last 3 years of the considered period of more than 9 years; another possible contributing factor is an improved use of health care services in general thanks to the community based health insurance as it is evidenced by the large proportion of its holders in this study (72.8% or 190/261 known insured patients). An improved use of health care services may also be appreciated from the origins or residence places of diagnosed patients; although a clear discrepancy in the number of patients, diagnosed patients come from the City of Kigali and all the 4 provinces of Rwanda with 29/30 districts having at least 1 representing patient. Larger proportions are mainly seen in Kigali city districts, Huye district, but also in some big towns like Musanze in North. The higher numbers in Kigali and Huye district may be explained by the presence of genetic services there. The existing transfer system allows for patients from any corner in the country to reach referral hospitals where genetic services are available, but it is likely that number of patients may fail to consult given different social economic reasons especially for those from remote areas. The department services are also delivered to patients from other countries in the sub-region, showing an increasing and high demand within but also outside Rwanda. If there is an improved awareness about DS, one may wonder whether the current health system, other institutions in the country and the general public are prepared or empowered enough to face the burden imposed by Down syndrome morbidity and associated social-economic and financial requirements or expenses for the optimal care of affected individuals from early infancy to adult life. In our experience, there is still a long way to go starting with advocacy and psychosocial accompaniment of affected child parents. Helping in creation of parental groups or one organ where they can meet to share experiences and channel their problems may advance the welfare of affected children. One example is access to corrective surgery for congenital heart defects affecting around half of DS infants. Today in the country, cardiac surgery is only possible thanks to visiting teams from Australia, Belgium or the United States of America coming twice a year on average; there is always a long waiting list and only from those considered to have a “good prognosis” are selected patients to benefit from surgery. Down syndrome patients are not part of priority in these programs and are almost totally excluded. In our sampled population, it was documented that 172 patients (53.7%) had had echocardiography done and 113 of them (65.7%)

had abnormal anatomical findings or congenital defects. These figures are much higher than what it is already known from other studies because it is possible that those with suspected heart defects from physical exam were more likely to have echocardiography requested, and no systematic screening of heart defects was done; if they are taken from the total group (i.e. 113/320 or 35.3%), it may underestimate the magnitude of the problem. In any case we may say with some certainty that CHDs frequency ranges from 35.3% to 65.7% in our studied population of patients with DS, which confirms, like in other researches that CHDs are a major birth defect among the Down's. To the best of our knowledge and from the families we've maintained a regular follow-up, only five patients benefited from cardiovascular surgery and three of them had to go out of Africa (1 infant underwent open heart surgery in Germany, the second one in India, and the 3<sup>rd</sup> in the USA); 2 children had their surgery at King Faisal Hospital, Rwanda (one underwent a PDA closure, the 2<sup>nd</sup> had open heart surgery). Another family reported their child died while they were in process to take him abroad for heart surgery. This shows how parents are ready and committed for the best of their affected children but majority is those who cannot afford means to have their children sent abroad for such a costly procedure; there is need to advocate for these patients for the optimal health care and proper integration in the society. Since these are first pediatric patients, pediatricians should play a central role of coordinating a multidisciplinary and patient-centered team required for the management of these children.

The incidence and/or prevalence of DS births are not likely to change since no specific surveillance programs are present in Rwanda. With an increasing awareness of the general public, prenatal diagnosis is going to be more and more demanded. The medical professionals, especially obstetricians have to get prepared and think of setting up required equipment and importantly technical expertise. Though advanced maternal age is (in our study and many others done before) an important major risk factor for DS pregnancies, there is always a big proportion of children born to young mothers for which risk factors are yet to be determined.

### **Significance of the study**

The present study has allowed comparing trends in maternal age at birth for DS syndrome children diagnosed at the Center for Medical Genetics in Rwanda with what has been described in other parts of the world. It has allowed answering some questions regarding DS pregnancy and

maternal age in Rwanda. It is important information to the scientific community from a resource-limited area of Sub-Saharan Africa where research data are poor. It is particularly helpful for medical professionals in Rwanda to deliver education and counselling to patients' families and can be used in establishing future plans like prenatal diagnosis and other studies. This is the first study comparing maternal age at birth for DS children with a reference population.

### **Limitations of the study**

This was not a population-based study and only patients who presented to health facilities, which were at the referral level, could be recognized. Since most of data were collected retrospectively, some files were not complete and necessary information could not be found. Sometimes, there have been technical issues (absence of mitoses, clotted sample, broken machine, etc.) and no results were found in case blood specimen was not retaken. Mothers for the sampled population were part of the reference population but their number was too small to modify the distribution of the target population. The findings in this study should be interpreted in the limit of the sample size and bigger samples may yield different information.

