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Research Article

Gender Differences in Eye-Tracking Parameters in Children on the Autism Spectrum Disorder

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Abstract

Using eye-tracking paradigms, gender differences in gaze patterns for Autism Spectrum Disorder (ASD) patients were determined. This study included 155 subjects: 41 ASD subjects, 30 of their siblings and 84 neuro-Typically Developing (TD) controls. The female gender distribution of this cohort was 39% of ASD group, 37% of siblings and 57% of control groups. The five paradigms used were: 1-Horizontal and vertical motion video; 2-Face image; 3-Mute video of a talking face; 4-Animate/ inanimate object; 5-An inverted scene. In the horizontal motion video, ASD male subjects took longer to fixate compared to the siblings and controls. The male ASD group had deficits in horizontal motion tracking with prolonged time to first fixation and decreased duration compared to controls, while the female ASD group had similar parameters to controls. In the vertical gaze experiment, male and female ASD subjects both followed the moving target for a shorter time. Paradigms with social content revealed that male and female ASD patients preferred looking at the eyes rather than the mouth of a smiling face with longer duration and shorter time to first fixation, and this was significant. When looking at the silently talking face, female ASD subjects fixated faster on eyes vs mouth and with a greater percentage of fixation, despite the mouth movements. This, however, was not the case in males. When compared to siblings and TD controls, both genders had decreased duration of fixation on both eyes and mouth. The animate/ inanimate paradigm revealed a preference to the animate element for both genders and the inverted scene image had similar patterns across groups without differences. In conclusion, gender differences appeared in ASD subjects, specifically, when gazing at horizontally moving targets. Female ASD patterns resembled TD controls while males had decreased attention. When looking at a mute, talking face, females had preferential fixation to eyes despite the moving mouth element. Decreased general attention to all paradigms was observed for both genders.

Keywords: Autism spectrum disorder; Eye-tracking; Gender; Vision screening

Introduction

Autism Spectrum Disorders (ASD) are a group of neurodevelopmental syndromes characterized by variable degrees of impaired social behaviors, communication and language [1]. Individuals with ASD tend to perform Restrictive Repetitive Behaviors (RRBs) in an almost ritualistic manner and to have a contracted range of interests [1]. The prevalence of ASD has been increasing worldwide in the past few decades and has reached 1:68 in Lebanon in 2018 [2,3]. There are notable differences in prevalence rates of ASD in different countries and in different populations, most likely due to genetic factors [2]. Nonetheless, what holds true across borders is the male-biased prevalence of the disease with reported ratios ranging from 4.3:1 female and up to 16:1 [4,5]. Most research on ASD symptoms was conducted in males with no general consensus about specific gender differences in ASD phenotypes [6]. Physicians tend to face difficulties and delays in diagnosing ASD in females [7,8]. Tools used for the diagnosis of ASD, such as the Autism Diagnostic Observation Schedule (ADOS) and Autism Diagnostic Interview-Revised (ADI-R), are more sensitive for males than females with ASD [8,9]. Females are more likely to receive a diagnosis of atypical forms of autism [10]. McLennan et al. showed that socialization skills including understanding social clues and comforting others in males with ASD were more severely impaired than in female counterparts [11,12]. Females with ASD were more motivated to develop friendships, experience empathy, and participate in social activities than males with ASD [13,14]. In addition to stronger social skills, research also suggests that females with ASD exhibit more compensatory social behaviors than male counterparts and are less preoccupied by restrictive, repetitive behaviors [15-17]. Gender differences in symptomatology are not consistent in the literature. Also, no significant gender differences in disease severity, verbal and non-verbal skills, and cognitive abilities have been reported [18,19].

Independent of the etiology of gender-biased differences in prevalence and phenotypes, eye-tracking technology has emerged to qualitatively and quantitatively demonstrate different fixation and gaze patterns in ASD based on sex. Eye tracking technology allows the study of eye movements and helps measure the preferential distribution of observations [20]. It is non-invasive and uses corneal reflection to quantify and qualify fixations as well as interpret them [20]. Numerous eye tracking studies looking at children with ASD demonstrated consistent differential fixations and gaze compared to TD children [21,22]. Most eye-tracking experiments in the literature included a predominantly male ASD population and reported increased focusing on the mouth while looking at faces [23,24]. This group recently published results on eye movement recordings in ASD children using novel testing paradigms and reported significant differences in gaze patterns compared to controls [25]. The current study was conducted to detect differential gender-based eye tracking patterns in children with ASD compared to siblings (high risk group), and neuro-Typically Developing (TD) controls using these novel and validated testing paradigms.

Materials and Methods

Population

This study included 25 males and 16 females previously diagnosed with ASD aged 2-17 years, 19 male and 11 female siblings (high-risk subjects). Controls comprised 36 healthy age-matched males and 48 healthy age-matched females and were recruited from pediatric ophthalmology service. They were patients coming for routine visits with hyperopic or myopic spherical equivalent refraction up to 2 diopters. Informed consents obtained from parents or legal guardians were according to an Institutional Review Board approved protocol. Subjects excluded comprised those with gestational age below 36 weeks, or hearing loss/visual impairment determined at birth, if they had a history of non-febrile seizures, or known medical conditions associated with autistic features (Fragile X Syndrome, Tuberous Sclerosis, Rett syndrome) or any other identified genetic disorder. Also excluded were subjects with inaccurate calibration on the eye tracker due to poor cooperation. Participants with ASD and their siblings completed a neurologic assessment by a pediatric neurologist with diagnosis confirmed by DSM-5 criteria, Childhood Autism Rating Scale (CARS) and the Autism Diagnostic Observation Schedule (ADOS), the layer two to assess severity of ASD.

