Investigating the Associations between Autism Spectrum Disorder and Head Circumference

Bianca Vieira van der Net

A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master in Science, Child Health Neurodevelopment

Johannesburg, 17 May 2021

I, Bianca Vieira van der Net, declare that this Research Report is my own, unaided work. It is being submitted for the Degree of Master in Science, Child Health Neurodevelopment at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

Signature of Student.....

Date 12/05/2021

Name of Primary Supervisor Renate Strehlau

Signature of Primary Supervisor .....

Date 17 May 2021

Name of Co-Supervisor J.K. Bezuidenhout

Signature of Co-Supervisor .....

Date 12/5/2021

DEDICATION

Thank you for sparking my interest in Autism Spectrum Disorder, as well as this particular topic of research. Your passion in the field and your contribution to my personal family will forever be cherished. You inspired me to expand and enhance my knowledge and for that I will be eternally grateful.

In memory of

Professor Lorna Jacklin

1950 - 2019

ACKNOWLEDGEMENTS

1. My husband, Jared, was an unceasing source of encouragement and support, in multiple aspects. You were my pillar of strength in moments of weakness, and my biggest supporter in my proudest moments. My son, Alonzo, who entered this world during my Masters journey, was an additional source of motivation, to who I want to be a great example to.
2. To my Parents, Brothers and Sister, thank you for your interest and support in my journey to accomplishing my goal of obtaining my Masters. To my elder Brother and Sister who have completed their Masters and Doctorate respectively, thank you for being the role models that you are and for setting the bar so high! To my youngest Brother, you are the very reason I am in the profession I am today, and I will be eternally grateful to you, because every day I truly live out my passion.
3. Thank you to my Mother for taking care of Alonzo during my maternity leave so that I could complete my data capturing prior to my return to work. Katia, my cousin, thank you for being so enthusiastic and willing to assist in capturing data with me. I greatly appreciate you both!
4. To my Primary supervisor, Dr Renate Strehlau. From the first time I met you during our "research block" I appreciated and admired the way you imparted your knowledge and expertise. You are a mentor and have directed me gently, confidently and smoothly throughout the whole process. I really appreciate having you "on my team", and count myself lucky to have had our paths crossed.
5. To my Co-supervisor, colleague and friend, Dr Jacqui Bezuidenhout. Initially met as colleagues, introduced by the dear Professor Lorna Jacklin. Thank you for introducing and encouraging me to do this Masters course, initially I was reluctant to, for personal reasons, yet your encouragement and motivation assisted me in making one of the best decisions of my life! You have played an integral part in sparking my passion for Autism. The importance you place on, and the respect you have for, the Allied professions is so greatly appreciated and admired. Working closely with you every month is such a blessing as you gracefully impart your never-ending fountain of knowledge. Although no one could ever replace Professor Jacklin, you are most certainly making her proud!

6. Thank you to Jaryd Nesbitt for your unwavering commitment in helping me make my statistics component of this research perfect! Your knowledge and expertise in the field is unmatched – and I count myself so lucky having had the opportunity to work with you! Thank you Julia Nesbitt for your role in assisting Jaryd and I, as well as your consistent interest and encouragement in my project.
7. Although there are far too many to name, thank you to my loyal friends (and colleagues who are friends) for being an integral part of my greater support structure.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ADDM Autism and Developmental Disabilities Monitoring Network

ADI-R The Autism Diagnostic Interview–Revised

ADOS-2 The Autism Diagnostic Observation Schedule, Second Edition

ART Antiretroviral therapy

ASD Autism Spectrum Disorder

CARS-2 The Childhood Autism Rating Scale, Second Edition

CMJAH Charlotte Maxeke Johannesburg Academic Hospital

DSM-5 Diagnostic and Statistical Manual of Mental Disorders

DTI Diffusion Tensor Imaging

HC Head Circumference

HEU HIV-exposed and uninfected

HIV Human Immunodeficiency Virus

HUU HIV-unexposed uninfected

ID Intellectual Disability

M-CHAT Modified Checklist for Autism in Toddlers

MRI Magnetic Resonance Imaging

mtDNA leukocyte mitochondrial DNA

NDC Neurodevelopmental Clinic

RTHB Road-to-Health Booklet

SA South Africa

SD Standard Deviation

Stats SA Statistics South Africa

USA United States of America

WHO World Health Organization

ABSTRACT

Background: Autism Spectrum Disorder (ASD) is a complex neurodevelopmental disorder that is rapidly increasing in prevalence globally. An emerging field of research investigating the association between macrocephaly and ASD is developing. There is an absence of data emanating from the South African context. Currently the journey to an ASD diagnosis is lengthy, complex and costly within the South African context.

Objective: To investigate the head circumference measurement of children living with ASD and to describe their ASD severity and diagnosed comorbidities.

Methods: A retrospective record review of patients aged 0-5 years diagnosed with ASD, was conducted. All patients were attending a Neurodevelopmental Clinic at a South African, tertiary-level, state hospital during April to September 2019. Demographic data including head circumference measurements were collected. The World Health Organisation data set of head circumference norms were used as the comparator reference population. Statistical analysis was conducted using parametric, descriptive and inferential methods.

Results: Data from 135 children diagnosed with ASD were included. The sampled group had a mean age of 40.58 months (13 – 61 months) and consisted of 107 (79%) males. The majority, 49 (36.2%), of the patients were in the 4-5 year old age group. Thirty (22.2%) patients in the cohort had a head circumference which was classified as macrocephalic. Most, 63 (46%), of the patients had an ASD severity of three and 40 (29.6%), had not been diagnosed with any comorbidities. The most commonly diagnosed comorbidities were intellectual disability and attention deficit hyperactivity disorder. No significant associations were found between macrocephaly and ASD severity.

Conclusions: Head circumference measurements in ASD patients is a culturally-sensitive, cost-effective, simple and non-invasive procedure that can assist

professionals in raising suspicion of ASD at an early age. Early detection and intervention has the ability to optimise participation and integration for the individual living with this life-long neurodevelopmental disability.

Keywords: autism, head circumference, autism phenotype, autism severity, autism detection, early intervention, macrocephaly.

INTRODUCTION

Definition and Epidemiology

South Africa, in alignment with the World Health Organization (WHO), has prioritised coordinating and improving the management of individuals living with Autism Spectrum Disorder (ASD) as it is a complex, lifelong neurodevelopmental condition (1). The Diagnostic and Statistical Manual of Mental Disorders 5 (DSM-5) (2013) defines ASD as a developmental disorder comprising impairments in all three areas of social communication and interaction; namely (a) back-and-forth flow of communication, (b) non-verbal communication and (c) developing and maintaining relationships (2). The disorder is further defined by restricted and repetitive behaviours, interests or activities that manifest in at least two out of the following four criteria; (a) stereotype or repetitive motor movements or use of objects, (b) excessive adherence to routines, (c) intense interests and (d) unusual sensory interests or reactions (2). These behaviours must significantly affect functioning and performance of daily activities (2). In addition to the above mentioned criteria, the DSM-5 also requires the diagnostician to specify whether the ASD is accompanied by a language disorder or not, note the presence or absence of intellectual impairment, and, state if the ASD is associated with a primary medical and or genetic condition or environmental factor. Secondly, a rating is provided for social communication and the behaviours that were previously considered in the above mentioned criteria (2). With autism being a spectrum disorder, the amount of support required by the individual for the social-communicative as well as the restrictive behaviour subsection, must be specified in order to better understand the clinical severity of affected individuals. This is done by assigning severity levels: level one requires some support; level two requires substantial support, and lastly level three which requires very substantial support. Additionally, the impairments must be apparent during the period of early development and not be better explained by intellectual impairment or global developmental delay (2).

Due to the characteristics of ASD being spectral, a diagnostician truly needs to understand the disorder and not only adhere to a checklist approach. According to Voigt the overlap of several developmental-behavioural diagnoses with ASD makes it challenging to differentiate if specific areas of functioning are matching with the underlying cognitive capacity, or if the child does in fact have an ASD (3). Common disorders that may overlap and hence complicate a primary ASD diagnosis, are: language disorder, language-based learning disability, nonverbal learning disability, social communication disorder, attention-deficit/hyperactivity disorder (ADHD), and intellectual disability (ID) (3).

The Autism and Developmental Disabilities Monitoring Network (ADDM) – a collaborative network based in the United States of America (USA) which/that retrieves and reviews health records from medical and educational service providers - conducted a prevalence study across 11 sites in the USA (4). For the period of 2016, 1 in 54 children aged eight years had ASD (4). With regards to sex ratio, Baio et al. identified ASD prevalence to be higher in males than in females, with a sex ratio of 4:1, and the male-to-female prevalence ranged from 3.2 to 4.9 (5). There have been no racial or ethnic differences noted in prevalence studies (6).

There is a paucity of studies investigating the prevalence of ASD in the South African context, and it has been highlighted as an essential need in order to define the magnitude of the burden of ASD, ascertain risk factors and plan intervention strategies within this context (7, 8). Using the point prevalence data obtained in the USA as a benchmark, Springer et al. estimated there to be over 270 000 cases of ASD in South Africa (SA), with 5000 new cases annually (9). These are estimates, and it was therefore suggested that prevalence studies of ASD in SA are to be done. Van Biljon et al. suggested that ASD prevalence in SA may be higher than that of developed countries because of higher rates of poverty, illiteracy and HIV/AIDS, which are contributing factors (10). In an interview published in the website Africa Check, University of Cape Town autism expert, Professor Petrus de Vries, had a similar view in that although there are a lack of studies observing the total number of ASD individuals in SA, or even sub-Saharan Africa, there is

no reason to believe that numbers of cases in SA would be lower than the global figures (11). In the same interview, Ms Lamb, the national education facilitator of Autism South Africa, emphasised that there are too few ASD diagnoses made in SA and this may be due to the following two reasons; shortage of trained professionals and vast language and cultural variations amongst South Africans (11).

According to Statistics South Africa the disability prevalence in SA as measured in 2016, was 4.2% of all children under the age of nine (12). Disability was however measured using a broad definition of moderate to severe limitations in seeing, hearing, communication, remembering/concentrating, walking and self-care (12). Statistics South Africa has been criticised for inaccurately reflecting childhood disability as their statistics only include children from ages five years and older, and lack precision in determining numerical values for specific disabilities (13). Due to the dearth of published research it is not possible to draw reliable conclusions about the prevalence of ASD in the South African context.

Aetiology

The aetiology of ASD has historically been controversial. Subsequently, maternal ill-treatment, as well as vaccination has been disproven as a causative agent or action (14). Epidemiological studies worldwide have continued to refute a, now retracted, Lancet study conducted in 1998 linking the Measles, Mumps and Rubella vaccine to ASD (15). Genetic and environmental factors have both been deemed probable in playing a role in the manifestation of ASD (14). Additionally, epigenetics and gene-gene interactions may also play a role (16).

With regards to genetics, ASD is highly complex and patterns of inheritance are multifactorial (16). A single gene disorder has not been discovered, but an estimated 2500 susceptibility genes have been identified (16). These genes are known to have inconstant penetrance and expressions and are therefore often

associated with different neuropsychiatric phenotypes such as ASD (16). Familial patterns of ASD have been demonstrated as seen in twin studies, however the data appears complicated, as a "broader autism phenotype" has been identified that includes milder features that are similar to the current ASD definition (14). Various genetic conditions associated with higher rates of ASD have been identified, such as Fragile X, Rett Syndrome, Tuberous Sclerosis, Down Syndrome, Angelman Syndrome and a variety of other syndromes (14). It must however be emphasised that ASD is not associated with a specific, single "autism gene"; because currently, the majority of ASD diagnoses are not accompanied by syndromes, so the search for an "autism gene" has thus far been fruitless (14).

The epigenetic model with respect to ASD aetiology is one that proposes an individual with a genetic vulnerability that may be more sensitive to environmental influences that impact the early stages of neurodevelopment (6). The heritability risk has been deemed high, and environmental risk factors that have been identified include: advanced maternal and paternal age; gestational hypertension and diabetes; maternal immune activation; prematurity (14); maternal obesity; short interval between prior pregnancy; and certain prenatal infections (6).

Neurobiological research has revealed that rather than a structurally discrete anomaly, it is thought that ASD is associated with vast nonconforming neural activity and neuronal networks (14). The neurobiological and genetic nature of ASD are rapidly evolving and it is predicted that in the future ASD may potentially be sub-classified into conditions that relate to each other; however, are diagnosed and treated according to the identified aetiology (14).

Neurobiology of ASD

"Biomarkers are biological parameters that differ between normal and pathological processes and can be used as indicators for diagnosis, prognosis, risk assessment of a disease, and evaluation of therapeutic outcomes" as defined by

Abruzzo et al., who conducted a comprehensive search on the PubMed database investigating the literature on the neurobiological basis of ASD. A total of 869 papers were formally reviewed (17). Nine biological markers of ASD were identified, namely; [1] genetics, [2] function of proteins coded by risk candidate genes, [3] neuropathology, [4] redox system, [5] immunology, [6] oxytocin and social peptides, [7] environmental factors, [8] brain structure and function, and lastly [9] other (18). Focusing on ASD brain structure and function markers specifically [8], included was brain size increase, under and over-connectivity, difficulties in face and object recognition, lack of brain regions being integrated, modulated brain functions being disrupted, and lastly, ineffective information processing (18).

As early as 1938, Leo Kanner, a psychiatrist, physician and social activist, made observations on 11 children whom he deemed to have "infantile autism" and noted five of these children as having "relatively large heads" (19). This presumption has since lead to research investigations that have delved into the relationship between head circumference (HC) and ASD (20). Research has revealed a dynamic process of brain growth (demonstrated by HC) abnormalities as a function of age relative to the nature and severity of ASD symptoms (19). This novel insight has allowed for a non-invasive measurement such as HC, to be used as a consideration in early identification of ASD (20).

A systematic review and meta-analysis published in 2015 reviewed brain size from 261 records reporting HC and an additional 391 records reporting total brain volume from magnetic resonance imaging (MRI) studies in individuals with ASD (21). This review collectively established that the cerebral hemispheres, the cerebellum and the caudate nucleus were generally enlarged, whereas the corpus callosum and midbrain had smaller volumes in individuals with ASD (21). The authors did however emphasise that these abnormal anatomical characteristics are largely individualised and are therefore influenced by genetic and epigenetic factors. Similar findings were reported by Courchesne et al. who concluded that increased size, chemical composition, tissue structure and function of the

cerebellum, frontal lobes, temporal lobes and limbic system were most prominent, and that atypical grey and white cerebral matter was present (22). The brain areas mentioned above are seen as fundamental in higher order executive functions that have an influence on lower-order functions, such as socio-emotional development and information integration (22). Brown reports that numerous studies have demonstrated consistent neurological deviations of both white and grey matter in individuals with ASD, "as well as evidence of cortical dysgenesis" (14). Furthermore serotonin and γ-aminobutyric-acid (GABA) systems, as well as oxytocin, vasopressin, and androgens/estrogens are being investigated for abnormalities that could potentially influence neurodevelopment (14).