### **Recommendations**

- Since advanced maternal age is, until proven otherwise an important risk factor for DS, one strategy of prevention recommended to the general population is to complete their families when they are still relatively young, especially for women.
- There should be an organized mechanism for a multidisciplinary follow-up management of DS patients
- Families with DS children should be guided and helped to form parental support groups to share experiences and have strong advocacy for patients care
- Prenatal screening and diagnosis services should start as soon as possible to respond to parents demand likely to increase in the near future; this would not base on the advanced maternal age alone since there is a big percentage of affected children from young mothers
- Further studies to find out the exact etiology for non-disjunction trisomy 21 (age-dependent or not) are needed; there is also a need to determine the incidence/prevalence of Down syndrome at national level and studies, similar to the present one, with much bigger sample size can be more informative.

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## APPENDICES

### Appendix A: Data Collection form



**COLLEGE OF MEDICINE AND HEALTH SCIENCES**

School of Medicine and Pharmacy, Department of Pediatrics and Child Health

Research project comparing the maternal age for infants with Down syndrome at the Center for Medical Genetics with the maternal age at childbirth in the Rwanda general population

#### Data collection form

Study Number					
Patient names	.....		date of birth	(dd/mm/yyyy).....	
Sex	Male	Female			
Karyotype (encircle)	Free trisomy 21	translocation	Mosaic	Formula .....	
Test request date	(dd/mm/yyyy).....				
Site of consultation					
Site Visit No					
Clinical information					
mother's name	.....	mother's DoB	(dd/mm/yyyy).....		
Number of pregnancy/parity					
Birth order for the patient					
Origin :	District.....		Sector .....		
Phone contact					
Father's DoB	(dd/mm/yyyy).....				
mother's education level:	No schooling	primary	High school	university	Unknown
Father's education level:	No schooling	primary	High school	university	Unknown
Other information/ comments					

Investigator: Dr Janvier HITAYEZU

Registrar, Department of Pediatrics and Child Health

**Appendix B: Table showing birth order for DS patients**

Patient birth order	Freq.	Percent	Cum.
10 <sup>th</sup>	4	1.51	1.51
11 <sup>th</sup>	4	1.51	3.02
12 <sup>th</sup>	1	0.38	3.4
1 <sup>st</sup>	43	16.23	19.62
2 <sup>nd</sup>	45	16.98	36.6
3 <sup>rd</sup>	42	15.85	52.45
4 <sup>th</sup>	39	14.72	67.17
5 <sup>th</sup>	24	9.06	76.23
6 <sup>th</sup>	26	9.81	86.04
7 <sup>th</sup>	20	7.55	93.58
8 <sup>th</sup>	11	4.15	97.74
9 <sup>th</sup>	6	2.26	100
Total	265	100	

**Appendix C: Table showing number of births (P=parity) for mothers of DS patients**

mother's number of births	Freq.	Percent	Cum.
P1	42	15.97	15.97
P10	4	1.52	17.49
P11	4	1.52	19.01
P12	1	0.38	19.39
P2	45	17.11	36.5
P3	42	15.97	52.47
P4	38	14.45	66.92
P5	23	8.75	75.67
P6	27	10.27	85.93
P7	20	7.6	93.54
P8	11	4.18	97.72
P9	6	2.28	100
Total	263	100	

## Appendix D: Origin of diagnosed Down syndrome patients

District of origin	Freq.	Percent	Cum.
Bugesera	7	2.3	2.3
Burera	2	0.66	2.96
Gakenke	2	0.66	3.62
Gasabo	59	19.41	23.03
Gatsibo	8	2.63	25.66
Gicumbi	5	1.64	27.3
Gisagara	6	1.97	29.28
Huye	21	6.91	36.18
Kamonyi	8	2.63	38.82
Karongi	2	0.66	39.47
Kayonza	7	2.3	41.78
Kicukiro	35	11.51	53.29
Kirehe	6	1.97	55.26
Muhanga	9	2.96	58.22
Musanze	14	4.61	62.83
Ngoma	1	0.33	63.16
Ngororero	2	0.66	63.82
Nyabihu	4	1.32	65.13
Nyagatare	4	1.32	66.45
Nyamagabe	6	1.97	68.42
Nyamasheke	3	0.99	69.41
Nyanza	8	2.63	72.04
Nyarugenge	40	13.16	85.2
Nyaruguru	9	2.96	88.16
Rubavu	9	2.96	91.12
Ruhango	4	1.32	92.43
Rulindo	2	0.66	93.09
Rusizi	4	1.32	94.41
Rwamagana	9	2.96	97.37
Foreign	8	2.63	100
Total	304	100	

## Appendix E: Copy of CMHS IRB approval



UNIVERSITY OF  
**RWANDA** COLLEGE OF MEDICINE AND HEALTH SCIENCES

CMHS INSTITUTIONAL REVIEW BOARD (IRB)

Kigali, 28/01/2016

Dr HITAYEZU Janvier  
School of Medicine and Pharmacy, CMHS, UR

### Approval Notice: No 036 /CMHS IRB/2016

Your Project title *“Does Down Syndrome occur more frequently in mothers with advanced age for patients diagnosed at Rwanda Center for Medical Genetics ”* has been evaluated by CMHS Institutional Review Board.