All participants underwent a complete pediatric ophthalmic examination in order to rule out confounders potentially affecting eye-tracking findings. They first underwent vision screening using the PlusoptiX S12 vision screener (Plusoptix GmbH, Atlanta, GA) (Figure 1) as well as age-appropriate visual acuity testing, including, “fix and follow” testing for infants, “central steady maintained” testing for older preverbal children and vision charts (Allen pictures and ETDRS charts) for verbal children. The anterior segment examination, motility check for eye misalignment, and cycloplegic manual retinoscopy to detect refractive errors 30 minutes after pupillary dilation (with Mydriacyl 1% and Cyclopentolate 1%, applied twice 10 minutes apart), were carried out in addition to posterior segment examination using indirect ophthalmoloscopy.

Figure 1: PlusoptiX S12 vision screener.

Eye tracking testing

Subjects underwent eye-tracking examination with the Tobii 1750 Eye Tracker (Danderyd, Sweden) with recordings integrated on a 17-inch monitor. Infant testing took place while seated on a parent’s lap and older subjects sat approximately 65 cm from the monitor. Cameras with a frequency of 50 Hz placed under the monitor recorded reflections from an infrared light. The tracker compensated for head motion and movements faster than 10 cm/s. ClearView software was used to display stimuli from static and dynamic images with a five-point calibration prior to the assessment with use of the formal ClearView filter for both eyes. Fixation was defined as gaze within a radius of 30 pixels for at least 100 ms. Successful calibration was a prerequisite for proceeding with the full testing paradigm. When patients calibrated well, the trial commenced. Poor fixation resulted in repetition of the trial with help of parents and other examiners. Exclusion of 2 patients from the ASD group prior to analysis resulted from calibration issues and/or a low proportion of valid data points. Out of 43 patients recruited, 41 were included in the final analysis. Description of the 5 paradigms studied are detailed in a previous publication in this cohort of patients [25].

1. Horizontal and vertical motion paradigms evaluated subject tracking of a moving element (cartoon of a dog) in horizontal and vertical directions (Figure 2a). This is an example of event-related design, where latency and duration of fixations on an object, orientation of gaze, and oculomotor functioning provide information about attentional functioning.

2. Still image of a smiling face paradigm with two areas of interest, the eyes and mouth of a smiling girl. It assessed preferential gaze of the three groups on one of the two anatomic areas while looking at a still face image (Figure 2b).

3. Video of a talking face (same face as the still image) without sound, which also contained the same areas of interest as in the still image paradigm. This served to assesses the possible effect of mouth movement on eye tracking parameters.

4. Image containing an animate and inanimate object and included two elements of interest, the animate monkey and the inanimate laptop (Figure 2c). This is an example of a paired visual preference paradigm traditionally used to demonstrate that neuro-typically developing children prefer looking at biological motion and social scenes compared to children with ASD.

5. An inverted image of a man in a room (Figure 2d). Distraction due to the inversion was compared between the three groups. The 'inverted face effect' previously described in eye-tracking experiments used the perceptual approach of looking at inverted versus upright faces.

The total duration of the experiment was 47 seconds (Figure 3) with six parameters analyzed for every paradigm:

- Time to first fixation in seconds, which represents the time from the start of the stimulus display until the participant fixates on an area of interest (AOI) for the first time.
- First fixation duration in seconds or duration of the first fixation on an AOI.
- Total fixation duration in seconds or duration of all fixations within an AOI.
- Fixation count or the number of times the participant fixated on an AOI.
- Percentage of fixation or number of times participants fixated at least once within an AOI divided by the total number of fixation recordings.Average fixation duration, obtained by dividing the total fixation duration by fixation counts.

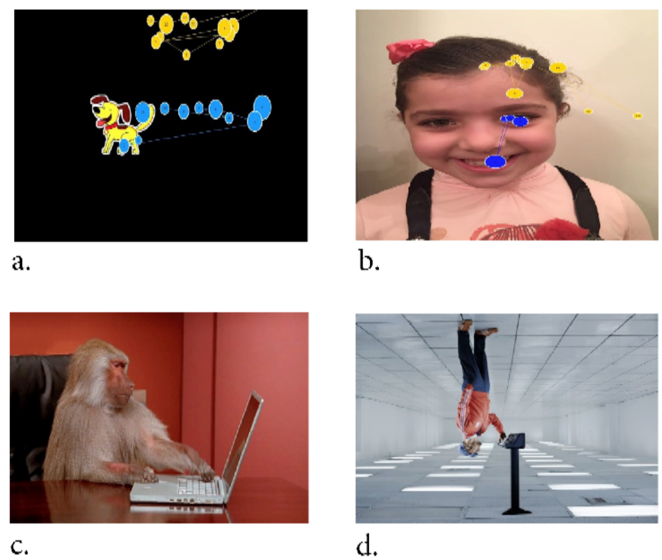

Figure 2a-2d: a. Horizontal and vertical motion paradigm showing the subject fixation points. Blue: control; Yellow: subject with ASD **b.** Still face image and subject fixation points on eye tracking testing. Blue: control; Yellow: subject with ASD **c.** Animate/inanimate image paradigm **d.** Inverted scene paradigm.