Parellada et al. identified [7] environmental factors such as exposure to drugs, viruses or toxins that are notable risk factors to consider in individuals who are diagnosed with ASD within the context of SA (18). Recent global statistics reveal there are 1.4 million woman living with HIV, who give birth annually to approximately 1.25 million infants who are HIV-exposed and uninfected (HEU) (23). These numbers are substantial and therefore have to be considered when investigating neurobiological markers such as exposure to drugs, viruses or toxins in ASD. Antiretroviral therapy (ART) for Human Immunodeficiency Virus (HIV) and ASD has been linked in literature with alterations to mitochondria (24). Budd et al. propose that ASD symptoms may have an increased penetrance in these diagnosed individuals due to the following sequence of events: potential genetic predisposition to ASD that is mediated by mitochondrial dysfunction and aggravated by HIV / ARV exposure which further increases the levels of mitochondrial dysfunction (24). The results of this study stress the necessity for further research into the prevalence of ASD within HEU children (24).

Kanner's original observations have thus led to researchers utilising various methods of brain size investigations, including MRI, to ascertain the justification of [3] neuropathology in individuals with ASD (19). Chawarska et al. made an important proposal; that skeletal growth, and not only neuronal growth, influences the conclusions of these growth related studies (25). Recently ASD has been

viewed in light of a “disconnectivity” disorder and thus brain networks instead of brain regions, have been the focus (18). A widespread Medline database search was conducted and narrowed down to review 869 papers published over five years (2008 – 2012), revealed that functional MRI (fMRI) and Diffusion Tensor Imaging (DTI) are being used due to the “disconnective” nature of the disorder (18). Functional MRI examines the functional connectivity, and the DTI technique studies the structural integrity of the brain (18). Both these techniques have revealed both under- and over-connectivity in adults with ASD (18). Brain region connectivity, in terms of activation and synchronization appears to be most impaired when applying these techniques (14, 18). The narrative of the neurobiology of ASD is still unresolved, however, it must be recognized that the ASD phenotype is the final expression of early-onset brain deviations as described above (18). Collectively it can be noted that macrocephaly in ASD can result from several mechanisms, which are not exactly understood at this time (21).

Signs and Symptoms

A literature review conducted by clinical practitioners of various disciplines published multiple summary statements with regard to the earliest possible signs and symptoms of ASD in children aged 24 months and younger (26). The authors draw attention to the crucial necessity to decrease the amount of time families spend awaiting the often drawn-out journey to a diagnosis of ASD (26). Using parental report, retrospective home video analyses, longitudinal studies, as well as assessments of high-risk infants, the following early markers for ASD were identified; “reduced levels of social attention and social communication”, “repetitive behaviour with objects”, “atypical body movements and motor development” and “temperamental profile” (26).

Reduced levels of social attention and social communication are characterised by decreased ability to be oriented to name, reduced use of communicative gestures such as shared focus as well as infrequent visual attention to socially-rich

environments (26). These are examples of some of the earliest concerns reported by parents of children later diagnosed with ASD (26). As children on the autism spectrum develop language, it may be a fluctuating gain and loss of skills, and is often described as "odd"; with variable intonation and volume of speech (27). In social contexts, these children can usually be found alone, and do not engage in or seek social interactions (27). Therefore, friendships are difficult to start and maintain and this becomes more apparent at school-going age (27). Verbal and non-verbal communication is disconnected and can be observed by limited facial expressions and failure to use eye contact for purpose, such as requesting (14). On the other extreme, an individual with ASD may have difficulty with restraint from a stranger and battle to interpret social cues received from them (28). Regardless of the individuals' language proficiency, social aspects of communication are impaired (28). These aspects of socialisation often hinder the individuals' opportunities for meaningful and successful relationships and hence these children are subjected to bullying (28).

As early as 12 months of age, repetitive and atypical behaviour with objects was observed in children who were later diagnosed with ASD, these atypical behaviours included lining up, spinning, and peculiar visual inspection of objects (26). Resistance to change and fixation on sameness can be demonstrated in repetitive routines, travelling a precise route to a destination, specific clothing and even linen selections (28). Preoccupations may become an obsession, with either the interest or the intensity thereof being inappropriate (28). Atypical, restricted and repetitive movements and postures are often characterised by delayed echolalic speech, hand-clapping, arm-flapping, lining up objects, rocking, spinning and toe-walking, all of which are pathological after the age of 24 months (28). Body movements and motor development in children who later develop ASD are less well understood, however, atypical body movements and posturing of the extremities are early indicators, and generally observed in children aged less than 24 months of life (26). Sensory stimuli can either be underreacted or overreacted to, and how children on the spectrum modulate these experiences vary; some may have little or no reaction to pain or temperature, whereas others

may demonstrate increased distress to everyday stimuli such as noises or food textures (14, 28).

Lastly, a marker of temperament can be observed as early as six months of age, for example, decreased understanding of positive reinforcement, with other areas of temperament being impaired such as difficulty in maintaining attention and behaviour as well as evidence of emotional dysregulation (26). Emotional instability can often be observed in response to "sameness" not being adhered to, which can potentially prompt an episode of distress which is displayed as a tantrum (28). As described by Frye, outbursts and tantrums can be a daily occurrence across the spectrum of ASD individuals and is often instigated by the areas of impairment mentioned above (27). Garon et al. investigated temperamental profiles of children who were later diagnosed with ASD and recognised that they had a low positive affect, a high negative affect and difficulties in controlling their behaviour and attention (29).

Due to the diverse and spectral nature of ASD expression in each individual there is no conclusive behavioural or diagnostic indicator of ASD prior to the age of 12 months (26). Bridgemohan echoes this sentiment that symptoms may very well be identified before the age of 12 months, however, these symptoms are not as reliable to predict a later diagnosis of ASD (6). This is especially true in children who present on the spectrum with very mild symptoms and may not be diagnosed until approximately preschool to school-going age (6). It is hypothesised that these individuals with mild ASD are recognised at this late stage due to the increased social demands that occur in these contexts, such as friendships and group participation (6).

Screening and Diagnosis

The social, affective and behavioural nature of ASD makes identifying and diagnosing the condition intricate and that much more multifaceted (30), resource-

intense and time-consuming (31). The outcomes of diagnostic measures for ASD are only as good as the professionals administering them, and only as good as the assessment instrument selected (30). The instrument selected is of utmost importance as it is the conduit for obtaining significant information regarding the individual possibly being diagnosed with ASD (30). Specialist clinicians should conduct standardised screening and assessments to evaluate social functioning, communicative abilities, cognitive abilities, emotional regulation and adaptive behaviour within their disciplines (30). Best practice is said to be the presence of a multidisciplinary team who have experience with ASD, and in conducting the diagnostic assessments necessary (6). Qualified professionals such as developmental-behavioural paediatricians, neurodevelopmental disability specialists, neurologists, psychiatrists, and psychologists are able to make a formal diagnosis of ASD (6). Additionally, other professionals involved in diagnosis and management are not limited to Speech-Language Pathologists and Occupational Therapists, and is dependent on the child's developmental concerns which require their services (6). A diagnostic profile can only be conclusive when the above mentioned domains are thoroughly assessed, as well as the consideration and differential diagnosis of co-occurring conditions have taken place (30). Assessments that have been suggested to assist in formulating a differential diagnosis include: a diagnostic hearing evaluation by an Audiologist; HC measurement and serial monitoring; identification of dysmorphic features; and Tuberous Sclerosis screening (6).

The relevant information that is required includes case history information on developmental, medical and behavioural aspects (30). Direct or informal observations can allow the assessor insight into the child's social skills, behaviour, communication and play (6). These informal observations can be accompanied by diagnostic tools such as The Autism Diagnostic Observation Schedule, Second Edition (ADOS-2) (32), The Childhood Autism Rating Scale, Second Edition (CARS-2) (33) or The Autism Diagnostic Interview–Revised (ADI-R) (34) which will be described (6).

The ADOS-2 is a robust instrument that has proven to be both reliable and valid psychometrically and can assess individuals over their lifetime (30). Practically, the ADOS-2 is a semi-structured, standardised assessment of communication, social interaction, behaviour and various forms of play (35). ADOS-2 administration requires costly specialised training as well as sophisticated scoring and interpretation of other observations (30, 35). A benefit of using the ADOS-2 is that the specified overall scores can be directly related to the diagnostic criteria of ASD from the DSM-5 (30). The ADOS-2 assessment is currently being used as a diagnostic tool in SA, however, it is limited to use in English-speaking individuals as it has not yet been translated into languages native to SA (36). A research study conducted in the Western Cape translated the ADOS-2 into Afrikaans and concluded that it was appropriate to be used in Afrikaans-speaking individuals accompanied with specific considerations which need to be applied in order to ensure cultural and socioeconomic sensitivity as well as reduce method bias (36).The ADOS-2 is often used in combination with another diagnostic tool, the ADI-R (35).

The ADI-R is a clinical, semi-structured caregiver interview tool, and is capable of capturing and integrating relevant information from the past and present and is seen to be a necessary part of an ASD diagnosis (30). Although this necessity has been realised, the ADI-R seems to be predominantly used in research intensive settings due to the lengthy administration time (6). The interview questions focus on the age period of four to five years and consists of 93 comprehensive questions which can take up to three hours to administer (30). A score is then calculated using an algorithm which classifies the individual as having ASD, or non-spectrum behaviours (30). The utilisation of the ADI-R tool in SA appears to be uncommon.

Lastly, the CARS-2 is a 15-item direct clinical observational tool which is based on a scoring system that classifies ASD on a rating scale for individuals over the age of two years old (6). When the severity of the disorder is established by the rating scale, the clinician must decide if an all-inclusive diagnostic tool such as the ADOS-2 (discussed above) is justified or indicated (30). The use of CARS-2

in the South African context is not well documented, and the reason may be that it was designed for use in the USA and requires payment for use, as well as brief training to administer. A study conducted in Tanzania to assess the cultural relevance of the CARS-2 concluded that it was valid, sensitive and specific at acceptable levels and could be used in that setting. Some cultural adaptations were however suggested; such as ensuring familiarity of the stimulus items, social routines and play scenarios (37).

Schulman et al. (30) reviewed diagnostic instruments specific to ASD and only included the instruments in a survey if at least one empirical article proving their reliability and validity was available in the literature. These diagnostic instruments included ADOS-2, ADI-R and the CARS-2. The ADOS-2 was deemed to be robust, reliable and valid for the diagnostic assessment of ASD across the lifespan (30). Screening tools and rating scales such as the Modified Checklist for Autism in Toddlers (M-CHAT) are recommended for use as a beneficial adjunct to the formal diagnostic process, as confirmatory or complementary tools (30). Although it requires further validatory research in SA, a South African student's Master's thesis conducted in 2012 concluded that the M-CHAT proves to be a successful screening tool in the South African population (38).

There is a dearth of research emanating from Africa with regards to ASD diagnosis, and Abubakar et al. has noted in the systematic review they conducted that ASD research has predominantly been undertaken in SA and Nigeria on the African continent (8). This paucity of research is suggested to be caused by the non-existence of ASD screening and diagnostics tools validated in the African context (8). This sentiment was echoed in a study conducted in the Western Cape evaluating perspectives of relevant ASD stakeholders, such as senior managers from the Departments of Health, Education and Social Development, who reiterated that ASD tools need to be translated and validated locally (1). Zwaigenbaum et al. stated that underrepresented and minority groups need to be included in rigorous ASD-specific screening research so that screening tools can be adapted and be made culturally and socio-economically appropriate (39).

Compounding this issue, Smith, highlighted that reputable journals will only publish research on ASD if the research participants have been diagnosed by the gold-standard ASD diagnostic assessment tool, ADOS-2 (36). A literature search revealed an absence of validity studies conducted on the ADOS-2 in SA. This is a worrisome topic, as research being conducted in SA may remain unpublished as not all clinicians can use ADOS-2 for diagnostic purposes. This is for numerous reasons, one being the cultural appropriateness as elucidated above, as well as training accessibility and affordability for professionals. ASD diagnostic and screening tools developed in high-income settings, for use in low-income settings requires clinicians to carefully consider the language used in the tools, physical resource requirements, levels of literacy, as well as the tasks expected of the child being assessed (8).

Early Identification

Typical age at Diagnosis

The age of the child at ASD diagnosis is highly variable due to multiple and diverse factors. As highlighted by Courchesne et al., ASD is usually only identified and formally diagnosed in individuals between two and four years of age (22). Data obtained from two large family research databases that registered children who were diagnosed with ASD between 2004 and 2014 in the United Kingdom (UK), concluded that the median age of ASD diagnosis was 55 months (four years and seven months) (40). Similarly, in the USA, Zwaigenbaum et al. (26) identified various large-scale epidemiological study findings indicating a mean age of ASD diagnosis between four and five years of age. Springer et al. conducted a study to describe the demographics, among other factors, of children diagnosed with ASD at a developmental clinic at Tygerberg Hospital, Western Cape, South Africa over a two year period (2008 - 2010) (9). They concluded from a total of 58 participants that the median age of ASD diagnosis was 42 months (3 years and 6 months), with the range being 15 – 106 months (1 year and 3 months – 8 years 10 months). In a South African study aiming to compare

profiles of ASD learners in private and public schools, the authors deduced that the mean age of diagnosis of 126 learners in public ASD specific schools was, on average, 47.9 months (3 years, 11 months) (41). Although not statistically significant (p=0.368), 24 learners in private ASD specific schools mean age of diagnosis was, on average, 42.7 months (3 years, 6 months) (41). Although there is increasing evidence that ASD can be diagnosed around 14 months (16), Erasmus et al. considered that South Africa's mean age of diagnosis may be later due to limited parental knowledge of typical developmental milestones, limited awareness of ASD as well as inaccessible diagnostic services for ASD (41). Franz et al. emphasises that improved statistics of prevalence as well as burden and cost may alter the South African government's approach to ASD (1).