Name of Members	Institute	Involved in the decision		
		Yes	No ( Reason)	
			Absent	Withdrawn from the proceeding
Prof Kato J. Njunwa	UR-CMHS	X		
Prof Jean Bosco Gahutu	UR-CMHS	X		
Dr Brenda Asimwe-Kateera	UR-CMHS	X		
Prof Ntaganira Joseph	UR-CMHS		X	
Dr Tumusiime K. David	UR-CMHS		X	
Dr Kayonga N. Egide	UR-CMHS	X		
Mr Kanyoni Maurice	UR-CMHS	X		
Prof Munyanshongore Cyprien	UR-CMHS	X		
Mrs Ruzindana Landrine	Kicukiro district		X	
Dr Gishoma Darius	UR-CMHS	X		
Dr Donatilla Mukamana	UR-CMHS		X	
Prof Kyamanywa Patrick	UR-CMHS		X	
Prof Condo Umutesi Jeannine	UR-CMHS		X	
Dr Nyirazinyoye Laetitia	UR-CMHS	X		
Dr Nkeramihigo Emmanuel	UR-CMHS		X	
Sr Maliboli Marie Josee	CHUK	X		
Dr Mudenge Charles	Centre Psycho-Social	X		

After reviewing your protocol during the IRB meeting of where quorum was met and revisions made on the advice of the CMHS IRB submitted on 26<sup>th</sup> January 2016, **Approval letter has been granted to your study.**

Please note that approval of the protocol and consent form is valid for **12 months.**

You are responsible for fulfilling the following requirements:

1. Changes, amendments, and addenda to the protocol or consent form must be submitted to the committee for review and approval, prior to activation of the changes.
2. Only approved consent forms are to be used in the enrolment of participants.

3. All consent forms signed by subjects should be retained on file. The IRB may conduct audits of all study records, and consent documentation may be part of such audits.
4. A continuing review application must be submitted to the IRB in a timely fashion and before expiry of this approval
5. Failure to submit a continuing review application will result in termination of the study
6. Notify the IRB committee once the study is finished

Sincerely,

Date of Approval: The 28<sup>th</sup> January 2016  
Expiration date: The 28<sup>th</sup> January 2017

  
Professor Kato J. NJUNWA  
**Chairperson Institutional Review Board,  
College of Medicine and Health Sciences, UR**

**Cc:**

- Principal College of Medicine and Health Sciences, UR
- University Director of Research and Postgraduate studies, UR

## Appendix F: Article from the study findings and published in a peer-reviewed journal

Research Article

# Advanced Maternal Age, also an Important Risk Factor for Down Syndrome in African Black Population. A Nine - Year Experience in Rwanda and Burden Outcome

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### Keywords

- Down syndrome
- Maternal age
- Non-disjunction
- Rwanda

### Abstract

**Background:** Down syndrome is the most common chromosome abnormality recognized to cause intellectual disability in all human ethnic groups worldwide. Advanced maternal age has been identified as risk factor associated with underlying mechanism of chromosome 21 non-disjunction error leading to free trisomy 21, the most frequent form of Down syndrome. A shift to younger maternal age for Down syndrome births appreciated in some recent studies, earlier data showing majority of Down syndrome infants born to young mothers in Rwanda and regularly diagnosed cases from young mothers have prompted us to conduct a research to verify the magnitude of maternal age effect on Down syndrome births.

**Methods:** A cross-sectional survey was conducted at the Rwanda Center for Human Genetics. Cases of Down syndrome patients diagnosed from December 2006 till February 2016 were identified with respective maternal age at birth. Using Data SE 12, the Wilcoxon signed test allowed to compare the maternal age for these patients with the reference median age from the 2010 Rwanda Demographic and Health Survey (RDHS).

**Results and Conclusions:** Of 320 patients diagnosed over this period, maternal age was recorded only for 386 patients, of them 276 patients had free trisomy 21 and the mean maternal age at which they were born was 34.6 years [95% CI: 33.8-35.5]. The z test statistic calculated at the reference median maternal age gave a p-value < 0.0001.

That is the maternal age at birth of Down syndrome patients was significantly higher than the maternal age for child birth in the Rwanda general population.