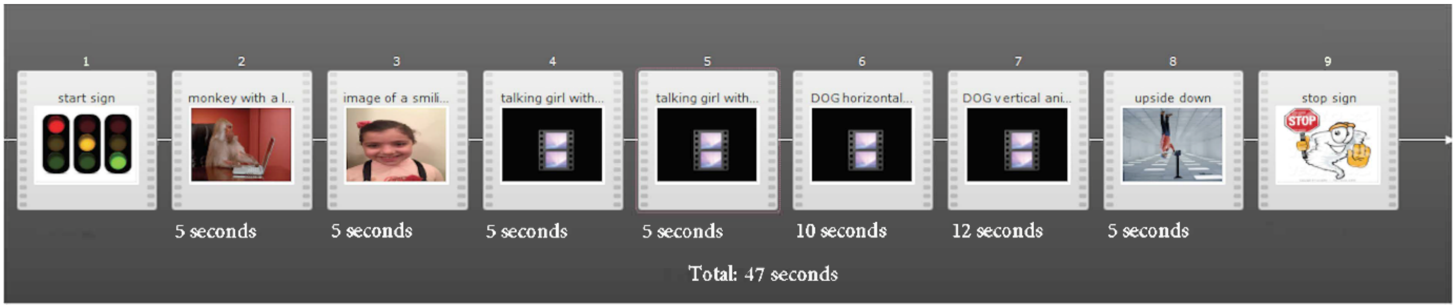

Figure 3: Overview of the experiment delineating all testing paradigms and duration of each.

Statistical Analysis

Execution of a statistical power analysis provided the appropriate sample size for this study. Entry of data variables into SPSS V22 software occurred. Division of subjects was by gender and then subdivided into ASD, siblings and control subgroups. Vision screening results and demographic variables were analyzed and compared in and between the three subgroups of each gender using ANOVA and Pearson chi-square tests, depending on the type of variable. Recorded eye tracking parameters were analyzed comparing children with ASD to their siblings and neuro-typically developing children, in males and females separately, using One-Way ANOVA when data of eye tracking related scores fit the normal frequency distribution, and its non-parametric counterpart (Kruskal Wallis) if data did not fit the normal distribution. When the difference across the 3 groups was statistically significant, Post-Hoc tests determined which differences between specific groups were statistically significant. A Bonferroni correction and Tukey's HSD test helped account for multiple testing. In the same paradigm, comparison between two areas of interest (e.g. total fixation duration on laptop vs. monkey) used the paired t-test. Statistical significance was set at a p-value <0.05.

Results

Of 155 subjects, 41 with ASD and 30 siblings at high risk from 40 families were included as well as 84 controls. Female gender accounted for 39% (ASD), 37% (siblings) and 57% (controls). Mean age was similar in groups, but ASD males were slightly older than male siblings and male controls (8.84 vs 6.23 and 6.47 years, respectively, p=0.04, (Table 1). No significant difference in visual acuity among subgroups was present. For the mean spherical equivalent, a significant difference in the females, specifically in the SE of the left eye appeared. In the ASD group, 2 subjects had astigmatism [1 male (4%); 1 female (6.2%)] and were prescribed glasses. Eight had hyperopia [3 males (12%) and 5 females (31.2%)], 2 females had exotropia (12.5%)]. Siblings included 2/30 cases of astigmatism (7%), both males. Controls belonged to a group of children coming to clinic for refractive errors. Forty/84 controls (47.6%) had refractive errors (11 males and 29 females). The distribution of refractive errors in males were astigmatism: 6; myopia: 4; hyperopia: 1 and for females 4, 21 and 4, respectively. Errors did not affect eye-tracking performance. ASD severity distribution was similar in genders (p=0.51, Table 1).

	Males				Females			
	ASD (n=25)	**Siblings (n=19)**	**Controls (n=36)**	**p-value**	**ASD (n=16)**	**Siblings (n=11)**	**Controls (n=48)**	**p-value**
Mean age ± SD years	8.84 ± 4.82	6.23 ± 3.15	6.47 ± 3.59	**0.04**	9.44 ± 4.26	9.18 ± 4.38	7.94 ± 4.07	0.38
Mean vision± SD (logMAR) Right Left	 0.05 ± 0.07 0.05 ± 0.09	 0.04 ± 0.08 0.04 ± 0.06	 0.06 ± 0.11 1.72 ± 9.99	 0.71 0.56	 0.04 ± 0.07 0.05 ± 0.08	 0.08 ± 0.14 0.07 ± 0.12	 0.48 ± 2.88 0.87 ± 4.08	 0.76 0.61
Mean SE ± SD Diopters Right Left	 0.42 ± 0.55 1.35 ± 1.11	 0.41 ± 1.08 0.51 ± 1.17	 -0.02 ± 1.56 0.58 ± 1.81	 0.30 0.08	 0.59 ± 1.19 1.25 ± 1.53	 -0.14 ± 1.49 -0.07 ± 1.31	 -0.26 ± 1.68 0.25 ± 1.98	 0.21 **0.02**
ASD severity* Mild Moderate Severe No data	 11 5 7 2				 5 7 3 1			
SD: standard deviation; SE: spherical equivalent. *p-value of ASD severity distribution between the genders is 0.51 as calculated by Pearson Chi-square								

Table 1: Demographics of study subjects by gender.