A scoping review conducted by Zwaigenbaum et al. on multiple peer-reviewed articles concluded that an ASD diagnosis can be deemed stable when made from the age of 24 months and beyond (39). Fukumoto et al. suggested that non-invasive, routine body measurement data, for example HC measurement, along with early behavioural observations may assist in forming a diagnosis at a young age (20). Understanding differences in brain growth and development could optimise time windows for specific areas of intervention (21).

Benefits of Early Diagnosis

Earlier identification of ASD could possibly enable general practitioners to refer children on to critical interventions at a younger age (42). For example, early intervention may allow a child with an ASD diagnosis to attend mainstream educational institutions without any specialised services by school-going age (43). Early intervention maximises on the benefit of experience-dependent neuroplasticity, resulting in enhanced rates of learning for the child with ASD (44). Experience-dependent neuroplasticity refers to structural and functional changes in the developing brain dependent on experience or injury, whereas experience-independent neuroplasticity is the neurogenesis and apoptosis of synapses that occurs most actively during the first three years of life (45). Zwaigenbaum et al. stresses that early identification can minimise the amount of

stress placed on families before a diagnosis is given (26). In a study that reviewed meta-analyses and systematic reviews of early intervention in the ASD population from the year 2003 to 2018, it was concluded that early intervention can have favourable effects on child outcomes, with the level of change in a particular area being predicted by a variety of factors (44). Examples of positive predictors on outcomes include reduced ASD severity, maternal level of education, treatment approach as well as treatment intensity. It was reported that adaptive behaviour outcomes were hinged on cognitive functioning (44).

Growth Monitoring

South Africa has been utilising a Road-to-Health Booklet (RTHB) since 1973, and continues to do so in order to ensure monitoring of immunisations and growth. In public health service in SA, the World Health Organization (WHO) child growth standards are utilised. The monitoring of growth is very important in paediatric health care (46). The WHO growth standards are applicable in the South African context as the normative data was obtained from diverse populations from a range of countries, namely, Brazil, Ghana, India, Norway, Oman and the United States of America (46). According to the interpretation of the WHO HC growth charts, microcephaly may be diagnosed when the HC measurement plots two standard deviations (SD) below the mean for age (less than the second percentile), and conversely, macrocephaly is considered when the HC plots greater than two SD above the mean for age (greater than the 98th percentile) (46). Growth monitoring and attending well-child visits has resulted in better health status of South African children and as a result reduces the amount of emergency hospital appointments and hospital admissions (47). In SA this monitoring is conducted at Primary Healthcare Facilities, whose chief objective is to provide health care to children presenting with acute symptoms, as well as the prevention of poor health by monitoring milestones, feeding, growth and immunisations in children under five years of age (48). Early detection of atypical HC growth is critical so that specialist referrals, and as needed, specialised intervention programs, can be

arranged (49). If microcephaly is identified, possible disabilities such as intellectual delay can be further investigated. Similarly, if increased HC is detected, hydrocephalous could be further investigated (40). The routine well-child visits are therefore essential for detecting red flags in development and thus allows for a process of referral and further investigation. The RTHB not only has growth monitoring charts, but also a developmental milestone screening tool to be used by the caregiver as well as primary health care workers (50). The use of a simple measure such as HC as a possible biomarker of ASD could assist in raising the clinical suspicion and augment referrals of children with ASD features.

In conclusion, the literature searches on ASD research revealed that there is a paucity of published data with regards to HC and ASD in the South African context. Therefore, the aim of our study was to investigate HC measurements in a paediatric population diagnosed with ASD. The following section details the study objectives and the methods employed to investigate the research question.

OBJECTIVES

Primary Objective

The primary objective of this research report was to identify whether there exists a significant difference in the HC measurements of children diagnosed with ASD when compared to a reference population (WHO norms) of children aged less than five years attending a single clinic in Johannesburg, SA.

Secondary Objective

The secondary objectives of this study were to:

(a) Describe the demographics of the patients attending the ASD clinic.
(b) Describe the incidence of microcephaly, macrocephaly and normocephaly according to age bands, as compared to the standard reference population (WHO norms)
(c) Investigate the association between the severity rating of ASD and HC measurement.

METHODS

Type of Study

A retrospective, record review was conducted.

Patient Records

The subject population consisted of children diagnosed with ASD who were attending the Neurodevelopmental Clinic (NDC) at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), in Johannesburg, SA. Paper file records retrieved for review were from all the patients who had been seen in the ASD clinic for follow-up from the beginning of April 2019 to the end of September 2019, using the clinic's booking diary as a reference to facilitate accurate file withdrawal. This process was enabled by the nursing staff of the NDC who allowed access to the booking diary, and assisted in locating files. The filing system in the NDC is one in which all patient records are kept alphabetically, by surname, in filing cabinets. The patient records are retrieved per patient as they attend the NDC. The details of the patient's assessment, treatment or management are written in their file by the consulting physician.

Patient Record Number

The number of patient records eligible for inclusion in this study was estimated to be 240. This was based on an estimate of 10 ASD children being seen in the clinic per week. ASD patients seen monthly would equate to 40 patients, with a data collection period of 6 months, hence equating to 240.

Inclusion and Exclusion Criteria

The following inclusion criteria were applied when reviewing patient records:

(a) Any patient attending the CMJAH NDC with a diagnosis of ASD according to the DSM-5 criteria.
(b) The first HC measurement documented at the initial clinic visit, was before the age of five years.
(c) The patient had been seen in the clinic for a follow-up during the period 01 April 2019 through 30 September 2019.

The following exclusion criteria disqualified patient records from being included:

(a) Inconclusive DSM-5 ASD diagnosis.
(b) The presence of hydrocephalous as a diagnosis.
(c) Any diagnosed syndrome associated with macrocephaly.
(d) Documentation of refusal or poor cooperation for HC measurement at the initial clinic visit.

These criteria were applied and patient records were only reviewed if all the inclusion criteria, and none of the exclusion criteria, were met.

Subject Selection

Once it was established whether the information from a specific patient record could be included into the research database or not, the record reviews commenced. Necessary for interpretation of HC using the WHO growth charts was chronological age in years and months as well as the HC measurement in centimetres. The first HC measurement (from the initial clinic visit) in the patient's record was captured. The HC measurements were conducted by the same set of nursing staff employed in the NDC who had been trained accordingly. The HC measurement was obtained by using a flexible, non-stretchable measuring tape. The tape was placed at the level of the supraorbital ridges, above the ears, and around the occiput so that the maximum circumference was measured, as is standard practice (51). The HC was then routinely recorded in the patient's record as well as plotted on the WHO HC chart (46). These two recordings were used as a cross-check by the researcher so as to ensure that the measurement was recorded accurately in both instances by the nurses doing the measuring. The HC measurement as plotted on the WHO growth chart was recorded as being within 1SD of the mean (above and below), >1-2 above/below, >2-3 above/below and >3SD above/below the mean.

Data Variables

In addition to the HC measurement being taken from the patient record, demographical data was obtained from the patient's medical records. Variables collected included: date of birth, gender, ethnicity, comorbid conditions, special investigations conducted and related findings as well as ASD severity. Severity level one defined as requiring some support; level two defined as requiring substantial support, and lastly level three defined as requiring very substantial support (2). Once the HC measurement and abovementioned demographical information was obtained, according to the inclusion and exclusion criteria, the

data variables were captured electronically using a password protected excel spread sheet.

Subject Confidentiality

Confidentiality was ensured by patients being assigned study identification numbers for use during data capturing and statistical analysis. This was in accordance with the ethical guidelines as well as the management personnel of CMJAH when approving and granting permission for the research to be conducted.

Data analysis

Data analysis was facilitated by Microsoft Excel. Assistance with the statistical analysis was sort from a statistician. The data analysis in relation to each of the research objectives will be discussed further. The primary objective set to analyse the ordinal data obtained from two different populations, namely the reference population from the WHO data set, and the test population that comprised ASD patients. The inferential statistics used involved parametric testing as assumptions were being made and a hypothesis drawn. The z-score test was chosen as it is a statistical test that compares results (standard score) from a test population to a reference population. Critical values were used as our data was normally distributed. Critical values are defined as a point on the test distribution that is compared to the z-score to determine whether to reject the null hypothesis or not. The null hypothesis is rejected if the z-score is greater than the critical value. The ordinal data was further categorised into male and female.

The data analysis for the secondary objectives used categorical, ordinal variables. With respect to the demographics of patients the data pertaining to (a) ethnicity, (b) age, (c) sex, (d) comorbidities, as well as (e) special investigations were analysed quantitatively. Furthermore data were arranged within the sex category as macrocephaly and not macrocephaly using proportions. Comorbidity data were further stratified into total number of comorbidity in the sample, number of comorbidities per patient, as well as number of comorbidities per patient per age band. Special investigations were stratified into normal and abnormal findings. Descriptive and inferential statistics were used in order to draw conclusions about the data and this was visually represented in the form of column charts, tables and pie charts while making use of measures of central tendency in order to comment on any interesting findings.

When analysing the categorical data with regards to macrocephaly / not macrocephaly in specific age bands, the chi-squared, parametric test was used. It was chosen as it allows the testing of relationships between categorical variables by looking at the observed versus the expected pattern of responses. The presence of macrocephaly / not macrocephaly was stratified according to age bands, per participant and their resultant proportion.

Lastly, the HC measurement patterns of data in relation to ASD, as well as comorbidity data were analysed quantitatively. Stratification of data took place during analysis in order to further analyse the relationship between ASD severity, number of patients, the presence of macrocephaly and their resultant proportions. Descriptive and inferential statistics were used with the data represented visually via tabular format while making use of measures of central tendency in order to comment on any interesting findings from the collected sample.

RESULTS

During the data collection period of 01 April 2019 to 30 September 2019, a total of 378 NDC patient records were retrieved and reviewed for eligibility into the

study (Figure 1: Patient record retrieval process of inclusion and exclusion into the data set). A total of 135 (35.7%) patient records met the inclusion criteria. The main reasons for exclusion of patient records were comorbid syndromes, inconclusive ASD diagnoses as well as patients having their initial HC measurement after the age of five years. Inconclusive diagnoses were observed in patients who had features of ASD, but who did not fulfil the complete criteria for ASD on the DSM-5. No potential patients for record review presented with hydrocephalous, nor did any of them refuse or have poor cooperation for their HC measurement to be taken.

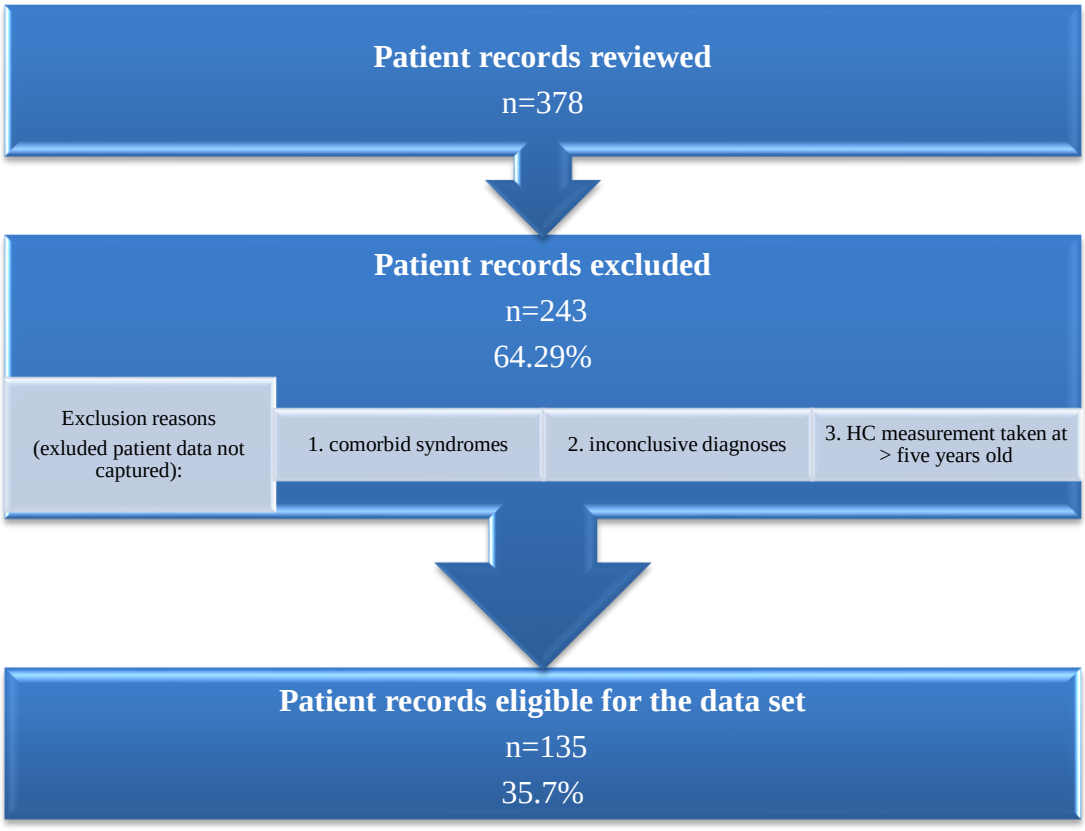


Figure 1: Patient record retrieval process of inclusion and exclusion into the data set

Table 1 below is a summarised version of the data collected for this research study, distributed among age bands. Table 1 shows the mean age in months, sex, ASD severity, mean HC, HC range, HC z-score, presence of macrocephaly,

comorbidities, ethnicity, special investigations and HC interpretation of the whole cohort stratified by age bands. The age bands featured are between the ages of one and five years. No patients less than one year were eligible to be included in the study.