### ABBREVIATIONS

CHDs: Congenital Heart Defects; CHUB: Centre Hospitalier Universitaire de Butare; CHUR: Centre Hospitalier Universitaire de Kigali; DS: Down syndrome; ND: Non Disjunction; RDHS: Rwanda Demographic and Health Survey

### INTRODUCTION

Down syndrome (DS), the most common chromosome abnormality among live born infants and the most frequent recognizable genetic cause of intellectual disability in all human ethnic groups across the globe, is caused by trisomy 21 due to non-disjunction (ND) error during meiosis at parental

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genetogenesis for the majority (about 95%) of cases [1]. With an estimated incidence of Down syndrome of 1 in 700 live births, advanced maternal age and altered meiotic recombination have been identified as 2 independent and strong maternal correlates associated with underlying mechanism of chromosome 21 non-disjunction, particularly for the mother owing to developmental differences of oogenesis compared with spermatogenesis making oocytes more vulnerable to missegregation during their arrested phase of cell division [2,3]. Advanced maternal age (defined as age 35 years or more) alone was initially used to screen pregnancies for Down syndrome [4,5]. Translocation and partial trisomy, other 2 chromosomal abnormalities responsible for Down syndrome, and accounting for about 5% of all Down syndrome cases are not related to maternal age; the paternal age also has no influence on chromosome 21 non disjunction [6].

Since the discovery that Down syndrome is caused by trisomy of chromosome 21, subsequent studies have ensued aiming at identifying the etiologic factors associated with the underlying mechanism of chromosome 21 NDJ. Chromosomal NDJ is considered complex and multi-factorial and its underlying mechanisms are associated with age dependent risk factors while others are age-independent. Other etiologic and risk factor hypotheses for Down syndrome have emerged like biological and/or genetic aging hypotheses, gene polymorphism in folate metabolic pathways, etc; and reported results have been conflicting or need further evidence [3,7,8].

A shift to younger ages for Down syndrome cases has been appreciated in some recent studies in India, and analyses of 294 cases in a retrospective study on maternal age and Down syndrome found its occurrence to be different in different age groups [9,10]. Researchers recommended further studies on larger samples to more characterize this relationship. Most of epidemiological data reported on Down syndrome are statistical estimates from developed countries [11]. Differences (genetic, racial, environmental, etc.) between black African population and developed world make these data hardly reliable [11], but very few studies and data specific for Africa can be found owing to the lack of genetic tests infrastructure, qualified personnel and poverty.

Since karyotype analyses have started in Rwanda from 2006, we have been observing number of younger mothers giving birth to infants with Down syndrome. This is in part thought to be related with the fact that Rwandan mothers give birth at young age in general on one hand, or on the other hand advanced maternal age has little impact on the occurrence of DS births as it has been widely accepted in the literature. To date, epidemiological data for DS are insufficient at national level since there has been no mechanism of its systematic screening/detection. However, the disorder is one of the most reasons to seek genetic consultation in Rwanda and available data are from cases seen at the center for human genetics with an advantage of being the only institution in the country where the diagnosis can be confirmed. These data reflect but underestimate the degree of the problem in the general population given a number of patients that may go undetected. Out of 345 patients consulted the department of genetics up to 2010, 65 cases of Down syndrome (18.8%) were confirmed on karyotype analyses [12]. A study

done earlier in 2007 had shown young women to represent the majority of mothers with free trisomy 21 births in Rwanda (72 ± 34.4%) years were old [12,13]; but this was done on small sample (n=29) before the beginning of genetic tests in the country.

These data, with regularly diagnosed cases of DS infants born to young mothers, have led us to think that DS births in Rwanda are more common in young mothers and may have other related risk factors than advanced maternal age. We conducted a research to examine whether those births occur more frequently as the maternal age increases in Rwanda or if other risk factors are yet to be discovered. Maternal ages for DS cases diagnosed at the Center for Human Genetics in Rwanda over a period of more than 9 years were compared with the maternal ages in the reference population of Rwanda using data from the Demographic and Health Survey.

## MATERIALS AND METHODS

### Study design, site and period

A cross-sectional survey was conducted at the Rwanda Center for Human Genetics. The center is unique in such a way that it is the only institution in the country that can offer karyotype testing thanks to its medical genetics laboratory located in Butare (School of Medicine/University of Rwanda), southern province. The study, mainly retrospective with some cases traced prospectively from December 2015 when the project was started, covers a period of more than 9 years for data collection since the beginning of genetic testing, mainly karyotype in Rwanda from December 2006 till February 2016. The study per se lasted 5 months from December 2015 till April 2016 with the completion of a final report. The center is still young with currently 2 medical geneticists (PhD holders) doing genetic outpatient clinics in 3 referral hospitals, the 2 University Teaching Hospitals in Kigali [CHUK] and Butare [CHUB] as well as the Rwanda Military Hospital in Kigali. The medical genetics laboratory can currently only perform karyotype locally while DNA is extracted and sent to partner laboratories (mainly at the Center for Human Genetics, University of Liege, Belgium) for molecular analyses when needed. With basic equipment now available, molecular analyses are expected to start in the near future at the center locally.