Horizontal and Vertical Paradigms (Table 2)

Males: Significant differences were noted in the male group on horizontal tracking motion when comparing the three subgroups, notably time to first fixation (p=0.01), total fixation duration (p<0.001) and fixation count (p<0.001). ASD male subjects took longer to fixate compared to siblings and controls groups. Furthermore, total fixation duration was shorter, and count was less than the control group. This meant that ASD children took a longer time to start following the moving target and tracked for shorter durations than siblings or controls. Similarly, vertical motion tracking showed significant differences between the three subgroups in total fixation duration (p=0.01) and fixation count (p=0.02). The total fixation duration of the ASD group was shorter than that in controls. Fixation counts in the ASD group were lower than counts in both sibling and control groups. This meant that in the vertical gaze experiment, males in the ASD group followed the moving target for shorter periods without a significant delay in the time to first fixation of the area of interest found when tracking horizontal motion.

Females: No significant differences in horizontal motion tracking paradigm in different female subgroups occurred. On the other hand, in the vertical motion paradigm, total fixation duration (p=0.04) and percentage of fixation (p<0.001) were lower for the ASD female group compared to controls.

	Males				Females			
	ASD	Siblings	Controls	P value	ASD	Siblings	Controls	P value
Horizontal Motion Time to first fixation (seconds)	4.51 ± 1.90	3.50 ± 0.84	3.96 ± 0.61	**0.001** ASD>S; ASD>C	4.01 ± 0.61	4.19 ± 1.65	3.51 ± 1.20	0.12
First fixation duration (seconds)	0.42 ± 0.49	0.28 ± 0.29	0.55 ± 0.48	0.06	0.38 ± 0.38	0.26 ± 0.27	0.36 ± 0.33	0.61
Total fixation duration (seconds)	2.63 ± 2.08	1.54 ± 4.40	4.40 ± 1.32	**<0.001 ASD>S; ASD<C**	2.76 ± 2.02	3.75 ± 1.93	3.86 ± 1.83	0.13
Fixation count	6.20 ± 3.96	11.00 ± 3.60	9.18 ± 4.24	**<0.001 ASD<S; ASD<C**	7.75 ± 4.56	9.64 ± 4.08	9.10 ± 4.23	0.46
Average fixation duration (seconds)	0.42 ± 0.44	0.37 ± 0.13	0.46 ± 0.20	0.06	0.63 ± 1.29	0.36 ± 0.16	0.43 ± 0.16	0.11
Percentage of fixation	0.96 ± 0.20	0.95 ± 0.22	1.00 ± 0.00	0.44	1.00 ± 0.00	1.00 ± 0.00	0.92 ± 0.27	0.31
Vertical Motion Time to first fixation (seconds)	2.72 ± 1.58	2.46 ± 0.87	2.46 ± 1.06	0.43	2.12 ± 1.53	2.72 ± 1.58	2.62 ± 1.59	0.95
First fixation duration (seconds)	0.46 ± 0.51	0.29 ± 0.30	0.31 ± 0.34	0.61	0.24 ± 0.23	0.22 ± 0.18	0.39 ± 0.39	0.13
Total fixation duration (seconds)	4.21 ± 2.95	5.07 ± 2.67	6.33 ± 2.09	**0.01 ASD<C**	3.85 ± 2.99	6.09 ± 2.79	5.59 ± 2.33	**0.04 ASD<C**
Fixation count	8.28 ± 5.68	11.84 ± 4.52	12.12 ± 5.26	**0.02 ASD<S; ASD<C**	8.00 ± 6.01	12.18 ± 4.64	10.35 ± 4.48	0.08
Average fixation duration: seconds	0.53 ± 0.26	0.45 ± 0.22	0.58 ± 0.29	0.34	0.49 ± 0.18	0.47 ± 0.19	0.63 ± 0.44	0.59
Percentage of fixation	0.92 ± 0.27	1.00 ± 0.00	1.00 ± 0.00	0.12	0.75 ± 0.44	1.00 ± 0.00	1.00 ± 0.00	**<0.001 ASD<C**
ASD= autism group, S= sibling group, C= controls								

Table 2: Eye tracking parameters for horizontal and vertical motion paradigms (mean ±SD).

Face Image Paradigm (Table 3)

Males: No differences occurred between the different subgroups when tracking eyes of the smiling girl in the image. On the other hand, when looking at the mouth, significant differences were noted between subgroups, specifically for the fixation count (p=0.03) and percentage of fixation (p=0.004). The fixation count and percentage fixation were significantly lower in the ASD group compared to controls and siblings. The male ASD group spent less time than controls looking at the mouth. When looking at the eyes versus the mouth, male ASD children fixated faster and for longer durations.

Females: A significant difference in total fixation duration when looking at the eyes of the smiling face (p=0.004) occurred being shorter in the ASD subjects compared to controls and siblings. When looking at the mouth, the time to first fixation, the first fixation duration and the percentage of fixation were low in the

ASD subjects compared to controls and siblings. This meant that females in the ASD group looked at the mouth slower than females in the other two groups, with less duration and lower percentage fixation. When comparing eyes vs mouth fixation, female ASD subjects fixated faster and longer on eyes.