Table 1: Distribution of general data collected, stratified by age bands

	12-23 months	24-36 months	36-48 months	48-60 months	>60 months
N	6	24	54	49	2
Mean age, months (SD)	16 (3.69)	31.46 (3.18)	42 (2.75)	52.94 (3.34)	60.5 (0.5)
Sex					
Male, n (%)	5 (83.3)	12 (50)	47 (87)	41 (83.7)	2 (100)
Female, n (%)	1 (16.6)	12 (50)	7 (13)	8 (16.3)	0
Ethnicity, n (%)					
Black	5 (83.33)	22 (91.67)	48 (88.89)	46 (93.88)	2 (100)
White	1 (16.67)	1 (4.17)	3 (5.55)	1 (2.04)	0
Indian	0	0	1 (1.85)	1 (2.04)	0
Coloured	0	1 (4.17)	2 (3.70)	1 (2.04)	0
ASD severity, n (%)					
1	1 (16.6)	1 (4.17)	5 (9.26)	4 (8.16)	0
2	5 (83.3)	11 (45.83)	22 (40.74)	22 (44.9)	1 (50)
3	0	12 (50)	27 (50)	23 (46.94)	1 (50)
Primary Comorbidity					
ADHD	2	2	14	10	0
Anxiety	0	0	0	1	0
Asthma	0	0	0	0	0
Behaviour problems	0	0	0	0	0
Chromosomal Abnormality	0	0	0	1	0
Developmental Delay	1	0	3	3	0
Epilepsy	0	2	3	3	1
Hyperactivity	0	1	1	0	0
Intellectual Impairment	2	5	13	17	0

	cont. 12-23 months	cont. 24-36 months	cont. 36-48 months	cont. 48-60 months	cont. >60 months
Otitis Media	0	0	0	0	0
None	1	14	20	13	1
Secondary Comorbidity					
ADHD	0	2	2	1	0
Anxiety	0	1	0	0	0
Asthma	0	1	1	0	0
Behaviour problems	0	4	7	2	0
Chromosomal Abnormality	0	0	0	0	0
Developmental delay	0	0	0	0	0
Epilepsy	0	0	0	1	0
Hyperactivity	0	0	1	1	0
Intellectual Impairment	3	2	9	9	0
Language Impairment	0	0	0	1	0
Otitis Media	0	1	0	0	0
None	3	13	34	34	2
Special Investigations (some participants had more than one)					
None	3	12	36	38	0
MRI	2	9	9	7	0
CT	0	2	4	3	0
EEG	2	3	5	4	0
ECG	0	0	0	0	0
Patients mean HC (cm), (SD)	46.64 (1.82)	49.88 (2.58)	51.06 (2.15)	51.99 (2.02)	51 (2)
Patients HC Range (cm)	45-49	45-55	46.5-58	48-56	49-53
HC z-score, n (%)					
>-2 to -3	0	2 (8.33)	2 (3.70)	0	0
>-1 to -2	2 (33.3)	0	3 (5.55)	4 (8.16)	1 (50)
Median	3 (50)	10 (41.67)	24 (44.44)	14 (28.57)	0
>1 to 2	1 (16.67)	6 (25)	14 (25.93)	18 (36.73)	1 (50)
>2 to 3	0	2 (8.33)	6 (11.11)	7 (14.29)	0
>3	0	4 (16.67)	5 (9.26)	6 (12.24)	0
HC interpretation, n (%)					
Macrocephaly	0	6 (26)	11 (20.37)	13 (26.53)	0
Microcephaly	0	2 (8.33)	2 (3.70)	0	0

	cont. 12-23 months	cont. 24-36 months	cont. 36-48 months	cont. 48-60 months	cont. >60 months
Normocephaly	6 (100)	16 (66.67)	41 (75.93)	36 (73.47)	2 (100)

Primary Objective Results

The primary objective was to identify whether there was a significant difference in HC of patients diagnosed with ASD when compared to a reference population (WHO norms) in children aged less than five years.

Firstly the data obtained will be represented using a column chart indicating the number of patients diagnosed with ASD who have HC measurements that were normal (within one SD of the mean), three SD below, 1-2 SD below, 0-1 SD below, three SD above, 1-2 SD above or 0-1 SD above as, characterised by the WHO HC charts.

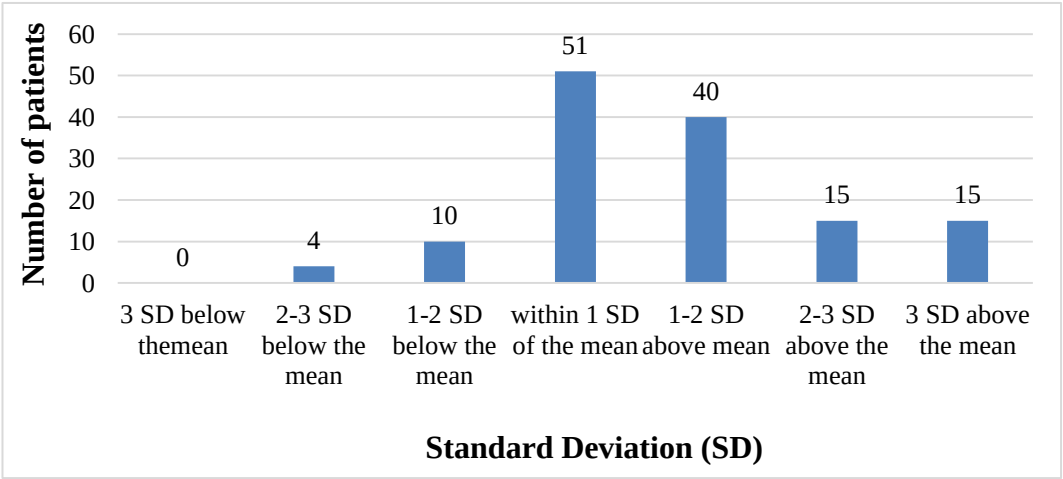


Figure 2: Column chart showing the distribution of patients' HC in relation to the mean WHO HC normative values

As depicted above in Figure 2, majority, 51 (37.7%) of the patients were classified as having a HC within normal limits (being within one SD of the mean). Following this, 40 (29.6%) patients had a HC 1-2 SD above the mean.

Additionally, 15 (11.1%) patients had a HC 2-3 SD above the mean, and another 15 (11.1%) had a HC three SD above the mean. Fewer patients had HC measurements which fell below the mean, being 10 (7.4%), four (2.9%) and zero patients with a HC of 1-2 SD, 2-3 SD and three SD below the mean, respectively. In accordance with the WHO chart interpretation, four (2.9%) patients had microcephaly (≥2 SD below the mean) and 30 (22.2%) patients had macrocephaly (≥2 SD above the mean for age). According to the WHO growth charts it is expected that 3.13% of children aged 0-5 years have macrocephaly, however in our sample it was observed that 22.2% had macrocephaly.

A z-score test was used to ascertain differences between mean HC in the patients with ASD (sample population) as compared to the mean HC of the reference population (WHO norms). The null hypothesis was that the proportion of the sample patients who have macrocephaly is equal to the proportion of the reference population who have macrocephaly. The alternate hypothesis was that the proportion of the sample patients who have macrocephaly is larger than the proportion of the reference population who have macrocephaly. The z-score value was 9.85. The results indicate that the null hypothesis can be rejected and it can therefore be inferred that the HC's of the sample patients are larger than in the reference population. Additionally, a z-score test was used for male and females separately. The male participants (107, 79.2%) had a z-score of 7.95 and a critical value of 1.65. The female participants (28, 20.7%) had a z-score of 6.10 and a critical value of 1.65. The z-scores are higher than the critical value of 1.65 for both male and female patients, and therefore reject the null hypothesis. Therefore, the proportion of the sample patients who have macrocephaly is higher than the proportion of the reference population who have macrocephaly. This is true in both male and female patients. Table 2below depicts the mean HC measurements of both the WHO reference population norms (expected), versus the observed measurements in our sample, stratified by sex and age band.

Table 2: WHO reference population's (expected) versus sample patient's (observed) mean HC measurement, stratified by sex and age band

Age band	N	Males mean HC (cm)		N	Females mean HC (cm)	
		Observed (sample)	Expected (reference population)		Observed (sample)	Expected (reference population)
12-23 months	5	46.10	46.94	1	48.20	45.80
24-35 months	12	50.21	49	12	49.54	48
36-47 months	47	51.28	49.77	7	49.67	48.85
48-59 months	41	51.99	50.41	8	52	49.55
≥60 months	2	53	50.74	0	No data	49.93

Figure 3 below shows a scatter graph indicating the median values of the reference population's HC measurements; males indicated in a green solid line, and females indicated in a pink solid line. The blue circles indicate each of the male sample patients' HCs, and the purple circles for the females in the sample. Of importance when interpreting this graph, is that a single circle may represent more than one patient in the sample, as some patients had identical ages and HC measurements. Additionally it can be described that the mean HC measurement of the sample's patients were 50.2 centimetres and the median was 50 centimetres. This demonstrates that data was normally distributed.

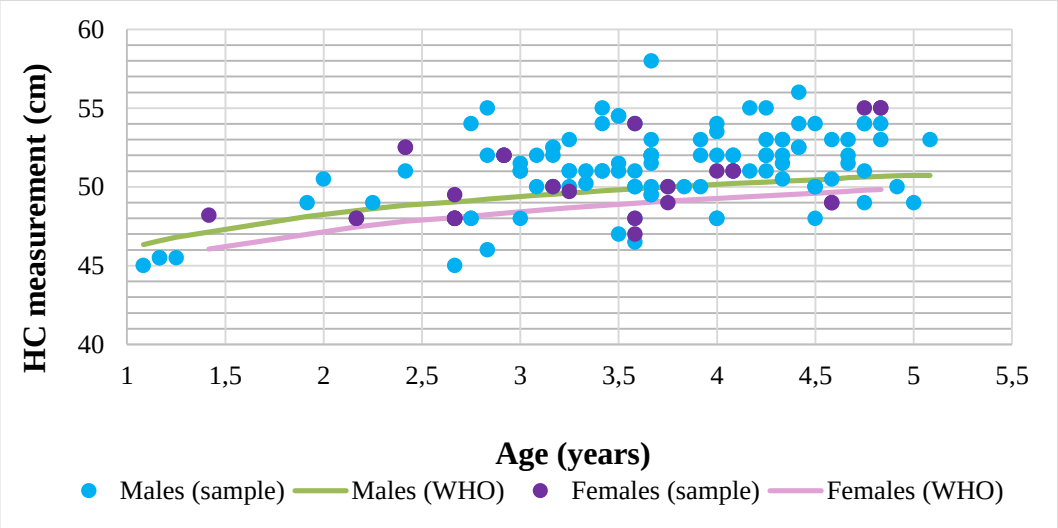


Figure 3: Graph showing HC measurement of each patient in the sample versus WHO norm medians

Secondary Objective Results

The secondary objectives were three pronged; and included

(a) Describing the demographics of the patients attending the ASD clinic.

(b) Describing the incidence of microcephaly, macrocephaly and normocephaly according to age bands, as compared to the standard reference population (WHO norms)

(c) Investigating the association between the severity rating of ASD and HC measurement.

(a) Demographics

Firstly, the demographics of the patients seen in the NDC and included in this analysis will be described. The highest percentage of patients were Black (123, 91.1%); followed by White (6, 4.4%); then Coloured (4, 2.9%) and lastly, Indian (2, 1.4%).

The data obtained for age and sex distribution within age bands is depicted using a column chart below (Figure 4). Ages were grouped into 12 month intervals. The age groups were planned to be grouped in six month intervals, but due to patient numbers being very low in specific age bands, the age band grouping was expanded to 12 months. The mean age of presentation to the NDC was 40.58 months. The highest distribution of patients fell into the ≥ 36 – 47 months age band, being 54 (40%) patients. The next highest distribution, 49 patients (36.2%) were in the ≥ 48 – 59 months age band. The ≥ 24 – 35 month age band had 24 (17.7%) patients. The least represented age bands were the following three. The ≥ 12 -23 month age band had six participants (4.4%), ≥ 60 months age band had two patients (1.9%), and zero participants were identified in the < 12 months age band. In terms of sex distribution, the majority of the sample was male (107, 79.2%). The observed ratio of female to male was therefore 1:3.8. Most male patients were in the age band of ≥ 36 - 47 months, and the most female patients were in the ≥ 24 - 35 month age band.

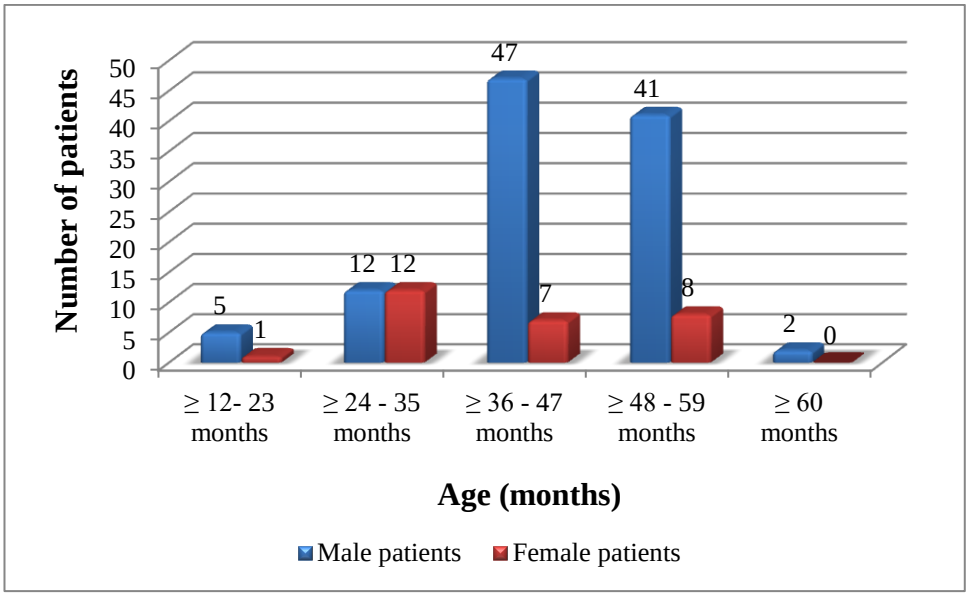


Figure 4: Bar chart depicting patients' age and sex distribution within age bands

ASD was the primary diagnosis reported on the problem list in the patient's record by the managing physician, and following the primary diagnosis the comorbidities were recorded. The listed comorbidities were captured from the patient's record and the treating physician ranked comorbidities in order of functional impairment. Where applicable DSM-5 criteria were used to confirm comorbid diagnoses. A maximum of two comorbidities were recorded per patient record for this analysis. A total of 11 different comorbidities were identified among the 135 patients. Just less than one third of patients did not have the presence of a comorbidity, 29.62% (n=40). The most common primary comorbidity present in the ASD patients used in our sample was ID (n=38) followed by ADHD (n=28). Additionally, epilepsy (n=9), behavioural problems (n=8) and developmental delay (n=7) were also identified. The least common primary comorbidities were hyperactivity (n=2), language impairment (n=1), anxiety (n=1), chromosomal abnormality (n=1) and otitis media (n=1). ID was the most common secondary comorbidity (n=22), followed by ADHD (n=5) and behavioural problems (n=5). Two patients had asthma as a secondary comorbidity, hyperactivity (n=2), epilepsy (n=1), language impairment (n=1) and lastly anxiety (n=1). Of the 30 macrocephalic patients, ID (13, 43.3%) and ADHD

(7, 23.3%) were the comorbidities most commonly present. Table 3 below provides a summary of the primary and secondary comorbidities.