### Patients

Three hundred and twenty patients (out of a total of 1560 tested at the center, or 20.5%) with DS were diagnosed over the period time considered for data collection. The male to female sex ratio was almost 1:1 (or 163 males over 157 females). The mean age at the time of testing was 20.5 months (615.6 days, 95% CI: 505.6- 725.5); 30 babies (9.4%) were tested by age 29 days, 173 (or 63.64%) by their 1<sup>st</sup> anniversary, and 246 (or 86.52%) by age 3 years, while 3.13% (or 10 patients) were aged above 10 years. The younger age at the time of consultation/test was the birth date for 1 baby, while the oldest was 17 years [Figure 1]. Of the 304 patients with identified origin, 8 of them were foreigners (mainly Congolese from refugee camps in Rwanda) while the remainder, 97.9% were nationals from all the provinces (in 29 out of the 30 districts) of the country.

### Genetic tests

Cytogenetic tests were performed on peripheral venous blood



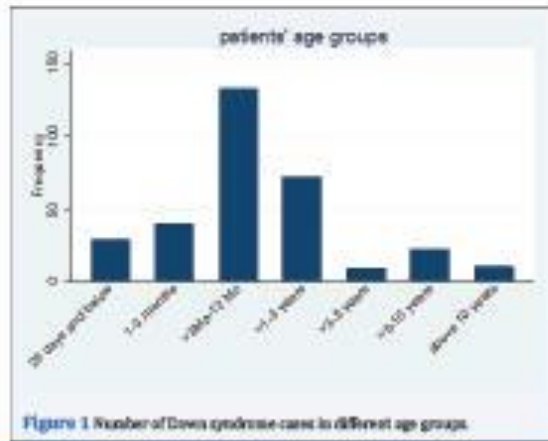


Figure 1 Number of Down syndrome cases in different age groups.

for each patient at the medical genetics laboratory in Butare. Chromosome preparations were made from 72-h peripheral blood lymphocyte cultures according to conventional protocols and routinely stained with Quinacrine. The standard karyotype was performed on Q-banded metaphase spreads and analyzed according to the International System for Human Cytogenetic Nomenclature guidelines. FISH techniques performed in the Belgium were used to detect a rare case of translocation between chromosome 21 and Y sex chromosome.

#### Reference population

With its more than 10,537,222 people as of 2012, Rwanda ranks among countries with the highest average annual population growth rate in Central and East Africa with 2.6% [14]. The total fertility rate remained relatively stable and fluctuated around 6 from 1992 through 2008 to decline to 4.6 and 4.2 children per woman in 2010 and 2014 respectively; these national demographic data are obtained thanks to two regular mechanisms for surveillance of the Rwandan population dynamics, the general population and Housing Census and the Demographic and Health Survey, DHS [15,16]. For DHS and regarding fertility and childbearing information, all women in their reproductive age between 15 and 49 years from selected households are eligible for the survey; the latest DHS in Rwanda conducted in 2014-2015 had preliminary results almost similar to that of its immediate preceding of 2010 [15,16]. We used data of the 2010 Rwanda DHS and obtained data on childbirth in Rwanda, which think reflect well the situation of our period considered for data collection. Mothers in our study are well part of this population but their number is considered negligible to impact the national trends.

#### Statistical analyses

With descriptive statistics, Stata SE 13 software was used to determine frequencies of DS cases according to different maternal age/age groups and the mean maternal age at birth for this group of children. Since there was no control group to test whether the maternal age at birth of these DS patients is different from that in the general population, we used data from the 2010 RDHS as mentioned above. During the 2010 RDHS, 13,671 women in the reproductive age from 15 years to 49 years were interviewed. Of them, 8,094 women had given birth to at least one child with

a total of 32,639 children. The mean and median ages at which each woman gave birth were calculated from different ages when her respective children were born and from individual maternal mean and median ages. And we were able to compute the mean and median ages for the whole population of women enrolled in the survey. The mean maternal age was 27.1 years, while the median was 26.3 years. When these parameters were calculated for the last childbirth, the mean maternal age was found to be 28.9 years, while the median was 29.3 years. After testing the normality, data in our sample were found to be not normally distributed and a non-parametric test (the Wilcoxon signed-rank test) applied to compare the maternal age in our study sample to reference medians  $\alpha = 0.05$ , in the 2010 RDHS. At a significance level the test statistic calculated has allowed statistical decision and conclusion to reject the null hypothesis.

#### Ethical considerations

The study protocol was submitted to and approved by Institutional Review Board of the University of Rwanda College of Medicine and Health Sciences (UR, CMHS IRB). Approval Notice: No 036/CMHS IRB/2016.