	Males				Females			
	ASD	Siblings	Controls	P value[a]	ASD	Siblings	Controls	P value[a]
Time to first fixation								
Eyes	0.67 ± 0.95	0.34 ± 0.47	0.34 ± 0.58	0.42	0.45 ± 0.69	0.18 ± 0.17	0.32 ± 0.49	0.60
Mouth	1.18 ± 1.47	1.27 ± 1.26	1.11 ± 1.02	0.67	0.57 ± 0.97	1.04 ± 1.39	1.33 ± 1.26	**0.01**
	p=0.13[b]	**p=0.01**	**p<0.001**		**p=0.001**	**p=0.01**	**p<0.001**	ASD<C
First fixation duration								
Eyes	0.24 ± 0.17	0.22 ± 0.12	0.29 ± 0.22	0.58	0.18 ± 0.13	0.21 ± 0.08	0.34 ± 0.71	0.48
Mouth	0.20 ± 0.21	0.24 ± 0.21	0.12 ± 0.18	0.71	0.12 ± 0.20	0.34 ± 0.28	0.29 ± 0.33	**0.01**
	p=0.40	p=0.75	p=0.32		p=0.32	p=0.15	p=0.67	ASD<S; ASD<C
Total fixation duration								
Eyes	1.54 ± 1.04	1.72 ± 1.10	2.13 ± 0.93	0.13	1.34 ± 1.06	2.61 ± 0.54	2.15 ± 1.05	**0.004** ASD<S; ASD<C
Mouth	0.41 ± 0.52	0.59 ± 0.45	0.63 ± 0.49	0.07	0.43 ± 0.64	0.74 ± 0.58	0.65 ± 0.55	0.07
	p=0.001	**p<0.001**	**p<0.001**		**p<0.001**	**p<0.001**	**p<0.001**	
Fixation count								
Eyes	5.56 ± 4.34	6.32 ± 4.13	7.74 ± 3.52	0.11	5.50 ± 4.01	8.55 ± 2.58	6.85 ± 3.12	0.06
Mouth	1.28 ± 1.54	2.16 ± 1.57	2.32 ± 1.53	**0.03**	1.31 ± 1.99	2.36 ± 1.91	1.90 ± 1.30	0.22
	p<0.001	**p<0.001**	**p<0.001**	ASD<S; ASD<C	**p<0.001**	**p<0.001**	**p<0.001**	
Average fixation duration								
Eyes	0.29 ± 0.13	0.27 ± 0.11	0.30 ± 0.31	0.91	0.23 ± 0.09	0.34 ± 0.14	0.42 ± 0.70	0.08
Mouth	0.35 ± 0.15	0.30 ± 0.19	0.27 ± 0.16	0.34	0.34 ± 0.13	0.35 ± 0.20	0.35 ± 0.28	0.88
	p=0.29	p=0.34	p=0.74		p=0.74	p=0.68	p=0.42	
Percentage of fixation								
Eyes	0.96 ± 0.20	0.95 ± 0.22	1.00 ± 0.00	0.44	0.87 ± 0.34	1.00 ± 0.00	0.98 ± 0.14	0.14
Mouth	0.55 ± 0.50	0.84 ± 0.37	0.91 ± 0.28	0.004	0.44 ± 0.51	0.91 ± 0.30	0.85 ± 0.35	0.001
	p<0.001	p=0.33	p =0.08	ASD<S; ASD<C	P=0.080	p=0.34	p=0.01	ASD<S; ASD<C
[a]p-value comparing difference in parameters among the subgroups: ASD, siblings and controls [b]p-value comparing difference in parameters within the same group, between fixation to eyes and fixation to mouth ASD= autism group, S= sibling group, C= controls								

Table 3: Eye tracking parameters for the still image of a smiling face paradigm (mean ± SD).

Video of a Talking Face without Sound (Table 4)

Males: When looking at eyes, percentage of fixation was significantly lower in the ASD group as compared to the sibling and control groups. Other parameters showed no differences. Looking at gaze patterns for the mouth, significant differences in the first fixation duration (p=0.02) and total fixation duration (p<0.01) occurred being shorter in the ASD group than sibling or control groups. Notable was that male ASD patients had a longer total fixation duration and higher fixation count at the eyes compared to the mouth.

Females: In the mute video, looking at the eyes of the talking girl, the time to first fixation was longer in ASD female subjects than controls, while total fixation duration was shorter, and the count was significantly lower. When looking at the mouth, a significant difference was seen only in the total fixation duration (p=0.05). Shorter total fixation duration was present in the ASD females compared to control females, which meant that ASD females looked at the mouth of the talking girl in the mute video for a shorter period. When comparing eyes to mouth AOI directly, female ASD subjects took less time to first fixate at the eyes than the mouth with increased total fixation duration, fixation count and percentage fixation. This meant that females were more attentive to the eye element in the face image, despite the moving mouth.