Table 3: Primary and Secondary comorbidities amongst patients

Comorbidity	Total Number of patients (n)	Patients with the condition as a primary comorbidity (n)	Patients with the condition as a secondary comorbidity (n)
ADHD	33	28	5
Anxiety	2	1	1
Asthma	2	0	2
Behavioural problems	13	8	5
Chromosomal abnormality	1	1	0
Developmental Delay	7	7	0
Epilepsy	10	9	1
Hyperactivity	4	2	2
Intellectual Impairment	60	37	23
Language Impairment	2	1	1
Otitis Media	1	1	0
No comorbidity	40	40	

The next important values to note with regards to comorbidities are the number of patients who had zero, one or two comorbidities. Forty patients (29.6%) did not present with any comorbidity, 55 patients (40.7%) had one recorded comorbidity and 40 (29.6%) patients had two recorded comorbidities. Furthermore and in relation to comorbidities, as depicted in

Table 4 below, patients were grouped into age bands. The age band with the highest number of comorbidities per patient was the ≥12-23 month age band (n=6) with 1.33

comorbidities per patient. The ≥60 month year age band (n=2) had the lowest number of comorbidity per patient (0.5).

Table 4: Number of patients, total number of comorbidities and number of comorbidities per patient, stratified by age

Age (months)	Number of patients n(%)	Total number of comorbidities (primary and secondary) (excluding "none" comorbidity)	Average number of comorbidities per patient
12-23	6 (4.4%)	8	1.33
24-35	24 (17.8%)	21	0.88
36-47	54 (40%)	54	1
48-59	49 (36.3%)	51	1.04
≥60	2 (1.5%)	1	0.5

In order to differentially diagnose comorbidities, the patients' managing physician often recommended special investigations to be conducted. The chosen investigations as well as their results were extracted from the patient records (as can be seen in Table 5 below). Of the 135 patients, 50 (37%) patients required further objective investigations. In 27 (54%) of the patients an MRI was indicated and 14 (28%) patients had an EEG. The least utilised objective investigation was the computerised tomography (CT) scan. Of the 50 patients, nine (18%) underwent a CT scan. All EEG and CT scan results were within normal limits and five of the MRI's conducted yielded abnormal results. None of the MRI abnormalities were common across participants. According to the MRI reports the following abnormalities were identified: MRI patient one had a structural malformation of the temporal lobe; patient two had a venous malformation of the temporal lobe; patient three had hyperintensity in the posterior deep white matter bilaterally; patient four had periventricular leukomalacia; and lastly, patient five,

had supratentorial ventriculomegaly with white matter volume loss and dilated perivascular spaces.

Table 5: Special medical investigations recommended and their findings

Special Investigation	Number of patients (n, %)	Normal Result	Abnormal Result
MRI	27 (54%)	22 (81.5%)	5 (18.5%)
EEG	14 (28%)	14 (100%)	0
CT scan	9 (18%)	9 (100%)	0
Total	50	45	5

(b) Incidence of microcephaly, macrocephaly and normocephaly per age band

In order to ascertain the incidence of microcephaly, macrocephaly and normocephaly within specific age bands, frequency counts were compared between age bands. As depicted in Table 6 below, the highest proportion (26.5%) of macrocephalic patients was in the age band of ≥48-59 months old, followed by the ≥24-35 month age band (25%). The age band of ≥36-47 months had the third highest proportion of macrocephalic participants, being 20.4%. The remaining two age bands had no children with HC measurements classified as macrocephalic.

Table 6: Proportions and frequency counts of macrocephalic patients per age band

Age (months)	Number of patients (n)	Number with macrocephaly (n)	Proportion (%)
12-23	6	0	0%
24-35	24	6	25%
36-47	54	11	20.4%

cont. Age (months)	cont. Number of patients (n)	cont. Number with macrocephaly (n)	cont. Proportion (%)
≥60	2	0	0%
Total	**135**	**30**	**22.2%**

Additionally a fisher exact probability test was used to ascertain whether a statistically significant association was apparent between the sample patients versus the reference population with regards to macrocephaly and age. A statistical value was calculated to be 203.33, and the critical value was 11.5, suggesting that there was a significant association between the proportion of the sample patients presenting with macrocepahly and their relative age. Furthermore, among the patients, 20.56% (n=22) of males and 28.57% (n=8) of females, were macrocephalic (as seen in Table 7 below).

Table 7: Patients' HC measurement interpretation stratified by sex

	Male n(%)	Female n(%)	p-value
Microcepahlic	4 (3.73%)	0	-
Normocephalic	81 (75.70%)	20 (71.42%)	0.45
Macrocephalic	22 (20.56%)	8 (28.57%)	

(c) Severity rating of ASD and HC measurement

In order to investigate the severity rating of the ASD diagnoses in association with the patients' HC category, the number of patients falling into one of the three severity categories was calculated.

Table 8: ASD severity stratified by age, macrocephaly incidence and proportions

ASD Severity	12-23 months n(%)	24-35 months n(%)	36-47 months n(%)	48-60 months n(%)	>60 months n(%)	Total Number of patients	Total Number of patients with macro-cephaly	Proportion of Macro-cephalic patients
1	1 (16.6)	1 (4.17)	5 (9.26)	4 (8.16)	0	11	1	9.1%
2	5 (83.3)	11 (45.83)	22 (40.74)	22 (44.9)	1 (50)	61	19	31.1%
3	0	12 (50)	27 (50)	23 (46.94)	1 (50)	63	10	15.9%
Total						**135**	**30**	**22.2%**

As depicted in Table 8 above, the patients were classified according to an ASD severity of one, two or three. In the entire cohort there were 11 patients who had an ASD severity of one, 61 with an ASD severity of two, and the remaining 63 an ASD severity of three. Of the 11 patients with an ASD severity of one, one had macrocephaly. Nineteen patients had macrocephaly with an ASD severity of two, and ten had macrocephaly in the ASD severity rating of three. A proportion within each ASD severity grouping was calculated and the following results were revealed. In total, there were 30 (22.2%) macrocephalic patients in the entire cohort. Of the macrocephalic patients, a proportion of 31.1% of patients presented with an ASD severity of two, 15.9% of patients presented with an ASD severity of three, and 9.1% of patients presented with an ASD severity of one. Using a chi-squared test the observed and expected values were calculated. The test statistic of 4.18 emerged, with a critical value of six. The test statistic was less than the critical value, indicating that there is no association between ASD severity and macrocephaly.

Below, Table 9 illustrates the number of patients with an ASD severity rating and number of comorbidities. There were 11 patients who had an ASD severity rating

of one, individually; seven patients had no comorbidity, two patients had a primary comorbidity, and two patients had a primary and secondary comorbidity. There were 61 patients who had an ASD severity rating of two; individually, 18 patients had no comorbidity, 22 patients had a primary comorbidity, and 21 patients had a primary and secondary comorbidity. There were 63 patients who had an ASD severity rating of three; individually, 15 patients had no comorbidity, 31 patients had a primary comorbidity, and 17 patients had a primary and secondary comorbidity. The highest proportion (22.9%) of patients presented with only a primary comorbidity with an ASD severity of three, followed by a proportion (16.3%) of patients with only a primary comorbidity with an ASD severity of two. The lowest proportion (1.4%) of patients who had an ASD severity of one, had only one primary comorbidity.

Table 9: ASD severity and number of comorbidities

ASD Severity	Patients with no comorbidity n(proportion)	Patients who had a primary comorbidity n(proportion)	Patients who had a primary and secondary comorbidity n(proportion)	Total patients n(proportion)
1	7 (5.1%)	2 (1.4%)	2 (5.1%)	**11 (11.6%)**
2	18 (13.3%)	22 (16.3%)	21 (13.3%)	**61 (42.9%)**
3	15 (11.1%)	31 (22.9%)	17 (11.1%)	**63 (45.1%)**
				135

DISCUSSION

Head Circumference in the ASD population

The primary objective of our research study was to identify whether there was a significant difference in HC of children diagnosed with ASD aged less than five years attending a single clinic in Johannesburg, SA, when compared to a reference

population using the WHO HC charts. Our study revealed that a statistically significant proportion of children in our sample (22.2%) diagnosed with ASD had macrocephaly. This is slightly higher than the percentage found in a systematic review and meta-analysis of 27 studies that concluded that 15.7% of ASD individuals displayed macrocephaly and 9.1% had increased brain volume detected using MRI (21). The results of this systematic review, however, must not be compared to the current study entirely as the systematic review and meta-analysis had varying age ranges in their samples; 2.9 to 33 years of age. The age range in samples is imperative to consider as it is understood that HC measurements in individuals with ASD are no longer different from typically developing peers after the fifth year of life (22). Not included in this systematic review and meta-analysis (due to time interval exclusion), was a South African retrospective record review that determined that 12.1% of their ASD sample presented with macrocephaly (9). Their study was similar to ours in that HC measurements in 58 children (mostly pre-schoolers) were recorded as a clinical characteristic from a NDC within a tertiary level state hospital between 2008 and 2010. During a synopsis of MRI studies, specifically total brain volume, in ASD children, Stigler et al. noted that by 4-5 years of age, 15-20% developed macrocephaly (19). Despite these variations of macrocephalic percentages of ASD individuals, in a recent publication, Bridgemohan, confirmed a high rate of macrocephaly in ASD individuals, up to 25% (6). This finding is most congruent with our study results.

Height, weight and HC in individuals with ASD, when compared to typically developing individuals, has been shown to differ significantly (14, 52, 53). To elaborate on a particular study, a longitudinal record review of a group of individuals with ASD (n=280) and a control group (n=609) was conducted in Japan using a RTHB equivalent. The researchers aimed to determine whether HC was larger in the ASD participants, and if so, did this growth occur along with general body overgrowth (height and weight) (20). Using analysis of covariance between the two groups, the mean HC was adjusted for height, weight and age in days (20). In male participants with ASD the mean HC was significantly greater

than the control group, whereas no statistical difference between the female participants was found (20).

During a retrospective record review, with similar methodology to the study conducted in Japan, findings indicated that in the ASD group (versus the community control group), somatic growth was increased in only the male ASD participants and was associated with an early generalised overgrowth (42). This overgrowth was first identified as increased length-for-age at four months, followed by increased HC and weight-for-age by 10 months (42). The researchers suggested that this somatic overgrowth at birth could be used as a biomarker for identifying individuals at high risk of ASD who would have potentially worse outcomes (42). In a nested-matched case control study in Finland, researchers investigated the dynamic features of HC growth (54). Their main finding from research involving 468 matched individuals indicated that there was a strong association between ASD and accelerated HC measurements (54). Most importantly, it was concluded that this accelerated growth was independent of acceleration in height and weight, which are deemed unassociated with ASD after six months of age (54). In 2007 Sacco et al. described that the height, weight and HC data they obtained from their sample of 241 ASD participants were significantly greater than the general population (52).

Despite these differing views of whether accelerated HC measurements are dependent on generalised body overgrowth in ASD individuals or not, in 2015, Sacco et al. reported in a systematic review and meta-analysis of 27 different studies spanning 20 years (1994-2014), that macrocephaly is consistent in ASD individuals, across nations (21). Consistent with Sacco et al. (21), Chawarska et al. (25) demonstrated in their study that indeed, male individuals with ASD do have an accelerated increase in HC during the first year of life, but this overgrowth was relative to overgrowth in height and weight. Furthermore, the development of macrocephaly underlying ASD is demonstrated very early on post-natally, and continues for several years (52). Multiple research investigations therefore conclude that brain growth, and thus HC, length and

weight in newborns during the early postnatal phase and infancy is accelerated in individuals with ASD (19-21, 42, 54).

With multiple literature sources addressing ASD and macrocephaly it is crucial to understand differences in brain growth and development (21) when contemplating a diagnosis of ASD for a child. With this realised, advantages can include guaranteeing early intervention by improving and anticipating diagnosis (17). Although diagnosis can be a lengthy process, as acknowledged by Brown, any child who has significant developmental delay should be referred promptly for the necessary interventions whilst awaiting a diagnosis (14). Early identification is fundamental when it comes to ensuring that individuals who have been diagnosed with ASD have access to not only specialised, but also evidence-based interventions that have been proven to enhance long term developmental outcomes (26). This is reiterated by Brown, who states that early identification and thus early intervention are directly related to improved outcomes (14).

On-going intervention needs to continue throughout the lifespan in majority of cases and in so doing, should optimise individual independence in activities of daily living (14). ASD interventions were recommended to hone in on areas such as social skills, social communication and behavioural therapy, achieved via structured education and supported employment (14). Early identification and intervention is valued by both the Department of Health and the National Integrated Early Childhood Development policy of SA which mandates various statutory institutions to implement early detection and intervention schemes (1). Additionally, parent and family education and support is critical, along with coordination of services that the family and individual have to undergo (14). Furthermore with regards to family involvement, not only is clinician-led intervention indicated; parent-implemented intervention is equally important and has been shown to enhance achievement of therapeutic goals (44). Parent-implemented intervention is defined as a "vital intervention component" in ASD and places significant importance on equipping parents and families with skills to manage their child's changing skills and behaviours (44). Our results contribute to and are in support of existing evidence of the importance of early identification.

Demographics

Demographic factors were explored as part of the secondary objectives of our study, namely; ethnicity, age, sex, comorbidities and special investigations.

Ethnicity

In a Western Cape study conducted with similar a population to ours (from a NDC), the following ethnicities were represented; black african (20.7%), white (20.7%), mixed ancestry (51.7%) and African immigrants (4%) (9). According to the authors, mixed ancestry related to ancestry originating in South Africa, black ethnicity referred to participants who originated in Africa and African immigrants included Somalian, Congalese and Angolan participants (9). In a Gauteng study exploring demographics in an ASD population at public and private schools, it was noted that black ethnicity was predominant (74.5%) with white (16.2%), coloured (1.9%) and Indian (6.5%) constituting the remaining ethnicities (41). The Black ethnicity was also found to be predominant in our study (91%). White, Coloured and Indian (9%) races made up the remaining ethnicities in our study. Our sample is therefore a close representation of the ethnicities present in South Africa as described by Stats SA (Statistics South Africa) (12). They reported that 80.7% of the South African population are Black, 8.1% White, 8.7% Coloured and 2.5% Indian (12).