#### RESULTS AND DISCUSSION

In present study, free trisomy 21 was found in 308 patients (or 96.2%) with one of them having double trisomy (chromosomes 21 and X: 48, XXX, + 21). Translocation cases were found in 11 patients (or 3.4%), 10 of them being robertsonian translocations (6 cases of isochromosome 21, two cases of translocations between chromosomes 21 and 22, one case between chromosomes 15 and 21 and one case between 14 and 21) with a rare translocation between chromosome 21 and the Y sex chromosome. One patient was found to have 2 cell lines with a standard karyotype 47, XY, + 21 and a robertsonian translocation involving chromosomes 13 and 21. There was also one patient with free/standard trisomy 21 associated with inversion of chromosome 9 segments [47, XY, + 21 inv9 (p11; q13)] (Table 1 A,B). More than 50% of patients were diagnosed only over the last 3 years between 2013 and 2015.

Maternal age/date of birth was recorded for only 286 patients. The youngest gave birth to a DS child at 16 years of age, while the oldest was aged 53 years (Figures 2,3). The mean maternal age in the whole group was found to be 34.5 years [95% CI: 33.7- 35.3]. In total, 46.85% were aged 34 and below, 44.4% were between 30 and 39 years, while 27.27% were aged between 40 and 53 years. When calculated only for those with free trisomy 21 DS (276 patients), the mean maternal age is 34.6 years [95% CI: 33.8-35.5].

Using the Wilcoxon signed-rank test, the z test statistic calculated at the median maternal age of 26.3 years was 12.9 [ $p > |z| = 0.0000$  or  $p$  value  $< 0.0001$ ] for those patients with free trisomy 21 DS; positive and negative observations were 233 and 43, respectively. Similar findings were obtained when comparison was made to the median maternal for the last-born; the z test statistic was 10.3 [ $p > |z| = 0.0000$  or  $p$  value  $< 0.0001$ ], while positive and negative observations were 200 and 76, respectively.

DS continues to be the most common identified genetic conditions in Rwanda as it is the case worldwide [1,11,17]. The average number has increased overtime since the beginning of genetic services with now 35 patients per year (320 patients over

Karyotype	Freq.	Percent	Cms.
47, XY, +21	157	49.86	49.06
47, XX, +21	151	47.39	96.25
46, XY, rob t(21;21)(q10;q10)	3	0.94	97.19
46, XX, rob t(21;21)(q10;q10)	3	0.94	98.13
Other	6	1.88	100
Total	320	100	448.63

Abbreviations: rob t = Robertsonian Translocation  
One patient with double trisomy 48, XXXX, +21 was intentionally registered as 47, XX + 21 for statistical purposes

Other karyotypes	Freq.	Percent	Cms.
46, X, der(Y)t(Y;21)(q11.21;q11.2)	1	14.29	14.29
46, XX, rob t(21;22)(q10;q10)	2	28.57	42.86
46, XX, t(14;21)(q10;q10)	1	14.29	57.14
46, XY, t(15;21)(q10;q10)	1	14.29	71.43
47, XY, t(13;21)(q10;q10)	1	14.29	85.71
47, XY, + 21 inv9 (p11;q13)	1	14.29	100
Total	7	100	371.43

Abbreviations: der = Derivative Chromosomes; t: Translocation

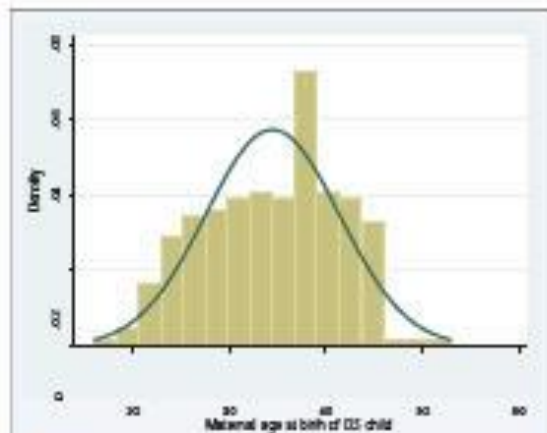


Figure 2 Maternal age distribution curve.

9 years and 3 months) while it was 22 patients per year in 2010. This probably results from increasing awareness of medical professionals and population. Findings of this study showed that large proportion (53.15%) of infants with DS diagnosed in Bouda were born to mothers with advanced age (35 years old and above) and statistically the maternal age for these children was significantly higher than the maternal age at childbirth in the Bouda general population [p value < 0.0001].