	Males				Females			
	ASD	Siblings	Controls	P value[a]	ASD	Siblings	Controls	P value[a]
Time to first fixation								
Eyes	0.32 ± 0.64	0.11 ± 0.19	0.10 ± 0.26	0.11	0.30 ± 0.66	0.26 ± 0.81	0.09 ± 0.26	**0.03** ASD>C
Mouth	0.61 ± 0.93 p[b]=0.23	0.86 ± 1.04 p=**0.005**	0.66 ± 0.66 p**<0.001**	0.45	1.25 ± 1.39 p**<0.001**	1.26 ± 1.15 **p=0.01**	0.57 ± 0.53 p**<0.001**	0.06
First fixation duration								
Eyes	0.37 ± 0.71	0.23 ± 0.20	0.32 ± 0.63	0.93	0.45 ± 1.12	0.18 ± 0.09	0.40 ± 0.89	0.99
Mouth	0.25 ± 0.36 p=0.43	0.96 ± 1.08 p=**0.01**	0.63 ± 0.63 p=0.09	**0.02** ASD<S; ASD<C	0.63 ± 0.98 p=0.09	0.92 ± 1.03 p=**0.04**	1.09 ± 1.11 p=**0.001**	0.31
Total fixation duration								
Eyes	2.87 ± 1.64	3.51 ± 1.04	3.49 ± 1.15	0.52	2.54 ± 1.68	3.80 ± 1.08	3.70 ± 1.21	**0.04** ASD<C
Mouth	0.70 ± 1.04 p**<0.001**	1.64 ± 1.42 p**<0.001**	1.56 ± 1.22 p**<0.001**	**<0.01** ASD<S; ASD<C	0.83 ± 1.04 p**<0.001**	1.54 ± 1.03 p**<0.001**	1.85 ± 1.40 p**<0.001**	**0.05** ASD<C
Fixation count								
Eyes	7.12 ± 4.40	7.37 ± 2.85	7.47 ± 2.88	0.93	5.56 ± 3.91	8.82 ± 2.48	6.90 ± 2.99	**0.04** ASD<S
Mouth	1.28 ± 1.40 p**<0.001**	2.16 ± 2.58 p**<0.001**	2.32 ± 1.77 p**<0.001**	0.10	1.31 ± 1.44 p**<0.001**	2.09 ± 1.22 p**<0.001**	1.88 ± 1.28 p**<0.001**	0.24
Average fixation duration								
Eyes	0.50 ± 0.44	0.55 ± 0.28	0.55 ± 0.38	0.36	0.76 ± 0.15	0.48 ± 0.28	0.73 ± 0.80	0.24
Mouth	0.57 ± 0.42 **p=0.05**	1.12 ± 0.90 p=**0.02**	0.75 ± 0.44 p**<0.001**	0.21	0.84 ± 1.09 p**<0.001**	0.90 ± 0.80 p=**0.04**	1.12 ± 0.78 p**<0.001**	0.10
Percentage of fixation								
Eyes	0.88 ± 0.33	1.00 ± 0.00	1.00 ± 0.00	**0.04** ASD<S	0.87 ± 0.34	1.00 ± 0.00	0.96 ± 0.20	0.31
Mouth	0.52 ± 0.51 p=0.71	0.74 ± 0.45 p=**0.02**	0.79 ± 0.41 p=**0.01**	0.07	0.69 ± 0.47 p**<0.001**	0.91 ± 0.30 p=0.34	0.81 ± 0.39 **p=0.01**	0.35

[a]p-value comparing difference in parameters among the subgroups: ASD, siblings and controls. [b]p-value comparing difference in parameters within the same group, between fixation to eyes and fixation to mouth. ASD= autism group, S= sibling group, C= controls

Table 4: Eye tracking parameters for video of a talking face without sound paradigm (mean ± SD).

Image of an Inanimate and Animate Object (Table 5)

Males: No significant difference was seen between the 3 subgroups in males while looking at either the monkey or the laptop AOI. Comparing the two AOIs, ASD males had a shorter total fixation duration and average fixation duration when looking at the inanimate object, meaning an increased interest in the living/animate element in the image.

Females: The gaze pattern was different between the 3 subgroups specifically for the total fixation duration for laptop and monkey (p=0.04 and p=0.03, respectively) with shorter duration in the ASD female group. Similar to males, females preferred looking at the animate object in the image as compared to the inanimate object with shorter time to first fixation and longer total fixation duration/count when looking at the monkey.

	Males				Females			
	ASD	**Siblings**	**Controls**	**P value[a]**	**ASD**	**Siblings**	**Controls**	**P value[a]**
Time to first fixation								
Laptop	0.97 ± 1.21	1.29 ± 1.19	1.21 ± 0.93	0.34	1.17 ± 1.42	0.96 ± 0.73	1.03 ± 1.00	0.99
Monkey	0.50 ± 0.65	1.13 ± 1.26	0.45 ± 0.55	0.37	0.62 ± 1.05	0.39 ± 0.13	0.37 ± 0.33	0.64
	p[b]=0.13	p=0.69	**p<0.001**		**p<0.001**	**p=0.03**	**p<0.001**	
First fixation duration								
Laptop	0.23 ± 0.19	0.21 ± 0.15	0.27 ± 0.22	0.80	0.19 ± 0.22	0.32 ± 0.28	0.28 ± 0.22	0.18
Monkey	0.31 ± 0.41	0.32 ± 0.30	0.36 ± 0.31	0.23	0.27 ± 0.34	0.35 ± 0.25	0.38 ± 0.29	0.12
	p=0.27	p=0.23	p=0.17		p=0.17	p=0.78	p=0.06	
Total fixation duration								
Laptop	0.77 ± 0.60	0.89 ± 0.78	0.87 ± 0.49	0.72	0.63 ± 0.65	1.34 ± 0.81	0.91 ± 0.69	**0.04** ASD<S
Monkey	1.41 ± 1.27	1.36 ± 1.08	1.78 ± 1.06	0.31	1.18 ± 1.98	1.56 ± 0.74	1.93 ± 0.95	**0.03** ASD<C
	p=0.03	p=0.16	**p<0.001**		**p<0.001**	p=0.60	**p<0.001**	
Fixation count								
Laptop	2.60 ± 1.97	3.21 ± 2.44	3.38 ± 2.11	0.38	2.31 ± 2.57	4.73 ± 3.77	3.10 ± 2.57	0.09
Monkey	3.00 ± 2.14	3.68 ± 2.66	4.35 ± 1.85	0.07	3.69 ± 3.13	4.00 ± 1.41	4.50 ± 2.23	0.46
	p=0.47	p=0.61	**p=0.04**		**p=0.04**	p=0.58	**p=0.01**	
Average fixation duration								
Laptop	0.31 ± 0.12	0.26 ± 0.11	0.29 ± 0.18	0.44	0.32 ± 0.19	0.32 ± 0.20	0.31 ± 0.16	0.99
Monkey	0.47 ± 0.42	0.37 ± 0.21	0.41 ± 0.18	0.73	0.35 ± 0.20	0.42 ± 0.19	0.47 ± 0.26	0.29
	p=0.02	**p=0.01**	**p=0.01**		**p=0.01**	p=0.17	**p<0.001**	
Percentage fixation								
Laptop	0.76 ± 0.43	0.84 ± 0.37	0.94 ± 0.23	0.14	0.69 ± 0.47	0.91 ± 0.30	0.88 ± 0.33	0.17
Monkey	0.80 ± 0.40	0.95 ± 0.22	0.94 ± 0.23	0.15	0.81 ± 0.40	1.00 ± 0.00	0.96 ± 0.20	0.08
	p=0.71	p=0.33	p=1.00		p=1.00	p=0.34	**p=0.04**	