Age

Three to four years of age was the age band that most patients were aged when meeting the inclusion criteria for this study. The mean age of presentation was 40.58 months which may be ascribed to the average age at formal ASD diagnosis in this particular NDC. Springer et al. found a mean age of ASD diagnosis in a study conducted in a Western Cape Hospital of 3.5 years of age (9), similarly a study conducted in a public South African ASD school, mean age of diagnosis was 3.9 years of age (41). Our sample is therefore consistent with these abovementioned local findings. A UK study found the median age of diagnosis being 4 years 7 months (40). Crane et al. conducted an online parental survey in

the UK, and reported a mean age of diagnosis in their sample of 7 years 5 months, with 82% of those being between the ages of 3-18 years old (55). Several epidemiological studies conducted across the USA reported mean age of diagnosis being between 4 and 5 years of age (26). Contrary to the most recent hypothesis that ASD can potentially be diagnosed as early as 14 months (56), this clearly differs in the reality of diagnosing ASD.

Our study findings indicated that most patients were diagnosed between three and four years of age and barriers to an earlier diagnosis should be explored. In an interview for the South African Medical Journal (2013) a South African general practitioner (whose son has been diagnosed with ASD), stated that general practitioners have a lack of knowledge regarding ASD, and consequently avoid making ASD diagnoses. Reflecting on her own tertiary education she stated that exposure to developmental disorders was very limited at medical school. Although medical school curricula is constantly updated and revised, this particular medical officer reported that ASD was only mentioned as a condition to consider when differentially diagnosing conditions (57). Franz et al. divulges on the typical, lengthy, diagnostic process in the Western Cape, South Africa, which is deemed to be one that is well-resourced in terms of health care (1). "Limited" is how Franz et al. describe the population's access to early identification of and intervention for ASD (1). To elaborate, when a caregiver suspects ASD in their child, they will be evaluated in a primary health care setting. A medical professional will then refer the case to a specialised neurodevelopmental clinic that is usually at a specialised or tertiary level hospital (1). This process is reported to take between 9 and 18 months, and in some instances, even longer (1). Once an ASD diagnosis is given, the child is often referred for therapy and school placement, and placed on the Western Cape Education Department's provincial ASD waiting list (1). Internationally, according to a UK research database, early parental concern was communicated to professionals in due time, but still, there were delays in diagnosing ASD in their children (40). The following reasons were proposed: parental postponement in pursuing a diagnosis; intricate pathways between initial parental concern and a diagnosis; and lastly, lack of awareness of ASD features by general practitioners (40).

Sex

The male predominance of 107 (79%) patients with a ratio of male-to-female being 3.8:1 is in keeping with multiple international literature findings (5, 6, 14, 28). Springer et al. reported a male-to-female ratio identical to ours (9); which was a local study conducted in a NDC setting similar to ours with a total sample size of 58 participants. Two further South African studies reported higher male-to-female ratios of 15:1 (38) and 6.8:1 in a public ASD school and 8:1 in a private ASD school (41). Although these studies had a greater discrepancy of male-to-female ratios when compared to this study; the comparison of the ratios should consider sample sizes; Stephens had a total sample of 16 participants (38) and Erasmus et al. had 126 participants in their private school sample and 24 participants in their public school sample (41).

Comorbidity

Co-existing ASD conditions have been increasingly recognised due to the DSM-5 now including further specifiers comorbid to ASD (30). Approximately 45% of individuals with ASD have ID (6), which is comparable to our sample which found that a total of 60 patients (44.4%) had been diagnosed with ID (as either a primary or secondary comorbidity). Baio et al. reported that 31% of their ASD sample in 11 different American states were classified with ID and varied by sex and ethnicity (4). Springer et al. did not include ID as a prominent health or behaviour comorbidity in their participants (9). They did however report that 42 (72%) participants were non-verbal. This is noteworthy, as delay in language can be a feature of ID (58). Discrepancies in study findings are however anticipated as performance on intellectual subtests are highly variable in ASD individuals (6) as well as the complex nature of differentially diagnosing ASD and ID in young children (30).

Identifying ADHD comorbidity is crucial as attention and hyperactivity can consequently form an imperative aspect of ASD intervention and therefore a separate diagnoses assists in highlighting the individual's specific areas of difficulty (30). ADHD comorbidity was present in 33 (24.4%) patients in our

sample. A study conducted in the Western Cape, reported that 25 participants (43%) of their sample presented with hyperactivity (9). Bridgemohan et al. estimate that ADHD is comorbid with ASD in approximately 28 – 44% of individuals (6). The percentage of behaviour-related comorbidities may be elevated in individuals diagnosed with the DSM-5 criteria as it became a specifier (to state if the ASD was associated with neurodevelopmental, mental or behavioural disorder) as did ID, in contrast to previous DSM's (2, 14). Therefore the DSM used at the time of diagnoses must be considered when investigating and comparing comorbidity data.). Strikingly, a further 9.6% of our sample had significant behaviour difficulties that did not fulfil the comprehensive criteria for ADHD. If such cases were to be included, behaviour related comorbidities would increase to 34%. It is therefore crucial to be vigilant of patients who present with ID and or ADHD for features of ASD, and vice versa.

Special Investigations

Special investigations in the current study sample included MRI, EEG and CT scans. During an assessment of ASD, multiple tiers of testing are recommended by Bridgemohan (6), firstly physical examination (HC for example), diagnostic testing (chromosomal microarray / Fragile X test / audiological evaluation), additional genetic testing (karyotype for example) and additional targeted diagnostic testing, such as electroencephalogram (EEG), metabolic testing or MRI. Choice of testing is dependent on the individuals history and clinical presentation (6). MRI scans were indicated in 27 (54%) of the patients in our sample who required any sort of special investigation, with 5 (9.2%) of those having abnormal MRI findings. Springer et al. had 5 (8.6%) participants who were referred for MRI scans, with 2 (40%) of them being abnormal (9). Clinical reasoning for conducting an MRI on an individual is based on lateralising signs or other symptomology discovered upon routine neurological examination (28). In the case of pure macrocephaly in ASD, MRI is not immediately indicated due to the high prevalence of macrocephaly in this population, whereas an MRI is indicated in microcephaly and developmental regression (major language regression for example) (6, 28). MRI has assisted greatly in investigating the

neurobiology underpinning ASD (19) and imaging findings of ASD individuals are highly heterogeneous (28). Hirtz et al. describe that when MRI's have been indicated as part of an ASD assessment battery, focal findings are very rare and if abnormalities are found, it is generally coincidental as ASD is not ordinarily associated with focal lesions (28). MRI research in ASD individuals has however contributed to our knowledge into the neurobiology of ASD, neurocircuitry and atypical cortical development (19); and therefore should not be disregarded.

Macrocephaly and Age

The secondary objectives in our study included describing the incidence of macrocephaly, microcephaly and normocephaly within age bands. The proportion of macrocephalic patients in our study within the age range of 2 – 5 years was statistically significant when compared with the reference population and is similar to HC measurements reported in numerous other studies (14, 22, 35, 42, 53). However, the number of patients and their respective HC measurements within age bands ≤ 11 months, ≥12-23 months old and ≥ 60 months old were too few to prove significance. Patients in our sample, more specifically, aged ≥ 48 – 59 months of age constituted the greatest overall proportion of macrocephalic patients. Brain growth trajectories of individuals who have ASD have been described in research literature. Extreme generalised brain overgrowth occurs within the first year of life (22, 52), with extreme brain overgrowth (including increasing HC) and macrosomy extending into and concluding in the second year of life (14, 22, 42). This increase seems to be apparent until approximately four years of age (35, 52), which is followed by a plateau of brain growth, or more easily described as an arrest of brain growth (19, 22). From approximately five years of age extending into adolescence, HC is no longer significantly different to that of typically developing peers (22), and adolescents with ASD have been reported as having normal total brain volume (19).

Furthermore Sacco et al. emphasised a crucial finding in their study, among others, regarding the significance of postnatal macrocephalic development in ASD individuals (52). They concluded that increasing HC is a clinical presentation of the active pathogenetic processes that underlie ASD, and can allow for it to be suspected and/or detected early on which is critical for intervention (20, 52). In line with this hypothesis our data can contribute a clearer understanding of what HC measurement at a particular age can be anticipated to be in an individual suspected of having ASD.

ASD Severity

ASD severity was the final secondary objective characteristic to investigate within our sample. As similarly found in a study conducted in France by Fombonne et al. the severity data obtained for each patient in our sample, found no association between ASD severity and macrocephaly (59). Contrary to our finding, Courchesne et al. observed in their research data that infants with "extreme brain growth" were diagnosed with a more severe form of ASD and had more atypical neuroanatomy (22).

However, the majority (45.1%) of patients in our sample had an ASD severity of three and additionally had the highest frequency of comorbidities. Springer et al. similarly found that patients with moderately-severe and severe ASD at an increased risk of poor cognitive performance, potentially due to their autistic features (9). When considering separating their sample of participants into low- and high-functioning groups and therefore interpreting statistics separately, Fukumoto et al. decided not to do so as multiple literature sources found no associations between cognition and ASD severity (20). Franz et al. did however recommend on the importance of investigating severity of ASD individuals in sub-Saharan Africa as this can contribute to an increased understanding of the phenotype of ASD as currently there is a dearth of such literature (60).

Differentially diagnosing ASD patients can be cumbersome, but Shulman et al. emphasise the necessity of this process, as co-existing conditions are commonplace in this population (30). Kliegman et al. further emphasises this importance as a lower symptom severity is a predictor of a more favourable prognosis (6) and can assist in an appropriate school placement (41).

LIMITATIONS AND R ECOMMENDATIONS

The WHO growth charts, although used throughout the public health sector in South Africa, are only available for ages 0-5 years old. Given the age limits of the growth charts, the number of patients that met inclusion criteria for our study was restricted. Vigilantly choosing such criteria would ensure patients with ASD of varying ages are included so that age bands can be better represented. This could enable statistical methods to be more easily utilised across all age bands. Despite these growth charts not being from the specific population in which this study was conducted, it is stated by the WHO that the standards can be applied to all children everywhere, regardless of ethnicity, socioeconomic status and type of feeding. Therefore, replicating this study with a matched control group and a more thorough data collection tool would allow the researcher to eliminate sources of variability and explore additional associations that may be specific to the South African population. For example, collecting data on HIV status' of ASD patients; uninfected, infected or exposed, would be valuable to gain insight into any potential associations between HIV and ASD. Additionally, collecting further data on comorbidities, and not limiting the data to a maximum of two comorbidities per patient, would have also supplied added understanding to the complexity of ASD presentation.

Additionally, an important consideration is that ASD is generally diagnosed in older children when the symptoms are more apparent, and therefore a range of patient ages, especially less than one year old, was challenging to obtain in this study. The targeted patient numbers were further negatively influenced due to staff shortages and therefore less patients being booked for ASD-related consultations. Secondly, the large number of South African public holidays within the data capturing period prevented the NDC from being open and hence resulted in less patients being booked during that time period.

It is evident that there is limited research in sub-Saharan Africa describing or quantifying knowledge about ASD among health care workers, policy-makers, faith healers or traditional practitioners (8). Studies conducted generally concur on limited ASD knowledge amongst health care practitioners, and the necessity of training for these professionals. Despite this, appropriate training programs have not been proposed or attempted by researchers (8). This reflects the limited knowledge of clinicians regarding ASD red flags, which may have potentially deleterious consequences for early identification and early intervention. Early intervention is a key objective in South Africa currently, and it is therefore critical that we utilize a screening tool that is simple and can be used at an early time in a child's development (38). With early identification being the key objective, it is fundamental to note that early intervention has the potential to decrease large expenses associated with ASD as it improves prospective outcomes (38).

In order to safeguard early diagnosis and thus intervention, it is imperative that a simple, effective and efficient method of raising suspicion for ASD in young children is established. A suggested approach would be firstly to encourage research to be conducted in NDCs across the county, in both urban and rural settings, to investigate the HC of their patients as well as mean age of ASD diagnosis per clinic. Furthermore, growth parameters of patients who are diagnosed with ASD should be scrutinized to investigate the potential of generalized overgrowth in conjunction with HC as a possible early indicator of ASD. Additionally, studies that can highlight and contribute toward early and

equitable ASD identification in SA, as well as the betterment of ASD intervention services, are encouraged to be conducted.