These results differ from those found earlier in 2007 at the same center [13]: the mean maternal age was 31.6 years in the 2007 study while it was 34.5 years for the current one [for all the types of DS karyotypes, and not for standard trisomy alone];

while the mothers 34 years and younger represented 72.4% in 2007, we found only 46.8% in this study. These findings differ largely because the sample size was much smaller in 2007 than in the current study (29 mothers in 2007 vs 286 mothers today). Similarly, our current findings contrast with some other previous studies like in India where majority of DS infants were born to mothers aged below 35 years [9,10]; these 2 Indian studies showed large proportions of mothers aged less 35 years with 92.7% ( or 64/69) and 85% (or 250/294) respectively. The sample size in the latter study is very similar to ours i.e. 294 vs 286 mothers though the 2 populations are quite different with respect to race, cultural and other social demographic aspects. However, the percentage of mothers aged below 35 years (85%) is much higher than what we found in our study (46.85%); the author did not do any comparison with a control to make inferential statistics but simply described the significant statistical difference between the 2 maternal age ranges (below 35 years and ≥ 35 years) with regard to the occurrence births ( $X^2 = 1.22$  and p value= 0.002). According to another Indian study with 69 cases of Down syndrome, the researchers managed to conduct a comparative analysis with a control from 200 families selected randomly from the same community, and they studied both parents and grandparents. Though the study was of small sample compared to ours, the authors found that in both cases and control, more children were born to young mothers; 75% of DS cases were born to mothers aged between 18 and 29 years, and in the control more children were born to young mothers (18-24 years) and father of advanced age (30-35 years). The authors thought it could be due to the fact that usually Indian women get married at young age. Interestingly, in the same study, the authors found that the advanced maternal grandmother age was a risk factor for DS births. In their multiple logistic regression model, only grandmother' age showed a significant difference in the odds ratios among the 4 variables (consanguineous marriage and ages of mother, father and maternal grandmother) analyzed: OR= 1.30, 95% CI 1.22; 1.39 and p value < 0.001. Most our data were obtained retrospectively and information about the age of maternal grandmothers and fathers were missing and their effect on the occurrence of DS could not be analyzed.

Our study would compare best with others in our region

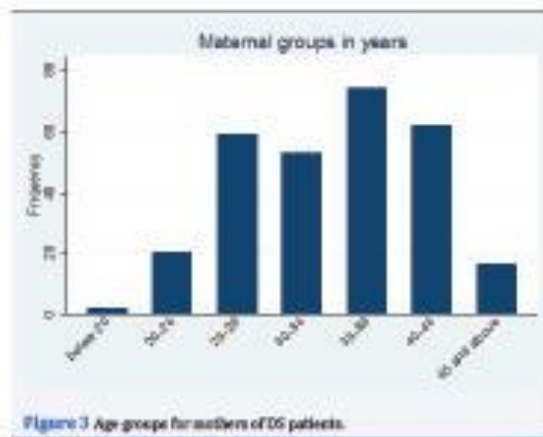


Figure 3 Age groups for mothers of DS patients.



where population presents similarities at least for race (black) and close cultures but there are almost no data. Nevertheless, the proportion of advanced maternal age in our study was comparable to findings in South African hospital-based birth prevalence studies; in Pretoria urban academic hospital, a rural hospital and Johannesburg academic hospital studies, DS infants born to mothers aged 35 years and above were 52%, 56% and 55%, respectively [18]. Similarly in the 20-year birth prevalence study in Cape Town, from available data on maternal age between years 1987 and 1993, DS infants born to mothers aged over 35 years were 35%, 52% and 60% for whites, colored and blacks respectively. Here, the researchers confirmed the increasing risk for DS with advancing maternal age and there was no significant difference in birth prevalence in the three race groups [19]. In this study, the number of mothers with known age (from 1987 to 1993) was 261 and similar to our sample size; again the researchers were interested in birth incidence/prevalence and no inference in the general population was made. In the 2012 annual report in England and Wales, the mean age for women at birth of their infants with a postnatal diagnosis of Down syndrome was 35.3 (95% CI: 34.8 - 35.9), with an overall (pre and postnatally diagnosed) 65% or 1163/1786 of the women aged 35 or older [20]; these results are comparable/consistent with our current findings. Similar results were found in other European countries from EUROCAT (European Surveillance of Congenital Anomalies) registries over 20 years between 1990 and 2009 [21]; over half of DS cases occurred in mothers aged 35 years and above in 10 of the 12 participating European countries (Table 2).

Whether studies mentioned above compare well or not our findings, this has given us a different trend from what we believed before (or at least from the few data we had) in maternal age vis-à-vis the occurrence of DS syndrome births in Rwanda. If we found similar results i.e. DS infants being born more to younger mothers, our future researches would focus on determining other etiologic risk factors like those stipulated in literature [3,7,8] and would recommend other policies like prenatal screening and diagnosis to consider and base on that situation.