[a]p-value comparing difference in parameters among the subgroups: ASD, siblings and controls. [b]p-value comparing difference in parameters within the same group, between fixation to monkey and fixation to laptop. ASD= autism group, S= sibling group, C= controls

Table 5: Eye tracking parameters for the inanimate/animate object paradigm (mean ± SD).

Inverted Scene Paradigm (Table 6)

The measured tracking parameters were similar between the 3 subgroups for male and female groups when looking at the inverted scene with no statistically significant differences observed, reflecting no perceptual or visual alterations due to image inversion.

	Males				Females			
	ASD	**Siblings**	**Controls**	**P value**	**ASD**	**Siblings**	**Controls**	**P value**
Time to first fixation (seconds)	0.63 ± 1.24	0.18 ± 0.43	0.11 ± 0.21	0.68	0.12 ± 0.31	0.08 ± 0.15	0.10 ± 0.23	0.97
First fixation duration (seconds)	0.23 ± 0.22	0.14 ± 0.08	0.15 ± 0.11	0.55	0.13 ± 0.10	0.16 ± 0.10	0.24 ± 0.70	0.90
Total fixation duration (seconds)	2.55 ± 1.24	2.81 ± 1.19	3.05 ± 1.32	0.32	2.96 ± 1.65	3.43 ± 1.26	3.36 ± 1.09	0.50
Fixation count	9.08 ± 4.64	10.95 ± 4.77	11.88 ± 5.05	0.10	9.69 ± 5.01	12.00 ± 5.00	11.83 ± 3.76	0.20
Average Fixation Duration (seconds)	0.30 ± 0.14	0.27 ± 0.10	0.25 ± 0.04	0.46	0.30 ± 0.08	0.30 ± 0.08	0.37 ± 0.69	0.37
Percentage of fixation	0.96 ± 0.20	0.95 ± 0.22	0.91 ± 0.28	0.74	0.87 ± 0.34	0.91 ± 0.30	1.00 ± 0.00	0.06

Table 6: Eye tracking parameters for the image of an inverted scene paradigm (mean ± SD).

Correlation between vision screening and eye examination:

Only a small percentage of the ASD group (5%) had refractive errors and were already wearing corrective lenses. These errors did not interfere with the subjects' eye tracking ability. The intraclass correlation coefficient (ICC) for spherical equivalents indicated excellent agreement between the Plusoptix S12 and manual cycloplegic retinoscopy upon comprehensive eye examination in the control group (ICC= 0.9) and moderate agreement in the high risk group of siblings (ICC =0.6) and ASD group (ICC=0.5). When correcting for SE by a regression model, no difference was determined in eye tracking results indicating that there was no association between eye-tracking parameters and refractive errors in our cohort.

Discussion

Using eye-tracking technology, gender differences in gaze preferences of ASD children were observed as compared to siblings and controls using novel testing paradigms. Significant alterations revealed themselves for male ASD subjects when looking at moving targets with horizontal motion showing more deficits than vertical motion. In female subjects, only a slight difference in vertical motion emerged. To assess subject interactions with scenes containing social contexts, the two paradigms used were an image of a smiling face and a silent video of a talking face. Both genders looked faster and for longer durations at eyes vs mouth. Using a mute video of a talking face showed similar longer fixation at the eyes compared to mouth for both genders. Female ASD subjects, however, attended faster at eyes compared to mouth and with an increased percentage of fixation, not so in males. Compared to siblings and controls, duration of fixation at the mouth diminished regardless of movement (still vs video). Finally, the paradigm with animate/inanimate parts revealed no differences for male and female ASD subjects across the three subgroups, but both genders preferred looking at animate (monkey) vs inanimate (laptop) elements. The inverted scene gaze patterns for both genders were similar.

There are published studies looking at gender differences in eye-tracking parameters in light of sex-biased ASD phenotypes. After recruiting a cohort of exclusively female ASD participants, Ketelaars et al. did not replicate the hyperfocus on the mouth area reported in previous studies but reported longer fixation duration on eyes vs mouth [26]. Gender differences have been explained by both inherent sex-related biological factors and by social modeling and reinforcement of gendered behaviors theories without a clear consensus [26]. Interestingly, using an eye-tracking experiment, Harrop et al. found that children with ASD had similar gender-typical preferences of fixation to different scenes and objects that were consistent with those of TD children [27]. Males with ASD were also more likely to be distracted from looking at faces when objects of circumscribed interests (trains, computers) were shown simultaneously in a paradigm [28]. Another study by the same author used two different scenes in their eye-tracking study: one with rich social context showing two children interactively playing, and one with poorer social context with two children playing side by side [29]. After adjusting for mental and chronological age, ASD females looked more at faces than ASD males in both videos and were comparable to neurotypically developing males [29]. High-risk children (siblings of ASD probands) sex-based differences express preferential fixations as well. Male siblings looked more at the mouth than male controls and female siblings [30]. On the other hand, female siblings looked less at the mouth than all groups including female controls, suggesting social compensatory mechanisms in females as early as infancy [30]. Female siblings at risk for ASD expressed an enhanced attention to social targets correlating with less severe social impairment at the age of 2 years [31].