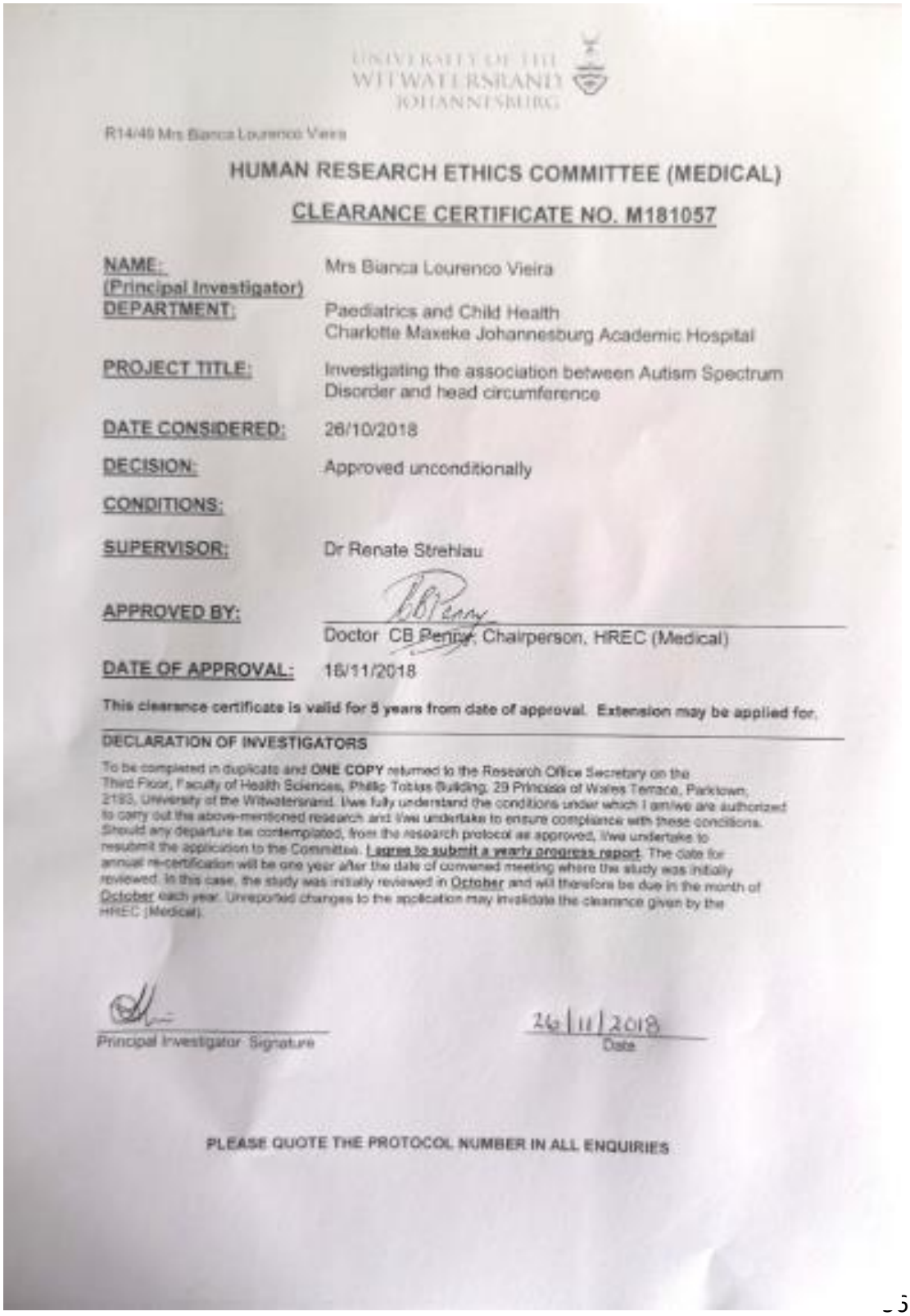
CONCLUSION

Autism is a lifelong, complex, neurodevelopmental disorder that impacts socialisation, communication and behaviour. ASD is considered one of the most expensive disabilities to diagnose (as agreed internationally), with intervention and support structures being unaffordable to the majority of South Africans (61). ASD prevalence has increased by 178% since 2000 and has no signs of abating (4). Identifying ASD early on, and with surety, is imperative and can safeguard a more favourable prognosis. The current study provided an invaluable insight and understanding of the phenotype of ASD in a selected population attending a NDC in Johannesburg, SA; which is currently lacking in literature. Our study found that routinely measuring and interpreting HC measurements in individuals suspected of having ASD can be a culturally-sensitive, non-invasive, cost-effective, time-efficient assessment. Using the interpretation of this predictor as a tool to raise suspicion of ASD can result in earlier diagnosis and thus prompt early intervention services. In achieving this, the pathological processes underlying the macrocephaly associated with ASD can be interrupted and mitigated by therapeutic interventions aimed to optimise participation and social integration.

APPENDICES

Appendix A

Human Research Ethics Committee Clearance Certificate



UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R14/49 Mrs Bianca Lourenco Vieira

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M181057

NAME: (Principal Investigator)	Mrs Bianca Lourenco Vieira
DEPARTMENT:	Paediatrics and Child Health Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	Investigating the association between Autism Spectrum Disorder and head circumference
DATE CONSIDERED:	26/10/2018
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Renate Strehlau
APPROVED BY:	Doctor CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	16/11/2018

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. I agree to submit a yearly progress report. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in October and will therefore be due in the month of October each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

26/11/2018
Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix B

Turnitin Report

09 February 2021

9/2/2021

0714106y:Submission_Version_-_Bianca_Vieira_-_090221_-_pdf.pdf

ORIGINALITY REPORT

10%	7%	7%	3%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	hdl.handle.net Internet Source	1%
2	mobile.wiredspace.wits.ac.za Internet Source	1%
3	www.tandfonline.com Internet Source	<1%
4	repository.up.ac.za Internet Source	<1%
5	archive.org Internet Source	<1%
6	pediatrics.aappublications.org Internet Source	<1%
7	"Handbook of Interdisciplinary Treatments for Autism Spectrum Disorder", Springer Science and Business Media LLC, 2019 Publication	<1%
8	Katijah Khoza-Shangase, Julia Anastasiou. "An exploration of recorded otological manifestations	<1%

	in South African children with HIV/AIDS: A pilot study", International Journal of Pediatric Otorhinolaryngology, 2020 Publication	
9	"Handbook of Assessment and Diagnosis of Autism Spectrum Disorder", Springer Science and Business Media LLC, 2016 Publication	<1 %
10	Health Care for People with Intellectual and Developmental Disabilities across the Lifespan, 2016. Publication	<1 %
11	Submitted to Master's University and Seminary Student Paper	<1 %
12	silo.pub Internet Source	<1 %
13	Handbook of Growth and Growth Monitoring in Health and Disease, 2012. Publication	<1 %
14	wiredspace.wits.ac.za Internet Source	<1 %
15	www.sajch.org.za Internet Source	<1 %
16	Submitted to Anglia Ruskin University Student Paper	<1 %

17	easynewswire.com Internet Source	<1%
18	"Comprehensive Guide to Autism", Springer Nature, 2014 Publication	<1%
19	neurosci.cn Internet Source	<1%
20	Submitted to Colorado State University, Global Campus Student Paper	<1%
21	P Godfrey-Faussett, P Sonnenberg, SC Shearer, MC Bruce, C Mee, L Morris, J Murray. "Tuberculosis control and molecular epidemiology in a South African gold-mining community", The Lancet, 2000 Publication	<1%
22	estudogeral.sib.uc.pt Internet Source	<1%
23	Submitted to Cardiff University Student Paper	<1%
24	www.um.edu.mt Internet Source	<1%
25	Blatchley, Barbara. "Statistics in Context", Oxford University Press Publication	<1%

26	Lauren Franz, Nola Chambers, Megan von Isenburg, Petrus J. de Vries. "Autism spectrum disorder in sub-saharan africa: A comprehensive scoping review", Autism Research, 2017 Publication	<1 %
27	www.science.gov Internet Source	<1 %
28	www.frontiersin.org Internet Source	<1 %
29	Submitted to Curtin University of Technology Student Paper	<1 %
30	Sébastien Jacquemont, Alexandre Reymond, Flore Zufferey, Louise Harewood et al. "Mirror extreme BMI phenotypes associated with gene dosage at the chromosome 16p11.2 locus", Nature, 2011 Publication	<1 %
31	mafiadoc.com Internet Source	<1 %
32	Sarosha Pillay, Madeleine Duncan, Petrus J de Vries. "Autism in the Western Cape province of South Africa: Rates, socio-demographics, disability and educational characteristics in one million school children", Autism, 2020 Publication	<1 %

33	doctorpenguin.com Internet Source	<1%
34	Submitted to University of Newcastle Student Paper	<1%
35	"Interprofessional Care Coordination for Pediatric Autism Spectrum Disorder", Springer Science and Business Media LLC, 2020 Publication	<1%
36	iacc.hhs.gov Internet Source	<1%
37	www.cdc.gov Internet Source	<1%
38	open.uct.ac.za Internet Source	<1%
39	www.bmj.com Internet Source	<1%
40	www.vachementmoi.be Internet Source	<1%
41	Submitted to Chester College of Higher Education Student Paper	<1%
42	www.dhcas.gov.hk Internet Source	<1%
43	Submitted to Endicott College	

	Student Paper	<1%
44	clinicalinfo.hiv.gov Internet Source	<1%
45	dspace.nwu.ac.za Internet Source	<1%
46	Submitted to University of Salford Student Paper	<1%
47	jmedicalcasereports.biomedcentral.com Internet Source	<1%
48	Dinyadarshini Johnson, Vengadesh Letchumanan, Sivakumar Thurairajasingam, Learn-Han Lee. "A Revolutionizing Approach to Autism Spectrum Disorder Using the Microbiome", Nutrients, 2020 Publication	<1%
49	www.biorxiv.org Internet Source	<1%
50	Lauren E. Libero, Christine W. Nordahl, Deana D. Li, Emilio Ferrer, Sally J. Rogers, David G. Amaral. "Persistence of megalencephaly in a subgroup of young boys with autism spectrum disorder", Autism Research, 2016 Publication	<1%
51	www.labome.org	

	Internet Source	<1%
52	files.shareholder.com Internet Source	<1%
53	sta.epa.gov.tw Internet Source	<1%
54	Oliver D Howes, Maria Rogdaki, James L Findon, Robert H Wichers et al. "Autism spectrum disorder: Consensus guidelines on assessment, treatment and research from the British Association for Psychopharmacology", Journal of Psychopharmacology, 2017 Publication	<1%
55	explora.unex.es Internet Source	<1%
56	www.dovepress.com Internet Source	<1%
57	doi.org Internet Source	<1%
58	ajod.org Internet Source	<1%
59	academic.oup.com Internet Source	<1%
60	www.cancer.gov Internet Source	<1%

61	hal.inrae.fr Internet Source	<1%
62	juns.nursing.arizona.edu Internet Source	<1%
63	worldwidescience.org Internet Source	<1%
64	coek.info Internet Source	<1%
65	Submitted to Kennesaw State University Student Paper	<1%
66	Iveta Bujnakova, Igor Ondrejka, Michal Mestanik, Ingrid Tonhajzerova. "Autonomic nervous system in children with autism spectrum disorder", Cognitive Remediation Journal, 2015 Publication	<1%
67	www.wihd.org Internet Source	<1%
68	www.noellesdefiningmoments.com Internet Source	<1%
69	Nur ATEŞ-ŞAHİNKAYA, Nilüfer ACAR-TEK, Emre DIGÜZEL. "The association between maternal features and nutritional problems in children with autism spectrum disorder", Revista de Nutrição, 2020 Publication	<1%

70	www.researchsquare.com Internet Source	<1%
71	www.hindawi.com Internet Source	<1%
72	"Spoken sessions", Thorax, 2002 Publication	<1%
73	Valerio Napolioni, Federica Lombardi, Roberto Sacco, Paolo Curatolo et al. "Family-based association study of ITGB3 in autism spectrum disorder and its endophenotypes", European Journal of Human Genetics, 2010 Publication	<1%
74	www.scielo.br Internet Source	<1%
75	link.springer.com Internet Source	<1%
76	Cory Shulman, Catherine E. Rice, Michael J. Morrier, Amy Esler. "The Role of Diagnostic Instruments in Dual and Differential Diagnosis in Autism Spectrum Disorder Across the Lifespan", Child and Adolescent Psychiatric Clinics of North America, 2020 Publication	<1%
77	Nancy D Spector. "Sleep disorders, immunizations, sports injuries, autism", Current	<1%

	Opinion in Pediatrics, 12/2005 Publication	
78	pubmed.ncbi.nlm.nih.gov Internet Source	<1%
79	www.yumpu.com Internet Source	<1%
80	minerva-access.unimelb.edu.au Internet Source	<1%
81	www.ncbi.nlm.nih.gov Internet Source	<1%
82	Matlis, Sean, Katica Boric, Catherine J. Chu, and Mark A. Kramer. "Robust disruptions in electroencephalogram cortical oscillations and large-scale functional networks in autism", BMC Neurology, 2015. Publication	<1%
83	Jamie Murdoch, Robyn Curran, Ruth Cornick, Sandy Picken, Max Bachmann, Eric Bateman, Makhosazana Lungile Simelane, Lara Fairall. "Addressing the quality and scope of paediatric primary care in South Africa: evaluating contextual impacts of the introduction of the Practical Approach to Care Kit for children (PACK Child)", BMC Health Services Research, 2020 Publication	<1%

84	Lauren Franz, Konyin Adewumi, Nola Chambers, Marisa Viljoen, Joy Noel Baumgartner, Petrus J. de Vries. "Providing early detection and early intervention for autism spectrum disorder in South Africa: stakeholder perspectives from the Western Cape province", Journal of Child & Adolescent Mental Health, 2018 Publication	<1%
85	Andrew J.O. Whitehouse. "Elizabeth Usher Memorial Lecture: Rethinking the clinical pathway for autism spectrum disorder and challenging the status quo", International Journal of Speech-Language Pathology, 2017 Publication	<1%
86	L. Zwaigenbaum, M. L. Bauman, W. L. Stone, N. Yirmiya et al. "Early Identification of Autism Spectrum Disorder: Recommendations for Practice and Research", PEDIATRICS, 2015 Publication	<1%
87	Manahil Alfuraydan, Jodie Croxall, Lisa Hurt, Mike Kerr, Sinead Brophy. "Use of telehealth for facilitating the diagnostic assessment of Autism Spectrum Disorder (ASD): A scoping review", PLOS ONE, 2020 Publication	<1%
88	Lauren H. Hampton, Michal Harty, Elizabeth A.	

Fuller, Ann P. Kaiser. "Enhanced milieu teaching for children with autism spectrum disorder in South Africa", International Journal of Speech-Language Pathology, 2019 <1%
Publication

Exclude quotes	On	Exclude matches	Off
Exclude bibliography	On		

REFERENCES

1. Franz L, Adewumi K, Chambers N, Viljoen M, Baumgartner JN, de Vries PJ. Providing early detection and early intervention for autism spectrum disorder in South Africa: stakeholder perspectives from the Western Cape province. J Child Adolesc Ment Health. 2018;30(3):149-65. DOI: 10.2989/17280583.2018.1525386.

2. American Psychiatric Association. Diagnostic and statistical manual of mental disorders, DSM-5. 5th ed. Washington, DC: Library of Congress Cataloging-in-Publication Data; 2013.

3. Voigt RG. Autism Spectrum Disorder. In: Kline MW, editor. Rudolph's Pediatrics. 23rd ed. New York, NY: McGraw-Hill Education; 2018. Available from: accesspediatrics.mhmedical.com/content.aspx?aid=1154116348.

4. Maenner MJ, Shaw KA, Baio J. Prevalence of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network, 11 sites, United States, 2016. MMWR Surveillance Summaries. 2020;69(4):1-14. DOI: 10.15585/mmwr.ss6706a1.

5. Baio J, Wiggins L, Christensen DL, Maenner MJ, Daniels J, Warren Z, et al. Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years - Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2014. MMWR Surveill Summ. 2018;67(6):1-23. DOI: 10.15585/mmwr.ss6706a1.

6. Kliegman RM, Geme JW, Blum NJ, Shah SS, Tasker RC, Wilson KM. Autism Spectrum Disorder. In: Bridgemohan CF, editor. Nelson Textbook of Pediatrics. 21st ed. Canada: Elsevier; 2020. p. 294-302.

7. Bakare MO, Munir KM. Autism spectrum disorders (ASD) in Africa: a perspective. Afr J Psychiatry (Johannesbg). 2011;14(3):208-10. DOI: 10.4314/ajpsy.v14i3.3.