The mean age at which the diagnosis of DS was confirmed with a karyotype (20.5 months) was markedly lower in this study compared to that in the 2007 study at the same center (9.2 years) [13]. Again, this may partly result from improved awareness of medical care providers vis-à-vis Down syndrome and use of genetic services which may explain a big percentage (>50%) of cases diagnosed only over the last 3 years of the considered period of more than 9 years; another possible contributing factor was an improved use of health care services in general thanks to the community based health insurance as it was evidenced by the large proportion of its holders in this study (72.8% or 190/261 known insured patients). The existing transfer system also allows for patients from any corner in the country to reach referral hospitals where genetic services are available, but it is likely that number of patients may fall to consult given different social economic reasons especially for those from remote areas.

DS morbidity and mortality associated social-economic and financial expenses are a big burden for both patients' families and the country and deserve a special attention for the optimal care of affected individuals from early infancy to adult life. A big challenge with these patients is access to corrective surgery for congenital

heart defects (CHDs) affecting around half of DS infants. In our study, 172 patients (53.7%) had echocardiography done and 113 of them (65.7%) had abnormal anatomical findings or congenital defects. These figures were much higher than what it is already known from other studies because it is possible that those with suspected heart defects from physical exam were more likely to have echocardiography requested, and no systematic screening of heart defects was done; if they are taken from the total group (i.e. 113/320 or 35.3%), it may underestimate the magnitude of the problem. In any case we may say with some certainty that CHDs frequency ranges from 35.3% to 65.7% in our studied population of patients with DS, which confirms, like in other researches that CHDs are a major birth defect among the Down's. The prevalence of CHDs in Rwanda is not known but this seems to be very important and DS is by far the known genetic disorder identified to be associated with these congenital anomalies in the Rwandan children as demonstrated in our previous study [22]. Today cardiac surgery is possible in Rwanda thanks to visiting teams from Australia, Belgium or the United States of America coming twice a year on average; there is always a long waiting list and only from those considered to have a "good prognosis" are selected patients to benefit from surgery. DS syndrome patients are not part of priority in these programs and are almost totally excluded. To the best of our knowledge and from the families we have maintained a regular follow-up, only five patients benefited from cardiovascular surgery and three of them had to go out of Africa (1 infant underwent open heart surgery in Germany, the second one in India, and the 3<sup>rd</sup> in the USA); 2 children had their surgery at King Faisal Hospital, Rwanda (one underwent a PDA closure, the 2<sup>nd</sup> had open heart surgery). Another family reported their child died while they were in process to take him abroad for heart surgery. Other associated birth defects were reported for 10 patients only. Four patients had gastrointestinal tube-related disorders (1 case of imperforate anus, 2 cases of Hirschsprung disease, 1 case of duodenal atresia), 1 patient had congenital cataract, 2 patients had urinary tract disorders (cryptorchidism and hypospadias, 1 anomaly for each patient), and hypothyroidism (not clear whether congenital or acquired), hydrocephalus and brachial cleft cyst were documented for 1 patient respectively. These disorders and others are probably underreported or simply overlooked since there no systematic

Table 2: Number of Down syndrome patients diagnosed in different years.

Year of test	Freq.	Percent	Cum
2007	10	5.63	5.63
2008	10	3.13	8.75
2009	13	4.06	12.81
2010	57	17.81	30.63
2011	33	10.31	40.94
2012	21	6.56	47.5
2013	35	10.94	58.44
2014	47	14.69	73.13
2015	79	24.69	97.81
2016	7	2.19	100
Total	320	100	475.64

screening for their detection which shows the need to build a well organized and strong multidisciplinary approach for the management and follow-up of this vulnerable group of patients as well as the improvement in documentation of health system of the country in general.

The incidence and/or prevalence of DS births are likely to change since initiatives for specific surveillance programs are being undertaken in Rwanda and also due to the awareness of the authorities and families. In developed world, majority of DS cases are prenatally diagnosed and are likely to result in termination of pregnancy for fetal anomalies  $\geq 80\%$  for (EUROCAT registries) where this practice is legally accepted [17,18]. This has prevented the live birth prevalence of DS from increasing as the number of affected pregnancies has increased due to the rise in average of maternal age [18]. Thus, with an increasing awareness of the general public, prenatal diagnosis is going to be inevitably more and more demanded in Rwanda. The medical professionals, especially obstetricians have to get prepared and think of setting up required equipment and technical expertise. Though advanced maternal age was (in our study and many others done before) an important major risk factor for DS pregnancies, there is always a big proportion of children born to young mothers for which risk factors are yet to be determined. If prenatal diagnosis services were started in Rwanda, their offer would not simply base on advanced maternal age as lonely criterion as it is no longer the case in developed world with a similar trend in maternal age for DS births.

In conclusion, DS is an important problem and deserves a special attention in Rwanda. Our findings showed that DS infants are born to more aged mothers than others in general population, a trend similar to that observed in developed world. Prenatal diagnosis is urgently needed to respond to a high demand likely to occur in a near future. And more organized, multidisciplinary and patient/family-centered approach is yet to be achieved for optimal management of those infants born with the condition.

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