The results of this study were consistent with others reported in the literature. In a similar eye-tracking study, female ASD subjects looked at the face (mouth and eyes) for a shorter duration than TD females and attended to the eyes for a longer duration than the mouth [26]. Our study also revealed a preference of ASD females to look at eyes rather than mouth, with faster initiation of fixation and increased percentage fixation. In another eye-tracking study, authors employed a paradigm containing multiple social settings, including an audible woman. ASD subjects were not fixating at the mouth of the lady, due to the presence of multiple social distractors like her sound and hand movements [31,32]. In our current paradigm, we used a muted video of a talking face to decrease any auditory influence and still could replicate the decreased duration of fixation to the mouth element in both genders. Mouth gaze preference existed in the literature among high-risk groups of toddlers (siblings of ASD subjects). Male siblings were more interested in looking at the mouth than the eyes as compared to male controls and female siblings [30]. This was not the case in female siblings. They used faces displaying various emotions in a static paradigm rather than videos as in ours [30].

The other paradigm showing gender-based differences in our study was that of the moving target where males with ASD had significant impairments compared to TD males, while females were mostly similar to TD females in their gaze patterns. Although not previously studied, it may fall under the socially demanding category requiring fast saccades and increased interest in visually pursuing a moving target. The alterations mostly observed in the male ASD group were in the horizontal motion paradigm. The testing paradigm with animate/inanimate elements revealed no differences for both male and female ASD subjects across the three subgroups, but both genders preferred looking at animate (monkey) vs inanimate (laptop) elements. This paired visual preference paradigm, although traditionally used to demonstrate that neurotypically developing children prefer looking at biological motion and social scenes compared to children with ASD [33], uncovered similar patterns in our ASD cohort, irrespective of gender. In a previous study gender differences were reported, where the male ASD group expressed gaze preference for inanimate objects (toys, cars and stoves) while females with ASD looked more at animate objects (real faces) or toys symbolizing social content (dolls) [33]. The inverted scene failed to uncover any differential gaze patterns for both genders. Although there is description of an 'inverted face effect' in eye-tracking experiments where the perceptual approach differed from looking at upright faces [24], we did not observe this in our study where we used a semi-naturalistic inverted scene. Gender differences did not appear, only general attention impacted, also observed with other paradigms. When patients with ASD face different stimuli during behavioral therapy, this must be taken into consideration.

Numerous theories exist to explain gender differences in gaze preferences in ASD patients. One supposes that eye contact of infants relates to the level of fetal testosterone as measured in amniotic fluid [34]. Others advocate a Female Protective Effect (FPE). They suggest that there are biological components of the female sex that protect girls from developing ASD [35]. Generally, females with ASD harbor larger genetic insults than males, and the FPE model predicts that siblings of female probands would be at higher risk of developing ASD than siblings of male probands [35,36,37]. Traditionally, researchers support the male-specific risk for ASD theory also known as the "extreme male brain" [36,37]. This theory suggests that there are inherent male biological factors like fetal testosterone, larger amygdala, and heavier brains encountered in typically developing (TD) males that put male infants at a higher risk for the development of ASD [36,37].

Limitations to this work included the relatively small sample size in each group. This did not allow solid subgrouping by age and severity of ASD, which precluded sub-analysis. Additionally, the age group was of a wide range, both for males and females, although mean age was similar between groups for females. For males, ASD subjects were significantly older than their siblings and male controls; however, this further confirmed that the tracking deficits encountered in this group were less likely due to any age confounder (as older subjects would be expected to cooperate better and possibly show less differences from controls). A larger percentage of the control group had pre-existing refractive errors, as they were a group of children regularly presenting to the ophthalmology clinic. However, refractive errors do not affect tracking abilities. This study nonetheless had additional strengths including a comprehensive baseline eye examination to rule out any confounding visual disturbances, and the use of novel testing paradigms including a muted video of a talking face, horizontal/vertical motion, an inverted scene and an image with animate/inanimate elements.

In conclusion, sex dimorphism in gaze preference among

ASD patients was demonstrated using novel eye tracking paradigms. The female ASD group were more interested in looking at social scenes and initiating eye contact, even in the presence of mouth movements in the muted video of a talking face. Males, on the other hand, showed decreased duration of fixation in static and dynamic paradigms. Gender differences for preferential gaze at inanimate vs. living elements in an image were not demonstrated. Male ASD subjects exhibited significant alterations when tracking horizontally moving targets. Deficits in tracking horizontal motion were larger than for vertical motion. These observations are valuable for developing gender specific ASD therapy strategies. Female ASD subjects interacted in a social context comparably to typically developing females, especially in initiating eye contact. They did maintain this eye contact for shorter periods and paid less attention to other social cues, such as a moving mouth. This may also guide future eye tracking research in the field of ASD while selecting differential gender-targeted stimuli for this population.

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Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent

Informed consent was obtained from all individual participants included in the study. Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

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