8. Abubakar A, Ssewanyana D, Newton CR. A Systematic Review of Research on Autism Spectrum Disorders in Sub-Saharan Africa. Behav Neurol. 2016;2016:3501910. DOI: 10.1155/2016/3501910.

9. Springer PE, van Toorn R, Laughton B, Kidd M. Characteristics of children with pervasive developmental disorders attending a developmental clinic in the Western Cape Province, South Africa. South African Journal of Child Health. 2013;7(3):95-9. DOI: 10.7196/sajch.530.

10. van Biljon S, Kritzinger A, Geertsema S. A retrospective case report on demographic changes of learners at a school for children with Autism Spectrum Disorders in the Gauteng Province. South African Journal of Childhood Education. 2015;5:01-26. DOI: 2223-7682.

11. Jeynes K, Ngassa Piotie P, Skosana I. Frequently asked questions about autism in South Africa South Africa: Africa Check; 2018 [Available from: https://africacheck.org/factsheets/factsheet-frequently-asked-questions-about-autism-in-south-africa/. Access date: 13/04/2020.

12. Lehohla P. Community Survey 2016 in Brief. In: Africa SS, editor. Pretoria: Stats SA Library Cataloguing-in-Publication (CIP) Data; 2016. p. 1-192.

13. Department of Social Development. White Paper on the Rights of Persons with Disabilities South Africa. 2016 [1 - 194]. Available from: https://www.gov.za/documents/white-paper-rights-persons-disabilities-official-publication-and-gazetting-white-paper. Access date: 02/04/2020.

14. Kellerman RD, Rakel D. Autism. In: Brown R, editor. Conn's current therapy 2020. Philadelphia: Elsevier; 2020. p. 767-70.

15. Smith MJ. Vaccine Safety and Risk Communication. In: Shah SS, Kemper AR, Ratner AJ, editors. Pediatric Infectious Diseases: Essentials for Practice. 2nd ed. New York, NY: McGraw-Hill Education; 2019. Available from: accesspediatrics.mhmedical.com/content.aspx?aid=1157318887.

16. Meeks NJL, Saenz M, Tsai AC-H, Elias ER. Genetics & Dysmorphology. In: Hay Jr WW, Levin MJ, Abzug MJ, Bunik M, editors. Current Diagnosis & Treatment: Pediatrics. 25th ed. New York, NY: McGraw-Hill Education; 2020. Available from: accesspediatrics.mhmedical.com/content.aspx?aid=1172112498.

17. Abruzzo PM, Ghezzo A, Bolotta A, Ferreri C, Minguzzi R, Vignini A, et al. Perspective Biological Markers for Autism Spectrum Disorders: Advantages of the Use of Receiver Operating Characteristic Curves in Evaluating Marker Sensitivity and Specificity. Dis Markers. 2015;2015:329607. DOI: 10.1155/2015/329607.

18. Parellada M, Penzol MJ, Pina L, Moreno C, Gonzalez-Vioque E, Zalsman G, et al. The neurobiology of autism spectrum disorders. Eur Psychiatry. 2014;29(1):11-9. DOI: 10.1016/j.eurpsy.2013.02.005.

19. Stigler KA, McDonald BC, Anand A, Saykin AJ, McDougle CJ. Structural and functional magnetic resonance imaging of autism spectrum disorders. Brain Res. 2011;1380:146-61. DOI: 10.1016/j.brainres.2010.11.076.

20. Fukumoto A, Hashimoto T, Mori K, Tsuda Y, Arisawa K, Kagami S. Head circumference and body growth in autism spectrum disorders. Brain Dev. 2011;33(7):569-75. DOI: 10.1016/j.braindev.2010.09.004.

21. Sacco R, Gabriele S, Persico AM. Head circumference and brain size in autism spectrum disorder: A systematic review and meta-analysis. Psychiatry Res. 2015;234(2):239-51. DOI: 10.1016/j.pscychresns.2015.08.016.

22. Courchesne E, Redcay E, Kennedy DP. The autistic brain: birth through adulthood. Curr Opin Neurol. 2004;17(4):489-96. DOI: 10.1097/01.wco.0000137542.14610.b4.

23. Slogrove AL, Powis KM, Johnson LF, Stover J, Mahy M. Estimates of the global population of children who are HIV-exposed and uninfected, 2000–18: a modelling study. The Lancet Global Health. 2020;8(1):e67-e75. DOI: 10.1016/s2214-109x(19)30448-6.

24. Budd MA, Calli K, Samson L, Bowes J, Hsieh AYY, Forbes JC, et al. Blood Mitochondrial DNA Content in HIV-Exposed Uninfected Children with Autism Spectrum Disorder. Viruses. 2018;10(2):77. DOI: 10.3390/v10020077.

25. Chawarska K, Campbell D, Chen L, Shic F, Klin A, Chang J. Early generalized overgrowth in boys with autism. Arch Gen Psychiatry. 2011;68(10):1021-31. DOI: 10.1001/archgenpsychiatry.2011.106.

26. Zwaigenbaum L, Bauman ML, Stone WL, Yirmiya N, Estes A, Hansen RL, et al. Early Identification of Autism Spectrum Disorder: Recommendations for Practice and Research. Pediatrics. 2015;136 Suppl 1(Supplement 1):S10-40. DOI: 10.1542/peds.2014-3667C.

27. Frye RE. Autism. In: Carney PR, Geyer JD, editors. Pediatric Practice: Neurology. New York, NY: The McGraw-Hill Companies; 2010. Available from: accesspediatrics.mhmedical.com/content.aspx?aid=6654714.

28. Hirtz DG, Wagner A, Filipek PA, Sherr EH. Autistic Spectrum Disorders. In: Swaiman KF, Ashwal S, Ferriero DM, Schor NF, Finkel RS, Gropman AL, et al., editors. Swaiman's Pediatric Neurology. 6th ed. Edinburgh: Elsevier Inc.; 2017. p. 1104-36.

29. Garon N, Bryson SE, Zwaigenbaum L, Smith IM, Brian J, Roberts W, et al. Temperament and its relationship to autistic symptoms in a high-risk infant sib cohort. J Abnorm Child Psychol. 2009;37(1):59-78. DOI: 10.1007/s10802-008-9258-0.

30. Shulman C, Rice CE, Morrier MJ, Esler A. The Role of Diagnostic Instruments in Dual and Differential Diagnosis in Autism Spectrum Disorder Across the Lifespan. Child Adolesc Psychiatr Clin N Am. 2020;29(2):275-99. DOI: 10.1016/j.chc.2020.01.002.

31. Lemay JF, Yohemas M, Langenberger S. Redesign of the autism spectrum screening and diagnostic process for children aged 12 to 36 months. Paediatr Child Health. 2018;23(5):308-13. DOI: 10.1093/pch/pxx187.

32. Carr T. Autism Diagnostic Observation Schedule. In: Volkmar FR, editor. Encyclopedia of Autism Spectrum Disorders. New York, NY: Springer New York; 2013. p. 349-56. Available from: https://doi.org/10.1007/978-1-4419-1698-3_896.

33. CARS, Second Edition, Standard Version. In: Volkmar FR, editor. Encyclopedia of Autism Spectrum Disorders. New York, NY: Springer New York; 2013. p. 530-. Available from: https://doi.org/10.1007/978-1-4419-1698-3_100246.

34. Kim SH, Hus V, Lord C. Autism Diagnostic Interview-Revised. In: Volkmar FR, editor. Encyclopedia of Autism Spectrum Disorders. New York, NY: Springer New York; 2013. p. 345-9. Available from: https://doi.org/10.1007/978-1-4419-1698-3_894.

35. Daroff R, Jankovic J, Mazziotta J, Pomeroy S, Bradley W. Autism and other Developmental Disabilities. In: Nass R, Sidhu R, Ross G, editors. Bradley's Neurology in Clinical Practice. 7th ed. United States of America: Elsevier; 2016. p. 1301-23.

36. Smith L. Afrikaans autism diagnostic observation schedule-2 : translation and cultural appropriateness for the coloured population from low-middle

socioeconomic backgrounds living in the Western Cape. [minor dissertation]. In press 2015.

37. Harrison AJ, Zimak EH, Sheinkopf SJ, Manji KP, Morrow EM. Observation-centered approach to ASD assessment in Tanzania. Intellectual and developmental disabilities. 2014;52(5):330-47. DOI: 10.1352/1934-9556-52.5.330.

38. Stephens M. Screening for Autism Spectrum Disorders in South Africa: Using the Modified Checklist for Autism in toddlers (M-CHAT). Department of Psychology. [minor dissertation]. In press 2012.

39. Zwaigenbaum L, Bauman ML, Fein D, Pierce K, Buie T, Davis PA, et al. Early Screening of Autism Spectrum Disorder: Recommendations for Practice and Research. Pediatrics. 2015;136 Suppl 1(Supplement 1):S41-59. DOI: 10.1542/peds.2014-3667D.

40. Brett D, Warnell F, McConachie H, Parr JR. Factors Affecting Age at ASD Diagnosis in UK: No Evidence that Diagnosis Age has Decreased Between 2004 and 2014. J Autism Dev Disord. 2016;46(6):1974-84. DOI: 10.1007/s10803-016-2716-6.

41. Erasmus S, Kritzinger A, van der Linde J. Profiles of public and private autism-specific schools in Gauteng. South African Journal of Childhood Education. 2019;9(1). DOI: 10.4102/sajce.v9i1.691.

42. Campbell DJ, Chang J, Chawarska K. Early generalized overgrowth in autism spectrum disorder: prevalence rates, gender effects, and clinical outcomes. J Am Acad Child Adolesc Psychiatry. 2014;53(10):1063-73 e5. DOI: 10.1016/j.jaac.2014.07.008.

43. Zitelli B, McIntire, SC, Nowalk, AJ. Developmental/Behavioral Pediatrics. In: Feldman H, Chaves-Gnecco, D., editor. Zitelli and Davis' Atlas of Pediatric Physical Diagnosis. 7th ed. Philadelphia: Elsevier; 2018. p. 71-100.

44. Landa RJ. Efficacy of early interventions for infants and young children with, and at risk for, autism spectrum disorders. Int Rev Psychiatry. 2018;30(1):25-39. DOI: 10.1080/09540261.2018.1432574.

45. Gomella TL, Eyal FG, Bany-Mohammed F. Follow-Up of High-Risk Infants. Gomella's Neonatology: Management, Procedures, On-Call Problems, Diseases, and Drugs. 8th ed. New York, NY: McGraw-Hill Education; 2020. Available from: accesspediatrics.mhmedical.com/content.aspx?aid=1168356149.

46. World Health Organization. WHO Child growth standards - methods and development China, Hong Kong: WHO Library Cataloguing-in-Publication Data; 2007 [1 - 237]. Available from: http://www.who.int/childgrowth/standards/second_set/technical_report_2.pdf?ua=1. Access date: 14/06/2020.

47. Shraga Y, Sarid O, Cwikel J, Reuveni H. Well-Child Visits Regarding Growth and Development of Infants: A Cross-Cultural Comparison. Harefuah. 2016;155(7):431-4. DOI: 28514134.

48. Murdoch J, Curran R, Cornick R, Picken S, Bachmann M, Bateman E, et al. Addressing the quality and scope of paediatric primary care in South Africa: evaluating contextual impacts of the introduction of the Practical Approach to Care Kit for children (PACK Child). BMC Health Serv Res. 2020;20(1):479. DOI: 10.1186/s12913-020-05201-w.

49. Harris SR. Measuring head circumference: Update on infant microcephaly. Can Fam Physician. 2015;61(8):680-4. DOI: 26505062.

50. Department of Health. Side-by-side on the road to health [Booklet]. South Africa: Department of Health; 2018 [updated 26/06/2020. Available from: https://sidebyside.co.za/resources/road-to-health-book/. Access date: 26/06/2020.

51. McKinney ES, James SR, Murray SS, Nelson K, J A. Maternal-Child Nursing. Missouri: Elsevier Health Sciences; 2018.

52. Sacco R, Militerni R, Frolli A, Bravaccio C, Gritti A, Elia M, et al. Clinical, morphological, and biochemical correlates of head circumference in autism. Biol Psychiatry. 2007;62(9):1038-47. DOI: 10.1016/j.biopsych.2007.04.039.

53. de Vinck-Baroody O, Shui A, Macklin EA, Hyman SL, Leventhal JM, Weitzman C. Overweight and Obesity in a Sample of Children With Autism Spectrum Disorder. Acad Pediatr. 2015;15(4):396-404. DOI: 10.1016/j.acap.2015.03.008.

54. McKeague IW, Brown AS, Bao Y, Hinkka-Yli-Salomaki S, Huttunen J, Sourander A. Autism with intellectual disability related to dynamics of head circumference growth during early infancy. Biol Psychiatry. 2015;77(9):833-40. DOI: 10.1016/j.biopsych.2014.08.008.

55. Crane L, Chester JW, Goddard L, Henry LA, Hill E. Experiences of autism diagnosis: A survey of over 1000 parents in the United Kingdom. Autism. 2016;20(2):153-62. DOI: 10.1177/1362361315573636.

56. Goldson E, Angulo AS, Reynolds A, Raz DM. Child Development & Behavior. In: Hay Jr WW, Levin MJ, Abzug MJ, Bunik M, editors. Current Diagnosis & Treatment: Pediatrics. 25th ed. New York, NY: McGraw-Hill Education; 2020. Available from: accesspediatrics.mhmedical.com/content.aspx?aid=1172100337.

57. Bateman C. Autism--mitigating a global epidemic. S Afr Med J. 2013;103(5):276-7. DOI: 10.7196/samj.6915.

58. Marrus N, Hall L. Intellectual Disability and Language Disorder. Child Adolesc Psychiatr Clin N Am. 2017;26(3):539-54. DOI: 10.1016/j.chc.2017.03.001.

59. Fombonne E, Roge B, Claverie J, Courty S, Fremolle J. Microcephaly and macrocephaly in autism. J Autism Dev Disord. 1999;29(2):113-9. DOI: 10.1023/a:1023036509476.

60. Franz L, Chambers N, von Isenburg M, de Vries PJ. Autism spectrum disorder in sub-saharan africa: A comprehensive scoping review. Autism research

: official journal of the International Society for Autism Research. 2017;10(5):723-49. DOI: 10.1002/aur.1766.

61. Clasquin-Johnson MG, Clasquin-Johnson M. 'How deep are your pockets?' Autoethnographic reflections on the cost of raising a child with autism. African Journal of Disability. 2018;7:1-8. DOI: 10.4102/ajod.v7i0.356.